Proceedings of the
ICABGEH-24

VIII. International Congress on Domestic Animal Breeding, Genetics and Husbandry

September 23 - 25 2024 in Antalya, TÜRKİYE

Editors:
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ISBN: 978-605-06447-4-6

Proceedings of the ICABGEH-24

Antalya, TÜRKİ YE

September 23 - 25, 2024

Organized by

Proceedings of the VIII. International Congress on Domestic Animal Breeding Genetics and Husbandry 2024 "ICABGEH-24"

Publisher:
Black Sea Journals

E-Book Layout, Preparation and Composition:
Hasan ONDER, Ugur SEN and Hasan Alp SAHIN

All published articles were peer-reviewed by Scientific Referee Committee
The organizers do not have any legal liability for to contents of the presentation texts

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PREFACE

This volume contains the papers presented at the VIII. International Congress on Domestic Animal Breeding Genetics and Husbandry - 2024 (ICABGEH-24) was held on September 23 - 25, 2024, in Antalya, TÜRKİ YE.

The ICABGEH-24 has been organized by the Agricultural Faculty of Ondokuz Mayıs University, Agricultural Faculty of Akdeniz University, Agricultural Faculty of Kahramanmaraş Sütçü İ mam University, Biology Faculty of Bydgoszcz University of Science and Technology, and University of Agriculture in Krakow. ICABGEH-24 is the eighth international event of the congress series with the participation of top-rated invited speakers; Prof. Dr. Zeynel CEBECİ (Çukurova University, Türkiye), Prof. Dr. Jørgen Steen AGERHOLM (Københavns University, Denmark), Prof. Dr. Edit Jona MIKO (University of Szeged, Hungary), Prof. Dr. Daniel ZABORSKI (West Pomeranian University of Technology in Szczecin, Poland), and Prof. Dr. Jose Pedro ARAUJO (The Polytechnic Institute of Viana do Castelo, Portugal). This event has been planned to bring together leading researchers, engineers, and scientists in animal science worldwide. It also provided opportunities for the delegates to exchange new ideas and application experiences, establish business or research relations, and find global partners for future collaboration. The organizing committee has done severe planning and preparation to ensure that the international animal science community meets the challenges and moves safely and successfully into the advanced information era. To this end, ICABGEH-2024 has focused on recent developments and research in animal science to protect the environment and food safety. Thus, ICABGEH-2024 has achieved its main twofold objective: Firstly, the presentation of current research works in the field of animal science, and secondly, connecting the animal science community.

Prof. Dr. Hasan ONDER,
President of ICABGEH-24

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How deep can deep learning learn genomic prediction?

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Abstract

Genomic selection is a revolutionizing breeding strategy based on prediction of breeding values of selection candidates using genome-wide markers data. Genomic prediction (GP), started with Genomic Best Linear Unbiased Prediction (GBLUP), later continued with Bayesian alphabet methods (BA), and today has shown a rapid change towards Machine Learning methods (ML) including Convolutional Neural Networks (CNN). However, the present performance comparisons over the last few years on classical statistical methods, ML methods, and deep learning methods suggest that there is no one-size-fits-all model. As a matter of fact, the comparative studies on the classical and state-of-the-arts methods on various farm animal and plant genomes report that the methods of ML and CNN are superior in some cases, but there are no significant differences between their performances in many cases. In this study, the predictive power of different GP methods on the up-to-date research results are reviewed and some future research topics are highlighted in GP area of study.

Key words: *Genomic prediction, GBLUP, Bayesian alphabet, Machine learning, Deep learning*

INTRODUCTION

In animal and plant breeding, the application of genomic selection has been one of the most significant developments in the last two decades. It is reported that genomic selection (GS) uses dense marker data to predict genetic breeding values with 30–40% higher accuracies when compared to pedigree based classical selection methods. GS is based on prediction of the marker effects by running the statistical models on known phenotypes and genotypes animals called reference population.

Genomic prediction (GP) involves many different methods or algorithms to predict breeding values of selection candidates. Appropriate models trained on reference population data are used to predict breeding values only from marker data without the need for phenotype data. When marker effects are estimated, the breeding values of young selection candidates can be estimated with some accuracies higher than 80% in most of breeding programs. Different GP methods and algorithms have been proposed used to achieve more accurate genomic estimated breeding values since the beginning of 90's. The accuracy of genomic predictions is highly dependent on the characteristics of the data such as the number of individuals, the number of markers, and the heritability of the trait. Of course, there are many others like the relationship between the individuals in the reference population and the test individuals being evaluated because the distribution of LD between markers and QTL may vary across generations (Calus, 2010).

Recently, there has been an increasing interest in investigating and comparing the predictive ability of deep learning algorithms such as CNN and RNN, especially statistical machine learning methods such as RF, SVM, GBM, XGB and many more versus traditional GP methods. For some cases it has been approved that machine learning methods can outperform standard GBLUP particularly for complex traits with non-linear relationships. Machine learning algorithms have a great potential capture non-linear structures and interactions among genes and environmental factors better than GBLUP.

Of course, since there are so many prediction methods available, some questions immediately come to mind. Is GBLUP really not good at all? Does Deep

Learning learn genomic prediction deeply enough? Or are the machine learning methods outperforming traditional GBLUP in genomic prediction? The present study seeks the answers to these question by reviewing the various up-to-date research comparing the predictive power of different GP methods, and aims to provide some highlights and suggestions for future research topics in GP area of study.

GENOMIC PREDICTION METHODS

The pioneer of the GP methods was the Genomic Best Linear Unbiased Prediction (GBLUP) as a genomic version of Henderson's pedigree-based BLUP method adapted to dense marker data. GBLUP and its various extended successors have been extensively applied for predicting the milk and meat related traits in genomic selection in livestock breeding. A usual mixed model for GBLUP can be written in matrix notation as shown Equation 1.

$$\boldsymbol{y} = \mathbf{1}\mu + \boldsymbol{Xb} + \boldsymbol{Zg} + \boldsymbol{e} \qquad (1)$$

Where $\mathbf{y} \in \mathbb{R}^n$ stands for the observed values of phenotypes. $\boldsymbol{X} \in \mathbb{R}^{n\times k}$ and $\boldsymbol{Z} \in \mathbb{R}^{n\times l}$ are the incidence matrices for the fixed effects $\boldsymbol{b} \in \mathbb{R}^k$ and the random effects $\boldsymbol{g} \in \mathbb{R}^l$. The genomic values $\boldsymbol{g}$ are assumed to be normally distributed as $\boldsymbol{g} \sim N(0, \boldsymbol{K}\sigma_g^2)$. Here, $\boldsymbol{K}$ is a genomic relationship matrix (GRM) providing variance-covariance structure for genotypes. The GRMs containing the genetic similarities or genomic relationships between the samples are calculated using various various methods like Van Raden, Astle-Balding, Yang etc. The final term $\boldsymbol{e} \in \mathbb{R}^n$ is a vector of random errors following a known probability distribution with zero mean and a common error variance, $\boldsymbol{e} \sim N(0, \sigma_e^2)$.

As a parametric method, the regular GBLUP assumes that all markers contribute the same to the genetic variance, and their effects are normally distributed. However, the assumption of equal variance for all marker effects may not be true for many traits because some of the SNPs are major having large effects. To account for the different effects of different SNP alleles, the Weighted GBLUP (WGBLUP) method has been developed to assign different weights to SNPs (Zhang et al., 2010). In this method, different weights are provided by replacing the numerator $\boldsymbol{ZZ'}$ with $\boldsymbol{ZDZ'}$ in the formula of GRM of the traditional GBLUP. Here $\boldsymbol{D}$ is a diagonal weight matrix, and the *Fst* values or the estimated effects of markers obtained in a previous study are placed as diagonal elements of it. Genomic-supported prediction methods, i.e. HBLUP, combined traditional quantitative genetics models with genomic data, allowing an integration of both approaches. These methods have become particularly useful in predicting traits in species with limited genomic resources, like endangered species management.

Various Bayesian methods have been developed to use in GP, where the differences between them arise from various prior distributions adapted according to the proportions of non-zero effect markers in genomic data. In GP a series of Bayesian methods is generally known as Bayesian alphabets (BA) include BayesA, BayesB, BayesBπ, BayesC, BayesCπ, BayesL (Gianola, 2013) which assume that marker effects are not equal and not normally distributed. BayesA uses a scaled t-distribution as prior while BayesBπ and BayesCπ work with two-component mixture priors with point mass at zero and either a scaled t-distribution or normal distribution respectively (A compact list found in Perez and de Los Campos, 2014). Here, the parameter π is the proportion of zero effect markers. Bayesian Lasso (Bayes L) utilizes a double-exponential prior (Park and Casella, 2008). A general form of a BA model can simply be written as in Equation 2.

$$\boldsymbol{y} = \mathbf{1}\mu + \sum_{j=1}^{m} x_j\beta_j + \mathbf{e} \qquad (2)$$

Where $\boldsymbol{y}$ is the vector of observed phenotypic values, μ is the overall phenotypic mean, $\boldsymbol{x}_j$ is the genotype vector for j^{th} marker and β_j is a vector for the effect of the j^{th} marker.

The BA models apply stronger shrinkage towards zero for markers with small effect while less shrinkage for relatively large effect markers, on the other hand they can be complex to construct and require careful selection of priors. Non-informative priors

can lead to misleading or suboptimal estimates. They often require longer running times than traditional GBLUP models, and the results are easily interpretable when compared to those from the frequentist methods.

Beyond the parametric methods described above in GP, the semiparametric methods such as Reproducing Kernel Hilbert Spaces (RKHS) regression, which use kernels based on distances between samples, have also been studied and are claimed to be better than GBLUP using GRMs because their abilities to capture nonlinear relationships too. In addition to use the prior assumptions for random components in mixed models the semi-parametric models also estimates the required hyperparameters from the genomic data simultaneously. As a widely used example of SPs, a RKHS model is built as in the Equation 1 with an exception using a kernel matrix ($\boldsymbol{K} = \{k(\boldsymbol{x}_{i'}\boldsymbol{x}_j)\}$) instead of using a GRM as the variance-covariance matrix in its random component. Although there are many kernels depending on the structure and distribution of the data and the target of the analysis, the most commonly used Gaussian kernel is calculated as in Equation 3 (Wang, Xing & Schaid, 2015; Montesinos-López et al., 2021; Fu et al., 2023).

$$k(\boldsymbol{x}_{i'}\boldsymbol{x}_j) = e^{-\frac{||x_j - x_k||^2}{h}} \qquad (3))$$

In Equation 3, h is a bandwidth parameter and $||\boldsymbol{x}_j - \boldsymbol{x}_k||^2$ is squared-Euclidean distance between genotypes. The parameter h sets the width of the kernel and is usually used as $2\sigma^2$, sometimes σ^2, and $\sigma^2 = 1$ is usually set as its default value. However, the choice of h is a critical issue and cross-validation (CV) may be required to search for a grid value of h that gives the lowest error or highest prediction accuracy. Kernel models can also be extended to capture the intricate genetic architecture underlying phenotypic traits incorporating the additive, dominance and epistatic genetic components altogether.

Nowadays, when artificial intelligence is popular in every field, it is not surprising that machine learning methods (ML) have become a research topic of widespread interest in genomic prediction studies. There are a plenty number of ML algorithms that can be used in GP, and it is seen that there are more than 180 algorithms available with the package caret in the R environment alone (Cebeci and Gökçe, 2023). In the GP world of ML, Support Vector Machine (SVM), Random Forests (RF), and Gradient Boosting Machines (GM) have been the most studied algorithms (Montesinos-López et al., 2022). Compared to GBLUP and BA models, ML models have fewer assumptions about normality and distribution of data. In many researches, thus, it was concluded that ML can capture nonlinear relationships for complex traits and therefore expected as more successful compared to the classical statistical methods.

RF, a type of Bagging Classification and Regression Trees (CART) algorithm, is a popular aggregation technique based on combining or aggregating various decision tree models, developed to solve the overfitting problem encountered in modeling with classical CART (Breiman, 2001). RF repeatedly draws bootstrap samples from the training set and creates trees from these samples using the Gini index. At each decision node, it creates the desired number of independent trees by working on samples consisting of randomly selected features and randomly selected observations. Since these trees increase the variance of the models, more trees are needed. Thus, a forest consisting of a large number of trees is worked on. After the forest is created, the final model is produced by taking the average of the output values of all trees in the forest (Cebeci et al., 2022). RF has a number of hyperparameters that need to be tuned, and the search for optimal parameters, typically done through grid searches, can take quite a long time.

SVM proposed by Boser et al. (1992) is a supervised learning algorithm that has been widely used in the field of bioinformatics for a long time. The adaptation of SVM for continuous traits or regression problems is called Support Vector Regression (SVR). SVR uses linear models to implement nonlinear regression by mapping the input space (markers) to a feature space of a different dimension using a nonlinear kernel function and then linear regression on this feature

space. An SVR model using a radial kernel can be written as in Equation 4 (Hastie et al., 2009).

$(x)=\beta_0+h(x)T\beta$ (4)

In the equation 4, the basis functions, $h(x)T$, are linear (or nonlinear) transformations of one (or more) independent variables (x) and are combined by adding the weight vector (β). The important tuning parameters in SVR are the cost parameter (λ) and gamma (γ). The values of these parameters are predefined by assigning a series of different values to the parameters and cross-validating them to determine the model that shows the best predictive performance. If this is not done, it is advisable to start by taking λ =2 and γ= 0.01.

Extreme Gradient Boosting (XGBoost) is a special adaptation of GBM algorithms. Unlike traditional GBM, which works with gradient descent in the function space, XGBoost works with the Newton-Raphson algorithm in the function space using a second-order Taylor approximation in the loss function. In some studies, XGBoost (https://xgboost.ai) has been shown to work much faster than many algorithms including RF and artificial neural networks, and has high predictive power. XGBoost can be used to effectively solve most tree-based data science and machine learning problems for both regression and classification purposes. The algorithm currently has implementations for R, Python, Perl, JVM, Ruby, Scala, Swift, Java, Julia, C++ with the ability to work in large distributed environments such as Hadoop, SGE, and MPI.

As alternative popular subject for GP, Neural Networks (NN) came into scene to model high-dimensional data with complex interactions since a couple of decades. Multilayer Perceptron (MLP) is a feedforward NN that learn by feedforward but also backpropagation. MLPs use forward propagation for inputs and backpropagation for updating weights. They consist of multiple hidden layers and use nonlinear activation functions such as sigmoid in classification problems. MLPs can be designed as shallow or deep with the number of hidden layers, which include at least one input layer, one hidden layer and one output layer as shown in Figure 1.

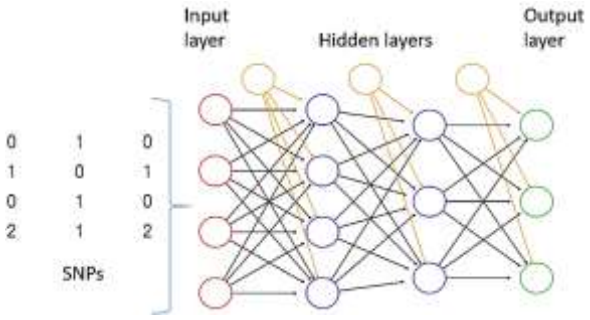

Figure 1. Architecture MLP.

Deep learning (DL) is becoming increasingly studied due to hypotheses for its success in finding complex patterns in large datasets. A popular example of DL is the use of convolutional neural networks (CNNs) to predict genomic values and phenotypes. CNNs (or ConvNets) are deep neural networks that learn from input data that has some kind of spatial structure using convolutional layers. CNNs consist of a series of convolutional layers, pooling layers, and smoothing layers, at least one of which replaces the hidden layers in MLP. Instead of matrix multiplication in feedforward deep neural networks, inputs are convolved by applying a set of filters of predefined dimensions, as shown in Figure 2.

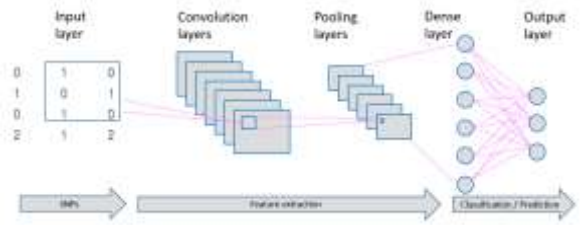

Figure 2. Architecture of CNN

CNNs work by first creating low-level data representations with filtering that reveals differences, and then using them to create higher-level representations such as outlines and contours (Montesinos López et al., 2022). The inputs of successive output layers are generated by the action of the activation function in the previous convolutional layer. Finally, the pooling operation is performed and the dimensions are reduced. In pooling, one of the values of the mean, maximum or

minimum is used, resulting in the concatenation of the kernel output. In addition to CNN's ability to reduce the number of hyper-parameters required to train the model through convolution, they also partially exploit the correlation between nearby data. Therefore, CNNs are expected to outperform deep feedforward neural networks when applied to the same problem.

As another method in the DL lane, Recurrent Neural Networks (RNN) are widely used networks for sequential data such as text, image sequences, and time series. RNNs are neural networks whose inputs are not fixed. They generate input sequences that can be used to transform the output sequence into a sequence by flexibly taking into account contextual information. Although RNNs are reported to outperform CNNs and other DLs on data that is highly dependent on sequence order in learning long-term information, through these iterations or iterations in networks, they have not yet been studied in the GP field. Input data is processed sequentially by RNNs. Past information can be implicitly stored in hidden state units through iterative computation where cyclic connections exist. The model output is then produced as an integrated result considering the current input and all previous inputs. Long Short-Term Memory (LSTM) is a network architecture that solves the problems of long time delays due to the exponential increase or decay of the error back propagated by analyzing the error stream in RNNs.

Although also not yet tried by the GP community, Graph Machine Learning (GML) algorithms have recently started to come into play in the GP scene. As an expanded DL, GML can reveal the intrinsic information about markers in addition to the relationships between them by using graphs. Some inspiring applications of GML in genomic prediction can be seen at *http://snap.stanford.edu/data/index.html*.

Finally, the ensemble methods have been studied to improve prediction accuracies by combining multiple prediction methods/models of GP For instance, a combination of GBLUP and machine learning called deepGBLUP has been recently developed as a joint deep learning networks and GBLUP framework for accurate genomic prediction in Korean native cattle (Lee et al., 2023).

COMPARATIVE STUDIES ON GP METHODS

Given a large number of options available for GP, many studies have been conducted to compare them, as some of them are shown in Table 1. In many of the benchmark scenarios examined, it was assumed that ML methods could automatically select the most relevant markers to improve prediction performance. It has been thought ML methods can manage high dimensional data better. In this regard, ML have been successfully used to predict yield traits in maize by processing vast genomic datasets, often yielding higher predictive accuracy compared to GBLUP.

Since there are a large number of SNPs per individual in genomic data, techniques such as Bayesian Lasso regression facilitate variable selection, which has been considered to lead to more robust models. Research in livestock breeding has demonstrated that these methods can outperform GBLUP by focusing on the most influential markers. In addition, many studies have been conducted suggesting that Bayesian models, in which non-zero effect major SNPs are considered with varying rates and different distribution priors, can be successful.

There are research findings that ML algorithms including CNN work better than traditional GBLUP, Bayesian alphabet methods including some of their extended versions. On the other hand, many studies report that there is no significant superiority of ML algorithms against the traditional ones (Fernandes et al., 2020; John et al., 2022; Lourenço et al., 2024). Indeed, comparing the predictive power of various classical and ML methods, it is reported that the predictive performance of the method depends on both the data and the target traits. Similarly, Chaafi et al (2023) stated that there's no universal method that can be applied to enhance the accuracy of prediction regardless of the domain of application.

Table 1. Research findings on comparison of the GP methods

Study	Species	Examined genomic prediction methods	Suggestions
Pedrosa et al (2024)	Dairy Cattle	BayesL, MLP, CNN, GBLUP	GBLUP
Da Costa et al (2022)	Simulation	GBLUP, MARS, RBF, DT, ANN (MLP)	MARS
Mota et al (2024)	Cattle	STGBLUP, MTGBLUP, BayesA, BayesB, BayesC, BRR, BLasso, SVR, MLP	SVR, MTGBLUP
Chen et al (2023)	Sugarcane	GBLUP,Ext.GBLUP RF, MLP, CNN	Ext. GBLUP, RF
Wan et al (2022)	Pig	GBLUP, ssGBLUP, BayesHE, SVR, RF, KRR, AdabootR2	Adaboost.R2, KRR, ssGBLUP
Sirsat et al. (2022)	Wheat	BayesA, BayesL, RF, GBM, MLP	GBM, RF
Bellot et al. (2018)	Human	GBLUP, BayesB, BayesRR, MLP, CNN	Slightly CNN

CONCLUSIONS

Accurate prediction of genomic breeding values lies at the heart of genomic selection in plant and animal breeding. In this regard, to answer the question in the title of the article, it is clear that despite some research results indicating that DL is better for GP, it still needs research for unquestionably revealing its higher performance for several traits. Montesinos-López et al (2021) stated that there are clear evidences that DL algorithms capture nonlinear patterns more efficiently than conventional genome based, but they should be applied to large training-testing data sets. On the other hand, it was also stated that fine tuning of various hyper-parameters should be taken into consideration because the performances of the algorithms might be highly sensitive to the dimensional character or linearity structure of the dataset of estimated traits (Wang et al., 2022). Similarly, Chen et al (2023) state that DL models require more effort for model optimization and parameterization. The interpretability of ML models is also questionable due to their black box nature. Indeed, the relationship between the input and output is not simple to explain. Recently, a great deal of research has emerged to address the need to design understandable ML systems that can be grasped by the human mind and to understand and explain the predictions made by opaque models such as CNNs (Marcinkevičs and Vogt, 2023). In their frontier works Conard et al. (2023) reviewed learning various approaches for genomic studies to understand variable importance in nonlinear and non-parametric models.

Another way to achieve success is to work with GWAS-assisted GP models by sub setting genomic data containing the reduced number of markers with the smallest *p*-value according to the GWAS results (Medina et al., 2021). A pruning procedure for the related LDs can be employed to reduce the computational costs. Moreover, a systematic sampling or random sampling can be applied to build a reduced dataset of markers to use with ML algorithms as proposed by Li et al. (2018).

The tools and systems are needed to drive ease the application of GP methods in an integrated framework. In this regard, the SKM library is a valuable tool for genomic selection as demonstrated by Montesinos-López et al. (2022). As another valuable tool, Pérez-Enciso and Zingaretti (2019) provides a pipeline in their DL implementation guide based on the Keras API available at *https://github.com/miguelperezenciso/DLpipeline* with all the codes on Jupyter notebook. Additionally, the BGLR package by Perez and de Los Campos (2014) is a comprehensive tool to fit various types of parametric and semi-parametric Bayesian regressions to continuous and categorical traits in R.

In GP context, the use of second or third order models such as Multivariate Adaptive Regression Splines (MARS) is recommended as a valuable option for GP because it takes into account gene interactions and provides more interpretable results compared to ML methods. In fact, Da Costa et al (2022) concluded that MARS was the most successful method and automatically

modeling nonlinearities and interactions of the predictor variables.

One of the main challenges is dealing with thousands of molecular markers spanning the entire genome. The intense processing costs because of ever-increasing marker dataset and capturing interactions and non-additive effects between genotypes lead to new efficient and fast techniques for hyper-parameter tuning. So further research is also needed for the practical applicability of ML and DL based methods in large breeding programs as reported by Pedrosa et al. (2024).

In the future, a special focus to the algorithms which able to efficiently handle high dimensional data or the data being bigger day to day.

In summary, although DL algorithms are hypothesized to have great potential in fitting and extracting patterns from large, noisy datasets, their use in breeding programs is not even in its infancy. Therefore, more research should be conducted to find new insights for GP. The limited research findings of ML for breeding programs have not yet fully explained the hypotheses of these models to improve genomic prediction of important traits. Therefore, further research is needed to consider the above-mentioned issues in larger datasets.

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Dystocia prediction in dairy cattle using machine learning methods

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Abstract

The aim of the study was to apply different machine learning methods for dystocia prediction in dairy cows. The first subset of methods included artificial neural networks (ANN), multivariate adaptive regression splines (MARS), naïve Bayes classifier (NBC), general discriminant analysis (GDA) and logistic regression (LR). The whole dataset of calving records (3041) was divided into two subsets: heifers (1342) and cows (1699). Each of them was further randomly split into a training (50%), validation (25%) and test (25%) set. The following predictor variables were used for model development: calving age, mean calving difficulty score for the daughters of the heifer's (cow's) sire, farm category, calving season, calf sex, preceding calving interval, mean daily milk yield for the previous lactation, preceding calving difficulty, and mastitis during pregnancy (the last four predictors only for cows). Two classification systems for the predicted variable (calving difficulty) were adopted: two categories (easy and difficult) and three categories (easy, moderate and difficult). The highest sensitivity on the test set (0.85 and 0.60 for the heifers and cows, respectively) was obtained for ANN. In the case of the three-class system, the highest percentage of correctly predicted difficult calvings (0.85 and 0.07 for the heifers and cows, respectively) was recorded for MARS. The most important predictors of calving difficulty were the mean calving difficulty score for the sire daughters and calf sex. The second subset of machine learning methods included: classification and regression trees (CART), chi-square automatic interaction detection (CHAID) trees, quick, unbiased, efficient, statistical trees (QUEST) and generalized linear model (GLZ). The whole dataset of heifer calving records was randomly divided into a training (75%) and test (25%) set. Additionally, model development involved a 10-fold cross-validation. The following predictor variables were used for model construction: calving age, mean calving difficulty score for the daughters of the heifer's sire, farm category, calving season and calf sex. The predicted variable was calving difficulty with three categories (easy, moderate or difficult). The highest percentage of correctly predicted difficult calvings on the test set (0.85) was obtained for CHAID and the most important predictors were the mean calving difficulty score for the sire's daughters, farm category and calving age. The last subset of machine learning methods included random forest (RF) and boosted trees (BT). Both datasets of calving records (362,635 and 883,744 for the heifers and cows, respectively) were randomly divided into a training (75%) and test (25%) set. The following predictor variables were used for model development: calving age, gestation length, calving season, and calf sex, breed of the calf's sire, previous calving difficulty and lactation number (the last two only for cows). Predicted variable (calving difficulty class) consisted of only two categories (easy and difficult). In addition, different proportions of difficult calvings were contained in the training and test sets (ranging from 50 to 3%). The highest sensitivity on the original test set (0.55 and 0.42 for the heifers and cows, respectively) was obtained for BT. The most important predictor variables were: calving age, gestation length and previous calving difficulty. It can be concluded that the above-mentioned methods were quite effective in predicting dystocia in dairy cattle based on the provided set of predictor variables but there is still room for improvement.

Key words: *Dystocia, prediction, dairy cattle, machine learning, model performance*

Heat stress in dairy cattle: *Impacts on welfare and productivity, and strategies for mitigation*

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Abstract

The recent rapid changes in climate have posed significant challenges to ecosystems, species survival, and the long-term sustainability of global animal husbandry systems. Climate change and global warming have notably increased the prevalence of heat stress, which presents a significant challenge to dairy cattle production. This phenomenon has led to adverse effects on both animal welfare and productivity. The physiological and behavioral consequences of heat stress negatively impact animal welfare, often resulting in health issues such as metabolic disorders, lameness, weakened immune function, and digestive disturbances. Additionally, heat stress reduces feed intake, leading to declines in milk protein, fat, and lactose levels, as well as impaired reproductive performance. Behavioral changes, including reduced activity, prolonged standing, and increased aggression driven by the need for shade and water, further underscore the animals' discomfort, raising ethical concerns. In light of these challenges, it is increasingly important for livestock managers to implement effective mitigation strategies that maintain animal health and welfare without compromising environmental sustainability. This presentation aims to provide a comprehensive overview of the effects of heat stress on dairy cattle welfare, focusing on the physiological, behavioral, psychological, and productive aspects. It will also highlight key mitigation strategies, such as environmental interventions (e.g., shading, cooling systems), nutritional adjustments, genetic and management adaptation, all of which are essential for promoting both the welfare and long-term productivity of dairy cattle in a changing climate.

Key words: *Climate change; Dairy farming; Animal welfare; Animal diseases; Sustainable animal husbandry*

Bovine genetic diseases – Old syndromes in a new perspective

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Abstract

Congenital malformations have always fascinated humans but it was not until recently, that deeper knowledge on their causes have become more evident. The development within genomic analysis has until now resolved the molecular cause of almost 200 bovine genetic diseases. However, the cause of certain well-known congenital syndromes in cattle have remained unexplained. The Danish Bovine Genetic Disease Program was in its current form established in 1989 and many genetic diseases have been investigated. Lately, we have focused on some well-known congenital disorders: schistosoma reflexum, congenital syndromic Chiari-like malformation and the bulldog calf syndrome. Genetic analyses of such cases sampled as part of the bovine genetic disease surveillance in Denmark have confirmed their genetic etiology. Investigations into a fourth congenital syndrome, perosomus elumbis, are underway.

Key words: *Schistosoma reflexum, Spina bifida, Bulldog calf, Chiari malformation; Perosomus elumbis*

INTRODUCTION

Congenital malformations have throughout history always puzzled and fascinated people. In the Middle Ages, occurrence of malformed animals was considered supernatural, while during the Enlightenment (17th and 18th centuries), scientists tried to understand why congenital malformations occurred. However, it was only after Gregor Johann Mendel's (1822-1884) description of the laws of heredity that a deeper understanding of the importance of genetics for the development of malformed fetuses grew. Still there was a long way to go to understand the causes themselves. In the 1920s, the first hereditary malformations in cattle were identified based on the Mendelian laws, but the actual understanding of *why* malformations develop had to await the breakthrough in genetic technology that enabled the identification of mutations, and thereby an understanding of the molecular mechanisms that lead to abnormal fetal development.

The number of recognized genetic disorders in cattle has raised steadily since Lerner in 1944 published a list of 44 lethal or sub-lethal condition in cattle until today (July 26, 2024), where 270 diseases with Mendelian inheritance are recognized and of which at least one likely causal variant is known for 189 (OMIA 2024).

The development within genetic technology is growing fast, which has made identification of mutations and other chromosomal abnormalities easier, more rapid and less costly.

Surveillance for genetic diseases in cattle was in Denmark formally established in 1989, which has led to the recognition and eradication of several genetic conditions. During recent years, we have tried to solve the riddle of bovine congenital syndromes that have been known for many decades or even longer. Here, an introduction to our recent research on a number of these classical bovine syndromes is given.

SCHISTOSOMA REFLEXUM

Schistosoma refleuxum (SR) is a congenital syndrome characterized by dorsal retroflexion of the spine. The spine will thereby get an almost U-shaped form with the consequence that the hindlimbs point in the direction of the head. The retroflexion of the spine, hinders ventral midline closure of the fetal body cavities and therefore their contents (intestines, etc.) protrude (Figure 1). Fetus affected by SR are born alive at the end of a normal length gestation period, but

die immediately due to impaired respiration. SR is associated with dystocia and affected fetuses must be released by cesarean section or fetotomy unless the size of the fetus/the size of the maternal birth canal allow assisted vaginal delivering.

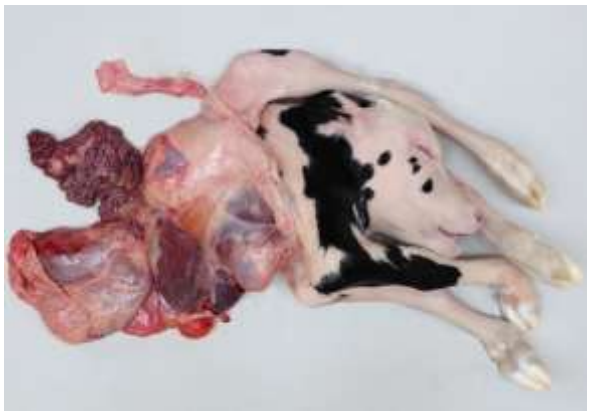

Figure 1. A typical case of schistosoma reflexum in a Holstein calf.

SR occurs sporadically in cattle but its etiology has not been known. Recessive inheritance has been proposed as some Holstein cases were genetically related (Citek 2012), but a genetic cause has not been proven. Genetic relationship in cattle breeding as evidence of inheritance should be interpreted with caution as some breeding lines have been widely used and therefore some sires may be present in the pedigree of most cattle of certain breeds.
We investigated DNA of 23 SR cases, mostly Holsteins (n=20) and when available, also their parents. Evidence of recessive inheritance was not found, but we were able to detected frameshift and missense variants in 12 cases. These genes belonged to the class of haploinsufficient loss-of-function genes, are involved in embryonic and pre-weaning lethality or are known to be associated with severe malformation syndromes in other species and therefore considered causal for development of SR. Study details have been published in Jacinto et al. (2024a).

CONGENITAL SYNDROMIC CHIARI-LIKE MALFORMATION

Congenital syndromic Chiari-like malformation (CSCM) is a sporadically occurring malformation that is often associated with dystocia (breech presentation). The most striking lesion is an almost bilateral symmetric arthrogryposis of the hindlimbs associated with hypo-/dysplasia of the associated muscles.

Figure 2. A case of congenital syndromic Chiari-like malformation. Notice the bilateral symmetric hindlimb arthrogryposis, lumbar lordosis and the flattened neurocranium. Reprinted from Jacinto et al. (2024b).

In many cases, a closer examination reveals a hairless skin lesion in the dorsal midline of the lumbar spine. This represents spina bifida, i.e. a lesion where the vertebral arch is not complete so the spinal cord is exposed. The spina bifida is sometimes covered by skin (a so-called spina bifida occulta).
The rostal aspect of neurocranium is often flattened. This causes compression of the developing brain and due to insufficient space, parts of the brain is dislocated caudally and some parts even protrude through the foramen magnum into the vertebral canal, a so-called Chiari type II like malformation. We performed a genomic analysis of 14 cases of CSCM (mainly Holsteins) and when available, also their parents and discovered that CSCM is due to extensive genetic heterogeneity, including both possible recessive alleles and dominant acting *de novo* mutations. The two recessive missense variants were identified in the genes *SHC4* and *WDR45B*, respectively.

Figure 3. Chiari type II-like malformation. Notice that the occipital lobes are dislocated towards the foramen magnum and the cerebelleum and parts of the brain stem protrudes into the vertebral canal.

Analysis of 1209 sires included in the 1000 Bull Genomes Project (Hayes and Daetwyler, 2019) revealed the presence of carriers of both variants. The *SHC4* variant may originate from the sire Wapa Arlinda Conductor (born 1970) or one of his parents, while the *WDR45B* variant could be traced to the sire Mascol (born 2000). Partial monosomy of chromosome 2 was identified in two other cases. Study details have been published in Jacinto et al. (2024b).

PEROSOMUS ELUMBIS

Perosomus elumbis (PE) is a congenital syndrome characterized by lack of development of the lumbar, sacral and coccygeal spinal segments (Figure 4). The spine and the spinal cord end near the thoraco-lumbar junction and the caudal part of the body therefore consists of a hypoplastic pelvis, hypoplastic hindlimbs with arthrogryposis, and an abdominal wall sac-like structure that encloses the abdominal organs (Agerholm et al., 2014).

This syndrome occurs sporadically and is associated with dystocia as the fetus is often in breech presentation.

We have recorded several cases in the Danish surveillance program for bovine genetic diseases. Some have occurred sporadically, but we have also encountered a cluster of cases after a Belgian Blue sire used for crossbreeding. Multiple cases after a single sire indicates that a dominant paternal germline mutation is the cause of PE in this particular family and that the sire is mosaic for a causal mutation, but as found for SR and CSCM, PE can probably develop due to a range of genetic scenarios.

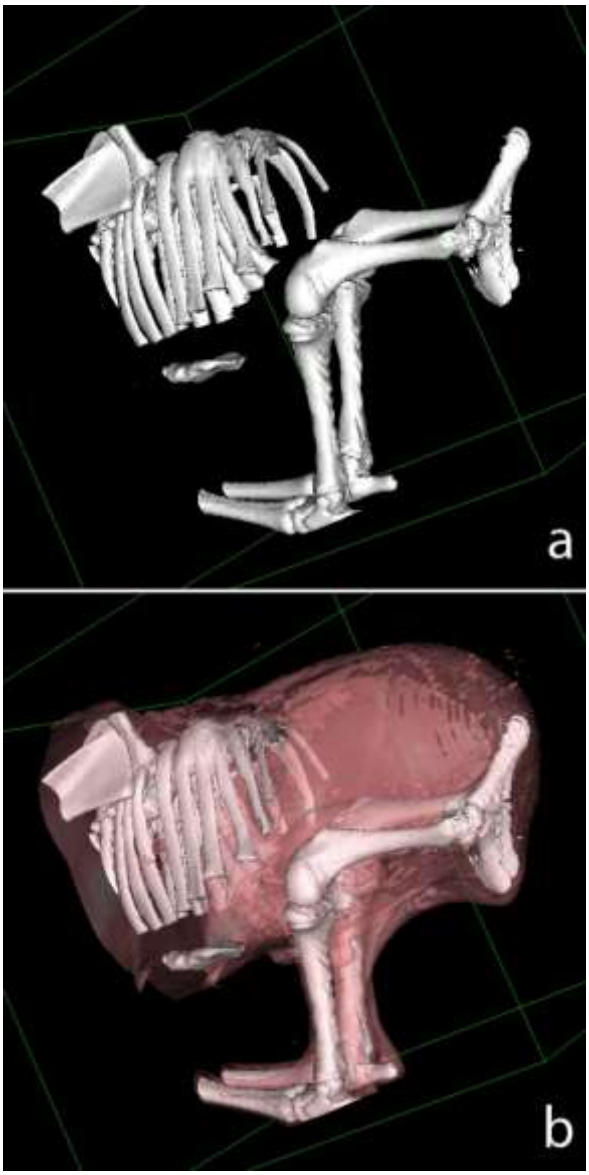

Figure 4. Surface rendered computed tomography image of perosomus elumbis. The lack of spinal development caudal to the thorax is visualized in part **a**, while part **b** also visualizes the calf's skin surface. Reprinted from Agerholm et al. (2014).

BULLDOG CALF SYNDROME

The bulldog calf syndrome (BCS) or bovine congenital generalized chondrodysplasia was the first hereditary disorder to be reported in cattle (Seligmann, 1904) and was therefore given the number A1 on Lerner's

list from 1944. BCS was originally reported in the Dexter breed, but BCS has turned out to consist of a very heterogeneous group of skeletal malformations that have disturbed endochondral ossification in common (Agerholm, 2007). The phenotype and mode of inheritance varies across different cases.
The "prototype" of BCS has dysplasia of the viscerocranium, a doomed neurocranium, a short compact corpus and short compact limbs (Figure 5). The tongue is often protruding because of the short viscerocranium and eventration of parts of the abdominal organs is a common finding, which is due to the short body that reduces the size of the body cavities.

Figure 5. Typical case of the bulldog calf syndrome. Holstein fetus. Reprinted from Jacinto et al. (2020).

Genetic analyses of BCS cases have in many cases identified a causal mutation in the *collagen type II alpha 1 chain* gene (*COL2A1*) as reviewed by Jacinto et al. (2020). Some cases have occurred in clusters related to a single phenotypically normal sire, who had then been mosaic for the causal mutation and carried the dominant mutation for BCS at a certain level in his spermatozoa (1-21%) (Bourneuf et al. 2017, Daetwyler et al. 2014). Other cases have been due to a *de novo* mutation occurring in the developing embryo.
Study details have been published in Jacinto et al. (2020).

CONCLUSIONS

Identifying the cause of a congenital syndrome in cattle is important to prevent spread of genetic disorders, to increase the breeders' economy by reducing loss of offspring and future breeding animals, and to reduce the critical animal welfare issues related to birth of defective offspring. However, as many important congenital syndromes share a common morphology (disease prototype), detailed genetic investigations are needed to differentiate between inherited and *de novo* mutations that have occurred in the developing embryo. While the former may require implementation of breeding restrictions, lethal mutations occurring in the developing embryo are not transmitted to the next generation.

ACKNOWLEDGEMENTS

Professor Cord Drögemüller and his research group at the University of Bern is greatly acknowledge for the genetic analyses.

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Minhota breed veal raised on a semi-ıntensive production system: sex effect ın carcass measurements

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Abstract

The Minhota cattle breed, located in the Northwest of Portugal has meat production as its main function. Cattle for slaughter are sold as veal at ages below 8 months and beef between 8 and 12 months. This product is in high demand by the industry, butchers and supermarket retailers. Although both intensive and extensive production systems are used with this breed, the semi-intensive system remains the most common. In this system, the calves are stabled and the cows graze whenever the weather allows. The calves suckle from dams twice a day, morning and night, and are supplemented from an early age with hay and concentrate or cornmeal. This breed is certified under the "Meat, Milk and Cheese Producers Group of Minhota Breed CRL, AGROMINHOTA", voluntary beef labelling system. For Minhota veal live weight (LW) varies between 180-425 kg for males and 150-350 kg for females. High-quality beef carcasses are essential in an increasingly demanding market. Carcass weight and measurements vary with the age of the animals, influenced equally by environmental and genetic factors. This study aims to evaluate the carcasses of Minhota veal commercialised by AGROMINHOTA. Involved 31 veal (11 males and 20 females) of the Minhota breed from 14 farms, slaughtered at PEC Nordeste in Penafiel. Live weight (LW) and cold carcass weight (CCW) were recorded, and dressing percentage (DP) was calculated. Carcass morphology was collected according to De Boer et al. (1974) and Franco et al. (2013) with the following parameters: carcass length (CL); leg length (LL); leg width (LW); and leg perimeter (LP). Carcass compactness index (CCI) (cold carcass weight / length of carcass) and hind-limb compactness index (HLCI) (leg length/leg width) were also calculated. The effect of sex was evaluated for the different characteristics using a general linear model (GLM), with IBM SPSS Statistics version 29.0.2.0.

These animals were slaughtered at an average age of 209.4±37.08 days for males and 229.0±27.83 days for females. Sex influenced veal LW, CCW and CCI, but not DP, CL, LL, LP, LW and HLCI. For males and females, following results were obtained respectively: LW of 311.8±35.92 and 300.5±24.85 kg (P<0.05); CCW of 180.4±24.61 and 162.3±16.36 kg (P<0.05); DP of 57.7%±2.14% and 54.0%±2.73% (P>0.05). Measurements (cm) were as follows: LC of 114.7±3.79 and 114.4±2.73 (P>0.05); LL of

73.4±2.27 and 73.2±2.06 (P>0.05); PL of 98.4±5.32 and 96.1±4.26 (P>0.05); WL of 22.1±1.08 and 21.6±1.52 (P>0.05). Concerning the indices: CCI, 1.6±0.19 and 1.4±0.13 (P<0.05); ILTP of 3.3±0.18 and 3.4±0.20 (P>0.05). Despite the variability on LW and CCW between the sexes, most of the remaining measurements show a reduced coefficient of variation (CV), revealing homogeneity. The results obtained are consistent with studies on other beef cattle breeds, where males tend to have heavier carcasses, larger leg perimeters, and more compact carcasses than females.

Key words: *Minhota breed, Semi-intensive production system, Carcass quality.*

Impact of breed and physiological status on blood content of goats in arid conditions of Algeria

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Abstract

The Damascus breed, known for its prolificacy and milking ability, is recently imported in Algeria. Farmers tend to improve the local native herds by crossbreeding with Damascus bucks. The aim of the current investigation was to study the effects of physiological status on blood progesterone and some biochemical parameters in Shami goats and their crosses with local breed in arid conditions of Algeria. Ten does with an age range of 1.5- 3 years and BSC between 2.5 and 3.5 were used. Female goats were divided into two groups of five animals each: Damascus, and crossbred (Damascus x Arbia). All females were estrus synchronized and naturally mated. Blood samples were collected before intravaginal sponge insertion (non- pregnant), in early (30 days after sponge removal), mid (90 days), late pregnancy (130 days) and after kidding (30 days post-partum). Results demonstrate a significant effect of the reproductive stage on progesterone (P4) levels in both groups, on glycemia and cholesterolemia in crossbred does ($p<0.05$) and on albuminemia and uremia in Damascus ones. Concentrations of triglycerides, total proteins, globulin and creatinine revealed no significant difference between physiological phases in both groups ($p>0.05$). Breed effect was detected in early and mid-pregnancy for P4, in early pregnancy and lactation for total proteins and in lactation for globulin with lower concentrations in Damascus compared to crossbred does. Changes in P4 and biochemical profiles of both groups reflect the female goat's adaptation to increased requirement of gestation and lactation in arid conditions of Algeria.

Key words: *Damascus goat, crossbred, reproductive status, progesterone, biochemical metabolites*

INTRODUCTION

Unlike the Damascus (Shami) breed, the Algerian Arbia goat has moderate prolificacy and low milk yield (Kouri *et al.*, 2018). Therefore, Algerian farmers tend to improve the local native herds by crossbreeding with imported Damascus bucks. Goats are highly seasonal breeders and their reproductive performance is affected by genetic, environmental, and physiological factors (Hussain, 2015). The assessment of progesterone levels at different reproductive stages is a valuable method for determining the female's fertility status (Talebi *et al.*, 2012). The blood biochemical parameters provide useful data for evaluating animal physiological, metabolic, nutritional, health status, and productivity. Nevertheless, results depend on many factors including breed, gender, age, nutrition, reproductive status (pregnancy and lactation), stress, disease, season, and farming system. The present study investigated the variations in blood progesterone and some biochemical indices at different reproductive stages in Damascus and Crossbred (Damascus x Arbia) does raised under Algerian arid conditions.

MATERIALS AND METHODS

The Ethical Committee of the Institute of Veterinary and Agronomic Sciences of Batna 1 University (Algeria) approved all procedures.

Experimental location

The experiment was conducted, in a private farm located at El-Doucen, Ouled Djellal, an arid region of southeastern Algeria (latitude 34° 06' N; longitude 5° 01' E) characterized by a dry climate, low rainfall, and dry

pastures. The study involved the period before estrus synchronization, the period of pregnancy, and 30 days post-partum.

Animals and Experimental Design

Ten clinically healthy female goats, aged 1.5 to 3 years with BSC ranging between 2.5 and 3.5, were divided into equal groups as Group 1: Damascus, and Group 2: Crossbred (Damascus x Arbia). The flock was bred under a semi-extensive farming system. Animals were grazed on natural pasture and received barley grain, wheat bran, and barley straw. Water was distributed once a day. Estrous cycles were synchronized by intravaginal impregnated sponges of 20mg Flurogestone acetate (Chronogest, Intervet) for 11 days, and 400 IU eCG (Folligon, Intervet)/doe was injected intramuscularly twenty-four hours before device removal. Damascus bucks were used for natural mating after the sponge withdrawal. The day of mating was considered as day 0 of pregnancy. Gestation was confirmed by transabdominal ultrasonography 45 days after mating.

Blood sampling, Progesterone, and biochemical metabolites assays

Samples were aseptically collected from animals through jugular venipuncture using vacutainers without anticoagulant before morning feeding, at five different times: before estrus synchronization, in early (30 days after sponge removal), mid (90 days), late pregnancy (130 days) and early lactation (30 days after kidding). The serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at –20°c till assayed for progesterone and biochemical parameters.

Progesterone (P4) concentration was quantitatively estimated using a chemiluminescence immunoassay kit (Progesterone II kit, Cobas®, Roche). Concentrations of biochemical metabolites (glucose GLU, cholesterol CHOL, triglycerides TG, Total Ptotein TP, albumin ALB urea, creatinine CRE) were measured by enzymatic colorimetric test using commercial kits (Spinreact, Spain) with an automated biochemical analyzer (Mindray BS 330E). Globulin (GLO) concentration was calculated as the difference between total protein and albumin concentrations.

Statistical analyses

Data of progesterone and biochemical parameters at different reproductive stages in Damascus and Crossbred goats were presented as means ± standard deviation (SD). The statistical analyses were carried out using the GraphPad Prism program (version 7.00). Two-way Analysis of Variance (ANOVA) and Tukey's post-hoc test was applied to evaluate the effects of the breed and reproductive status on the concentration of progesterone and biochemical indicators, and detection of significant differences between means concentrations. The results were considered statistically significant when $p<0.05$.

RESULTS

The mean values (±SD) of P4 and biochemical metabolites levels measured during different reproductive stages (premating, pregnancy, and lactation) in Damascus and crossbred goats are presented in Tables 1, 2, and 3. The P4 concentration (Table 1) increased with gestation progress and dropped to basal levels after parturition in both groups. The highest levels were registered in late gestation in crossbred does. The significant influence of physiological status ($p <0.01$, $p < 0.001$) was noted between the premating period and early lactation and stages of pregnancy (early, mid, late) in both groups, while significant differences between groups were observed in early ($p<0.01$) and mid-gestation ($p<0.001$).

No significant differences in glycemia were present among the different samples in Damascus goats (table 2) while it increased significantly in the crossbred group, in late pregnancy compared to mid-gestation. A significantly lower cholesterolemia ($p<0.05$) was noted at early lactation compared to the premating sample in crossbred goats. For uremia, a significant decrease (Table 3) was recorded in the Damascus group at early lactation compared to pregnancy stages. Triglycerides, creatinine, serum total protein and globulin profiles in both groups and albumin in the crossbred group showed no significant effect of reproductive status, while albuminemia of Damascus does was significantly higher at mid-gestation compared to other stages of pregnancy and

lactation. Breed effect was detected in early pregnancy and lactation for TP and in lactation for GLO.

Table 1. Effect of reproductive status on progesteronemia (mean ± SD) in Damascus and Crossbred goats

Parameter	*Group*	*Reproductive status*				
		S1	*S2*	*S3*	*S4*	*S5*
P4 (ng/ml)	*G1*	*0.29±0.15* ***a***b******	*9.20±0.64* ***e***f*** A*****	*14.84±1.50* ***h*** i*** A******	*22.04±0.46* ***c***j******	*0.56±0.27* ***g******
	G2	*0.40±0.16* ***a***b******	*11.74±1.12* ***e***f******	*20.26±0.36* ***h** i******	*22.38±1.89* ***c***j******	*0.49±0.15* ***g******

P4: *progesterone;* ***G1****: Damascus*(n=5)*;* ***G2:*** *Crossbred* (n=5)*;* ***S1****: premating;* ***S2****: early pregnancy;* ***S3:*** *mid-pregnancy;* ***S4****: late pregnancy;* ***S5****: early lactation;* ***A****: Damascus vs Crossbred;* ***a:*** *premating vs early pregnancy;* ***b****: premating vs Mid-pregnancy;* ***c:*** *premating vs late pregnancy;* ***d:*** *premating vs early lactation;* ***e****: early pregnancy vs mid-pregnancy;* ***f:*** *early pregnancy vs late pregnancy;,* ***g****: early pregnancy vs early lactation;* ***h****: Mid-pregnancy vs late-pregnancy;* ***i****: Mid-pregnancy vs early lactation;* ***j:*** *late pregnancy vs early lactation;* ********: *p<0.01;* ******:*** *p< 0.001.*

Table 2. Effect of reproductive status on serum energetic metabolite levels (mean ±SD) in Damascus and Crossbred goats

Blood indices	Group	Reproductive status				
		S1	S2	S3	S4	S5
GLU (g/l)	G1	0.53±0.05	0.42±0.07	0.47±0.07	0.46±0.04	0.51±0.07
	G2	0.49±0.05	0.52±0.07	0.43±0.05 **h***	0.56±0.03	0.49±0.09
CHOL (g/l)	G1	0.88±0.22	0.85±0.30	0.79±0.16	0.74±0.15	0.73±0.13
	G2	1.12±0.14 **d***	0.89±0.13	0.91±0.11	0.90±0.14	0.76±0.06
TG (g/l)	G1	0.13±0.04	0.09±0.01	0.08±0.02	0.12±0.03	0.12±0.02
	G2	0.18±0.08	0.12±0.04	0.11±0.03	0.16±0.05	0.14±0.04

GLU: *glucose;* ***CHOL****: cholesterol;* ***TG****: triglycerides;* ***d:*** *premating vs early lactation;* ***h****: Mid-pregnancy vs late-pregnancy;* **** :*** *p < 0.05.*

Table 3: Effect of reproductive status on serum Protein metabolite levels (mean ± SD) in Damascus and Crossbred goats

Blood parameters	Group	Reproductive status				
		S1	S2	S3	S4	S5
TP (g/l)	G1	70.40±3.44	69.40±5.13 **A***	70.00±4.18	70.40±3.51	64.20±3.27**A***
	G2	75.40±3.44	76.00±1.23	75.00±4.30	72.20±4.21	71.40±3.91
ALB (g/l)	G1	29.68±0.41	29.38±1.14 **e***	32.60±4.02 **h****	28.64±1.94	28.72±1.07 **i***
	G2	30.70±1.00	30.90±1.07	32.26±1.38	30.20±2.08	29.30±0.85
GLO (g/l)	G1	40.72±3.58	40.02±5.48	37.40±3.32	41.76±3.83	35.48±2.94 **A***
	G2	44.70±2.94	45.10±1.88	42.74±3.56	42.00±3.93	42.10±3.79
URE (g/l)	G1	0.40±0.08	0.48±0.04 **g****	0.49±0.06 **i****	0.45±0.03 **j***	0.31±0.06
	G2	0.38±0.10	0.45±0.08	0.46±0.08	0.42±0.07	0.40±0.08
CRE (mg/l)	G1	7.92±0.48	7.68±0.62	7.16±0.63	7.14±0.41	8.10±0.80
	G2	7.08±0.84	7.02±0.81	7.36±0.79	7.52±1.15	7.88±1.05

TP *: total proteins ;* ***ALB*** *: albumin ;* ***GLO*** *: globulin;* ***URE****: urea;* ***CRE:*** *creatinine;* ***A****: Damascus vs Crossbred ;* ***e****: early pregnancy vs mid-pregnancy;* ***g:*** *early pregnancy vs early lactation;* ***h:*** *Mid-pregnancy vs late-pregnancy;* ***i:*** *Mid-pregnancy vs early lactation;* ***j:*** *late pregnancy vs early lactation;* ****:*** *p < 0.05;* *********: p < 0.01.*

DISCUSSION

Mean P4 concentrations were at basal levels (< 1 ng/ml) in both groups before sponge insertion, suggesting that goats were in an anestrous period or early estrus (Pineda, 2003). The significant increase after mating indicated that estrus was efficiently induced in females. The rising trend with gestation advance observed in both groups and the decline to basal levels after kidding were reported earlier (Kadzere et al., 1996). Since the main site of production of P4 in pregnant goats is the ovary, the irregular increase of progesteronemia observed in literature during gestation may be attributed to the possible differences in corpus luteum composition and activity (El-Tarabany et al., 2020). On the other hand, the highly significant effect of pregnancy stage observed on blood P4 was previously

described by Sousa et al. (1999). After parturition, P4 dropped to its basal level as a result of corpus luteum regression (Talebi et al., 2012).

In the current experiment, the significant variation in P4 levels between Damascus and crossbred females at early and mid-gestation may be related to breed in agreement with Mmbengwa *et al.* (2009) who referred large variations within and between goat breeds and nutritional regimens. The investigations conducted by Abd El-Hamid et al. (2017) disagreed with these findings. The difference between groups could also be attributed to age, parity, or litter size (Hussain, 2015; Madan et al., 2020).

No significant effect of reproductive status on glycemia was recorded in Damascus does in agreement with Allaoua et al. (2021). The increasing trend during late pregnancy in crossbred does, also reported in sheep by Kandiel et al., (2016), indicates increased metabolic needs with advanced pregnancy. Additionally, rising levels noted in early lactating Damascus females compared to high pregnant ones were described earlier but with significant variation (Cepeda-Palacios et al., 2018). This rise may be related to the elevation of thyroid hormone during lactation which represents an adjustment to mobilize glucose for lactogenesis (Mbassa and Poulsen, 1991). It may be also ascribed to the recovery of feed intake and improving the energetic status of females after kidding (Mohammed et al., 2016). Moreover, there is no statistical difference between goat breeds' glycemia during different reproductive stages. In contrast, great variation was reported by Cepeda-Palacios et al., 2018). The difference could be attributed to age, feed intake, and differences in the animal's metabolism, litter size, season, or region (Khan et al., 2020).

Cholesterol levels were not significantly affected by physiological status in Damascus goats which conforms to earlier reports (Allaoua et al., 2021). On the contrary, crossbred does show lower values in the lactation period ($p < 0.05$) compared to the premating period in line with the findings of Berrani et al. (2021). Variations may be due to the direct involvement of cholesterol in reproductive processes and its intensive utilization by the mammary glands for milk synthesis. Additionally, there was no significant breed effect on cholesterolemia which is supported by the results of Al-Bulushi et al. (2017). The comparison among the different physiological periods revealed no significant change in TG content in Damascus and Crossbred does. Similar finding was reported by Jimoh et al. (2019). In contrast, Abdul-Rahaman et al. (2019) described an increasing profile in pregnant goats compared to non-pregnant ones, related to elevated hepatic synthesis or deficient energy intake. No such differences were registered throughout the current experiment suggesting adequate nutrient intake in both groups.

On the other hand, the studies by Waziri et al. (2010) and Abdul-Rahaman et al. (2019) did not find any notable differences in total protein concentrations between non-pregnant and pregnant does at various gestational stages in conformity with our results in both groups. On the contrary, Allaoua et al. (2021) observed a significant impact of reproductive status, showing higher total protein levels ($P<0.05$) during lactation compared to other stages. However, the lack of significant variations in albumin and globulin levels across different physiological stages aligns with the current results of GLO for both groups and ALB in the crossbred group. In Damascus goats, a notable decrease was observed in late gestation and early lactation compared to mid-gestation. The reduction of TP and ALB levels towards the end of pregnancy and during lactation is attributed to the rapid extraction of immunoglobulins by the mammary gland for colostrum production (Allaam et al. 2014). Concerning albumin levels, no breed effect was found in various sampling times, consistent with Mohammed et al. (2016), and Al-Bulushi et al. (2017) in Omani breeds. However, Damascus goats displayed statistically lower total protein levels ($P<0.05$) during early gestation and in early lactation, along with lower globulin levels in early lactation compared to crossbred does. Njidda et al. (2013) similarly found significant differences ($P<0.05$) in TP, ALB, and GLO levels among Nigerian indigenous goat breeds (Kano Brown, Borno White, and Sokoto Red), highlighting

comparable findings regarding total protein and globulin but not albumin.

No significant impact of reproductive status on uremia was registered in Crossbred females in support of observations of Waziri et al. (2010). The marked decline ($P< 0.05$) in early lactating Damascus goats compared to pregnant ones, is compatible with their significantly higher nitrogen requirements (Madan et al., 2020). In addition, the non-significant effect of the breed is consistent with the findings of Castagnino et al. (2015).

The creatinine results concur with those of Berrani et al. (2021), who found no significant variation related to the reproductive status. In contrast, Soares et al., 2018 recorded higher values in pregnant than lactating goats due to increased energy requirement, to maternal mobilization of protein for fetal muscle development, and the elimination of the fetal organic residues in maternal circulation. This evolution was not shown in the present study suggesting the absence of maternal muscle protein catabolism and indicative of the renal health of animals. On the other hand, results demonstrated no significant difference in CRE levels across all the reproductive stages between crossbred and Damascus does. A similar finding was reported by Abd El-Hamid et al. (2017) in Damascus and Baladi does.

CONCLUSIONS

Similar patterns of change of P4 were observed in Damascus and Crossbred females. The increased metabolic activities during gestation and lactation significantly affected certain biochemical indices, indicating an individual metabolic adaptation of females to meet the higher energy and protein demands of these critical stages. Further studies involving a larger number of goats are necessary to explore the productive, and reproductive performances of Damascus goats and their crosses in Algerian conditions. Additional research on various hormonal and biochemical parameters, considering potential sources of variability such as body condition, age, parity, litter size, genotype, season, management systems, and nutrition, is recommended to clarify the physiological adaptation mechanisms in this breed.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Dr. Loughraieb S., Dr. Hafdi W., Dr. Benali D., and Mr Zine B. for cooperating in the study.

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POU1F1 and DGAT1 gene polymorphism and Its relationship with milk yields in goats

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Abstract

Although the livestock sector has a huge potential today due to the increasing world population and income level, it still does not produce at the desired and required level. Researchers use various methods by taking advantage of the possibilities offered by science in order to make goat meat and milk consumption, which they see as having an important place in the human food chain, more effective, to minimize product losses by increasing disease resistance, and to increase fertility. When we look at the composition of goat milk, it substitutes the closest protein and fat values to human milk. At the same time, the amount of selenium, which has an important place in nutrition, is quite high in its composition. In addition to its high nutritional content, thanks to the small diameter fat globules it contains, the suitability of its consumption in terms of ease of digestion has been revealed in studies conducted in people with lactose intolerance. Due to the economic difficulties, the productivity of Hair and Honamlı goats, which have a strong adaptation ability and are easy to care for, is decreasing day by day. Considering the nutritional and therapeutic properties of goat milk, there is a need for more milk production. Studies have revealed that many genes affect milk yield. Megan et al. The two most basic candidate genes are DGAT1 and POU1F1. These two genes have an effect on milk yield in goats in the following processes.

Key words: *Milk yield, Milk composition, DGAT1, POU1F1, Goat*

Variation in the antibacterial activity of honeys of different origins against *Erwinia amylovora*

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Abstract

Beekeeping is an important agricultural activity that not only its crucial role in pollination but also useful products for human health. However, they can also lead to the spread of various diseases and pests, especially Erwinia amylovora. The hypothesis was that if the honey in the colony had antibacterial activity against that pathogen, the disease might be less likely to spread from these colonies. In this context, the current study investigated the antibacterial activity of honey of different origins against the Erwinia amylovora. In the study, honey samples obtained from Georgia, Erzurum, Konya, and Antalya, as well as a control group, were used. Firstly, to determine bacterial growth in honey, each honey sample was incubated on nutrient agar and then the macrobially were counted. Subsequently, honey samples, control group and Erwinia amylovora were added to the prepared liquid nutrient and incubated at +25 °C for 24 hours. To determine the antibacterial activity of each sample, microbial growth was examined on nutrient agar at 0 and 24 h after incubation. Although no bacterial growth was observed in the honey samples, the antibacterial efficacy rates for Georgia, Erzurum, Konya, Antalya, and the control group were 38.47%, 41.28%, 39.73%, 41.88%, and 34.95%, respectively. According to the results of analysis of variance, the antibacterial effect of Antalya honey was significantly higher than that of Georgia (P< 0.05). However, the differences between the other samples were considered statistically insignificant. As a result, this study suggests that if the honey within the colonies is high quality, Erwinia amylovora might be less likely to spread from these colonies. In addition, feeding bee colonies with high-quality honey can also reduce the incidence of Erwinia amylovora disease. Therefore, it is concluded that the health of bee colonies is not only important for the quality of bee products, but is also an important practice that needs to be considered in a broader context.

Key words: *Honey quality, Antibacterial activity, Erwinia amylovora, Colony welfare, Pollination*

INTRODUCTION

Erwinia amylovora is a gram-negative bacterium of the Enterobacteriaceae family that poses a global threat to commercial apple and pear production, and there are limited control options, the most effective of which is antibiotic application (Piqué et al., 2015). However, chemical applications offer temporary benefits and they pose significant risks to both the environment and human health. Therefore, the growing societal demand for sustainable control solutions has driven the need to explore biological methods, such as antagonists (Esteban-Herrero et al., 2023). Some studies conducted for biological control of fire blight; Turkish pollen and propolis extracts (Basim et al., 2006), Moringa oleifera leaf extracts (Fontana et al., 2022), bacterial isolates (Esteban-Herrero et al., 2023), silver nanoparticles (AgNPs) obtained using aqueous leaf and petal extracts of Tagetes erecta L. (Zarate-Escobedo et al., 2024). To create a healthier society, it is essential to focus on controlling the disease while simultaneously working on preventing it.

In a study, the role of honeybees in the dispersal of *Erwinia amylovora* in pear blossoms and the subsequent development of fire blight was investigated. Researchers have discovered that honeybees spread the disease from contaminated pear flowers or colonies during the first 48 hours. However, after this period, no live bacteria remain in the honeybee's body and intestine (Alexandrova et al., 2002a). In other study, the longevity of *Erwinia amylovora* in the beehive products (honey, beeswax, pollen,

and propolis), honeybee body, intestine, and beehives, was studied. The research determined that while the bacteria continued to survive at low temperatures, they died at high temperatures. However, the bacteria could not survive in propolis under any circumstances (Alexandrova et al., 2002b). In Korea, *Erwinia amylovora* was found to remain alive on the outside of bee bodies for 15 days and is most commonly found in the abdomen compared to other body parts. Bees contaminated with bacteria transferred them to healthy unripe apple fruits, shoots and flowers for 10 days. In addition, clean bees were also infected with bacteria from contaminated plant parts. Researchers reported that honeybees are vectors of *E. amylovora* and may play a role in causing new outbreaks of fire blight disease (Choi et al., 2022). Therefore, healthy colonies are of great importance for the control of *Erwinia amylovora*; colony health is closely related to nutrition and environmental conditions. Therefore, the hypothesis of the present study was that if the honey in the colony had antibacterial activity against that bacterium, the disease might be less likely to spread from these colonies. In this context, the current study investigated the antibacterial activity of honey of different origins against the *Erwinia amylovora*.

MATERIAL AND METHODS

The honey samples used in this study were purchased from roadside vendors in Georgia and Erzurum. Samples from Konya and Antalya were obtained directly from beekeepers. The beekeeping practices for the Georgia and Erzurum samples are unknown. However, the honey from Konya was produced in the highlands of Bozkır district, Konya, and the colonies were fed with syrup, including during the summer season. The Antalya honey sample was produced in Antalya without any feeding. Then, the bacteria growth and antibacterial activity in different origins of honey samples were determined. Firstly, to determine bacteria growth, the methodology described by Goszczynska et al. (2000), was used to determine the bacterial microbial load within the honey samples. Stock solutions were prepared from the honey samples at a ratio of 1:10 honey to Distilled Sterile Water. The obtained stock solutions were diluted 9-fold by adding 100 µl of each to microtubes containing 900 µl of sterile distilled water. Spot inoculation was performed with 10 µl of each dilution step onto Nutrient agar medium. The Petri dishes with spot inoculations were then incubated at 25 °C for 48 hours. Subsequently, the bacterial cell density forming colonies per milliliter was determined based on the developed colonies. Secondly, to detect the antibacterial activity, the methodology used by Fan et al. (2021), was modified to determine the antimicrobial properties of honey samples. Honey samples were added to Nurieth broth liquid medium at a concentration of 500 ppm equivalent for each honey sample, which had been sterilized in an autoclave. The control group did not receive any honey samples and was only treated with nutrient broth medium. Subsequently, an Erwinia amylovora isolate cultured for 24 hours in Nutrient agar medium was adjusted to a density of OD_{600}: 0.1 (10^8 cfu/ml) using a UV-visible spectrophotometer. The resulting suspension was added to 50 ml Nutrient broth media containing 500 ppm honey and to the control group, which received Nutrient broth medium, with 500 µl of the suspension containing 10^8 cfu/ml *Erwinia amylovora*. At 0 and 24 hours of incubation, suspensions prepared were diluted by adding 100 µl to microtubes containing 900 µl of sterile distilled water to achieve a 16-step dilution. Spot inoculation was performed with 10 µl of each dilution step onto Nutrient agar medium. The Petri dishes with spot inoculations were then incubated at 25°C for 48 hours. The developed colonies were counted, and the bacterial cell density forming colonies unit per milliliter was determined. The cfu/ml values of the developed colonies were subjected to logarithmic transformation, and the obtained data were analyzed using one-way ANOVA and Tukey HSD test with a 95% confidence level for multiple comparisons using the 'agricolae' package available in the R statistical software language (de Mendiburu and de Mendiburu, 2019).

RESULTS

Each honey sample was incubated on nutrient agar and then the macrobially were counted but no bacterial growth was observed in the honey samples. However, honey samples, control group and Erwinia amylovora were added to the prepared liquid nutrient and incubated at +25 °C for 24 hours the effect of samples on bacteria was statistically important (P< 0.05; Table 1).

Table 1. Variance analysis of the bacterial impact of honey samples

Source	df	$\sum_{i=1}^{n} x^2$	$\bar{X}^2$	F	(Pr>F)
Honeys	4	11.675	2.9188	5.6493	0.02223*
Error	10	6.278	0.6278		

*P< 0.05

Although no bacterial growth was observed in the honey samples, the antibacterial efficacy rates for Georgia, Erzurum, Konya, Antalya, and the control group were 38.47%, 41.28%, 39.73%, 41.88%, and 34.95%, respectively (Table 2).

Table 2. The effect of honey samples on *Erwinia amylovora* bacteria

Honey samples	0 hour (cfu/ml)	24 hours (cfu/ml)	Log transformation	Effect (%)
Georgia	0.4×10^{9}	1.25×10^{20}	20.09 ± 0.01^{a}	38.47
Control	0.6×10^{7}	3.4×10^{19}	19.44 ± 0.38^{ab}	34.95
Erzurum	0.1×10^{10}	3.13×10^{19}	19.28 ± 0.61^{ab}	41.28
Konya	0.1×10^{9}	1.6×10^{19}	19.20 ± 0.02^{ab}	39.73
Antalya	0.7×10^{8}	1.8×10^{18}	17.44 ± 1.67^{b}	41.88

[a, b]: The difference between the groups is important (P< 0.05)

According to the results of the analysis of variance, the antibacterial effect of Antalya honey was significantly higher than that of Georgia (P< 0.05). However, the differences between the other samples were considered statistically insignificant (Table 2). To better understand the differences between samples, the change in bacterial count in honey samples is visualized in Figure 1.

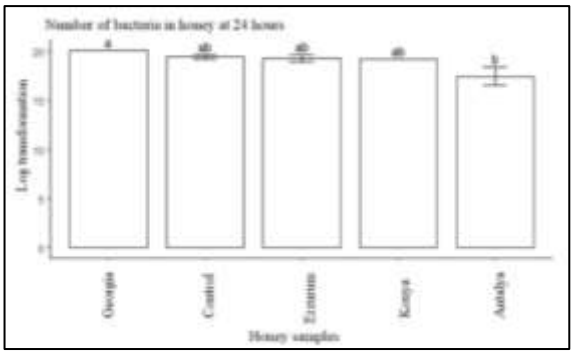

Figure 1. The change of number of bacteria in honey samples at 24 hours

DISCUSSION

Economic and ecological methods protect biodiversity by minimizing the use of chemicals and are critical for sustainable agriculture. Bee products may be significant for this purpose due to their bacterial effects. Hossain et al. (2022) reported that honey is a sweet and viscous food produced by honeybees from flower nectar and has an active antibacterial effect against clinically important pathogens. Honey is a compound that is rich in biochemical components, with over 180 different molecules from various families. This natural product is derived from the processing of nectar within the bee's abdomen. The antimicrobial properties of honey are attributed to its bioactive components, which exhibit potent antibacterial effects against both Gram-positive and Gram-negative bacteria (Szeda, 2017). Different types of honey, based on their floral and geographical origins, may possess varying antibacterial properties, which can be attributed to their unique chemical composition (Irish, 2011). Regarding the antibacterial effect of honey, the present study found that the effect of honey samples on *E. amylovora* is important. The difference between samples of Antalya and Georgia was important and Antalya honey had a stronger bacterial effect (P< 0.05). However, the differences between other honey samples were not significant. Alexandrova et al. (2002b) found that *Erwinia amylovora* survived in honey 42 days at +4 °C and less than two days at 28 and 35 °C. Karadal et al. (2018) stated that multifloral, chestnut and pine honey samples exhibited antibacterial activity against bacteria (*Listeria monocytogenes*, *Staphylococcus aureus*,

Escherichia coli O157: H7 and *Salmonella Enteritidis*) with different values. Tan et al. (2009) found that Tualang honey produced in Malaysia exhibited variable activities against *A. baumannii* and *S. maltophilia*, in the same range as manuka honey, and that tualang honey could potentially be used as an agent against these microorganisms. The results of the previous studies and the current study were found to be similar in that honey has an antibacterial effect and this may vary depending on the honey origin.

CONCLUSIONS

The study suggests that if the honey within the colonies is high quality, *Erwinia amylovora* might be less likely to spread from these colonies. In addition, feeding bee colonies with high-quality honey can also reduce the incidence of *Erwinia amylovora* disease. Therefore, it is concluded that the health of bee colonies is not only important for the quality of bee products, but is also for spread of disease.

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Animals disease and their prevention: livestock technologies

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Abstract

Animal disease control and the promotion and protection of animal health are essential elements of any effective animal production program. Despite remarkable progress in the diagnosis, prevention and control of animal diseases, animal health still leaves much to be desired in developing countries, which leads to significant economic losses and hinders the improvement of the productivity of the cattle. The purpose of this article is to bring together members of industry, researchers and veterinarians to share knowledge, exchange ideas and guide timely and targeted research to control infectious animal diseases. But it will also show the new means of technology used to fight against these diseases. The session will include production techniques, veterinary sanitary measures, different modern methods of animal vaccination, vaccination technologies, disinfection and veterinary sanitary control of livestock products. These brief presentations will lead to a cooperative discussion between the audience and panel members, identification of issues, discussion of different methods of the technology, and assimilation of information for future research strategies. Finally solutions to fight against animal diseases.

Key words: *Animals, disease, industry,prevention, production*

DNA methylation profile associated with fertility trait in goat using whole-genome bisulfite sequencing

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Abstract

This work designed to investigate the epigenetic regulation of ovulation rate through DNA methylation profile using WGBS. The whole genome DNA was extracted from ovaries of Zaraibi goats belonging to two different fertility groups: high (HFG) and low (LFG) fertility groups. The extracted DNA was subjected to WGS after treatment with bisulfite. The findings declared that a small difference in the DNA methylation levels is present among high and low fertility groups. The methylated C frequencies in contexts: CG, CHG and CHH were 89.89%, 2.39%, 7.72% and 90.19, 2.34%, 7.47% in high and low fertility groups, respectively. This finding showed that the level of methylated C in CG context is in directionally opposite with fertility trait where this level is lower in HFG than that in LFG. In contrast, the levels of methylated C in contexts CHG and CHH are higher in HFG than those in LFG groups. Despite this small difference in the methylation levels, there are many DMR and DMG were identified in the two groups. One-hundred and seventy fertility-related genes with different frequency in methylation levels were selected for functional enrichment analysis and the results declared the strong relation between methylation patterns of DMGs and fertility trait. It is concluded that DNA methylation patterns of Zaraibi goat ovaries may be responsible for difference in ovulation rate trait between high and low fertility groups through their important roles in folliculogenesis and oocyte ovulation rate. Also, this study declared the opposite association between the methylation levels and expressions of differentially methylated genes which are related to fertility phenomena in goats

Key words: *DNA methylation, Ovulation rate, Litter size, WGBS, Goat.*

Determination of the effects of different protein sources added to the maturing medium on glutation peroxidate enzyme activity in cattle oocytes

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Abstract

This study was conducted to determine the effect of protein sources added to the culture media during in vitro maturation (IVM) on the glutathione peroxidase (GPx) enzyme activity of bovine oocytes. Oocytes obtained from bovine ovaries were IVM cultured in bicarbonate buffered tissue culture media (TCM-199) containing 10% fetal calf serum (FBS), 4 mg/mL bovine serum albumin (SSA), 1 mg/mL polyvinyl alcohol (PVA) and without protein source for 22 hours in a humidified environment containing 38.5 °C and 5% CO2. Following the maturation period, cumulus-oocyte complexes (COCs) obtained were subjected to a procedure to separate the cumulus cells from the oocytes. The oocytes separated from the cumulus cells were washed with phosphate-buffered saline (PBS) containing polyvinyl alcohol and devoid of Ca2+ and Mg2+. Subsequently, the oocytes were divided into predetermined sample sizes and stored in microtubes for further processing. After IVM culture, using a commercial kit, glutathione peroxidase (GPx) enzyme activity in oocytes was determined spectrophotometrically at a wavelength of 340 nm. In the study, the levels of GPx enzyme activity in the cell extracts isolated from the oocytes in each experimental group did not change over time, and the GPx enzyme activity in the oocytes with PVA added to the culture medium was lower than the oocytes in the other experimental groups (P>0.05). As a result of this study, the effects of different protein sources on GPx enzyme levels, one of the indicators of antioxidant activity in bovine oocytes, were determined, and FBS and BSA, as protein sources, could be more effective on bovine oocyte development.

Key words: *Oocyte, Bovine, Antioxidant activity, Protein, Maturation*

INTRODUCTION

Intracellular ATP production occurs as a byproduct of oxidative metabolism; however, undesirable free oxygen radicals (ROS) are also produced as a result of this metabolic activity (Sen and Kuran, 2018; Sen, 2021; Sen et al., 2022). ROS production can vary depending on the rate of oxidative metabolism, and high metabolic activity can increase ROS production (Sturmey et al., 2009). High amounts of ROS can lead to apoptosis by causing cellular enzymes to become ineffective, membrane lipid peroxidation, and DNA damage in cells (Halliwell, 1996). Cellular damage can lead to apoptosis by increasing metabolic activity and producing more ROS with more nutrient consumption (Sturmey et al., 2009). Most media used during in vitro maturation of oocytes contain a protein source to support maturation. For example, the addition of various protein sources such as bovine serum albumin, fetal calf serum, etc. has been reported to be almost mandatory in the in vitro maturation of bovine oocytes (Sen, 2015; Kocyigit et al., 2015). Studies have shown that protein sources added to the in vitro culture medium can reduce the negative effects of substances that have toxic effects on the oocyte and embryo, protect cellular components by creating a defense against ROS, and regulate redox potential (Flood and Shirley, 1991; Koçyiğit et al., 2015). Protein supplementation has also been reported to provide growth factors that support oocyte maturation (Koçyiğit et al., 2015). Therefore, this study aims to understand how the addition of different protein sources to in vitro oocyte maturation cultures can affect glutathione peroxidase enzyme levels, an indicator of cellular antioxidant activity. It will also help to determine which protein source can

provide more effective antioxidant activity in the maturation of bovine oocytes.

MATERIAL AND METHOD

Obtaining Cumulus-Oocyte Complexes (COCs)

Bovine ovaries were obtained from a local slaughterhouse (Florya A.Ş.) after slaughter. A total of 200 oocytes were obtained from 50 ovaries during the study. The ovaries were transported to the laboratory within approximately 2 to 3 hours after slaughter in a medium containing 0.9% NaCl (S5886; w/v) at a temperature of 35-37 °C and supplemented with 0.1% v/v antibiotic solution (A5955; 10,000 IU penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL). Cumulus-oocyte complexes (COCs) were obtained from follicles 2 to 8 mm in diameter using a 10 mL disposable syringe attached to an 18-gauge needle (Figure 1). COCs were washed several times in Hepes-modified commercial culture medium (H-TCM–199; M7528) supplemented with 1% v/v antibiotic-antimycotic solution and 100 µg/mL L-glutamine. COCs were morphologically examined and only those with a non-atretic cumulus structure, compact and regularly granulated cytoplasm were selected for IVM (Figure 1) (Sen, 2022).

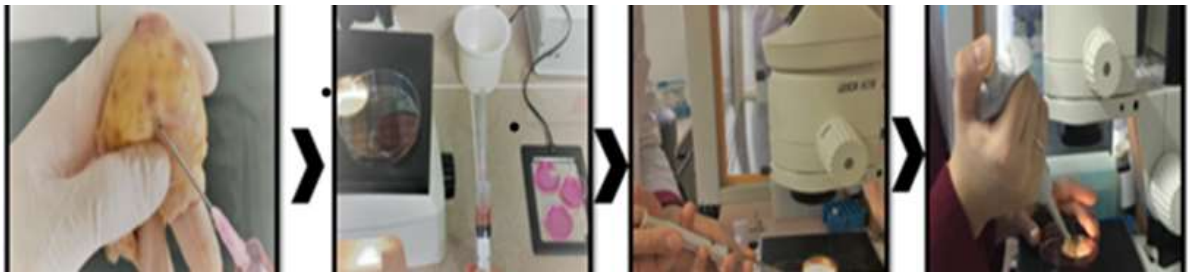

Figure 1. Aspiration and evaluation of oocytes using an injector.

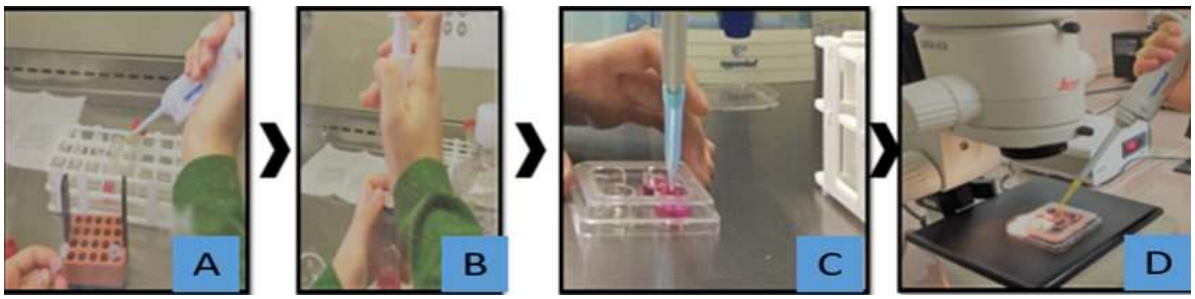

Figure 2. A) Addition of protein sources to the maturation medium. B) Filtration of the maturation medium. C) Transfer of the maturation medium to a four-well petri dish. D) Placement of oocytes to be matured into a four-well petri dish and incubation.

In Vitro Oocyte Maturation

Bovine oocyte IVM culture was performed as suggested by Sen (2021) (Figure 2). The IVM culture medium was prepared from a commercial tissue culture medium (TCM-199; M4530) supplemented with Earle's salts and L-glutamine, containing 5.5 µg/mL sodium pyruvate, 1% v/v antibiotic-antimycotic solution, and buffered with sodium bicarbonate. The selected COCs were washed three times in H-TCM–199 culture medium and then randomly distributed into bovine oocyte maturation culture groups. Bovine oocyte maturation culture groups were: (a) IVM + 4 mg/mL BSA, (b) IVM + 10% FCS, (c) IVM + 1 mg/mL PVA, and (d) no macromolecule supplementation. 500 mL of maturation medium was transferred to each well of a four-well petri dish (Nunc, Roskilde, Denmark), covered with 300 mL of mineral oil (Sigma M-5904), and left in the incubator to warm and gas. COCs in each medium group were cultured for 22 hours at 38.5 °C in an atmosphere containing 5% CO2 and

95% humidity (Cevik et al., 2011; Sen and Kuran, 2018).

Glutathione Peroxidase (GPx) Enzyme Activity

At the end of the maturation period, COCs were washed twice in H-TCM–199, then taken into 1.5 ml Eppendorf tubes and vortexed for 5 minutes to separate them from the surrounding cumulus cells (Labart Mult-Mixer). The oocytes separated from the cumulus cells and remaining bare were washed 3 times in phosphate-buffered saline (PBS) containing 1 mg/ml polyvinyl alcohol and devoid of Ca2+ and Mg2+, and stored in microtubes at -80°C until GPx enzyme activity analysis, with 25 oocytes in 10 µl PBS. The samples thawed on the day of analysis were sonicated at 50W for 2 minutes for enzymatic extraction (Sen et al., 2022). Then, 50 µl was taken from each of the samples and incubated in 2M 1X Assay Buffer (50 mM Tris-HCI, pH 7.6, 5 mM EDTA) and 2 µl DTT solution for 15 minutes on ice. Each sample was vortexed 3 times at 30-second intervals. Samples were centrifuged at 10,000 x g for 15 minutes at 4°C. Supernatants were collected using a pipette. GPx activity of oocytes was determined spectrophotometrically using a commercial kit (GSH-Px Assay, Northwest Life Science Specialties, LLC and Vancouver, WA USA) as recommended by the manufacturer. The activity determination of the GPx enzyme was observed at 340 nm by spectrophotometry (Shimadzu UV-1800) due to the increase in absorbance depending on the glutathione conversion. Spectrophotometric methods were performed at 340 nm for 3 minutes in the Kinatics rate setting (Sen, 2022).

Statistical Analysis

The data obtained throughout the study were analyzed using the SPSS 20.0 (2014) package program used with the license of Ondokuz Mayıs University. The normality assumption of the data on GPx enzyme activities of bovine oocytes matured in culture media containing different protein sources was determined by the Shapiro Wilk test, and it was determined that they exhibited a normal distribution. In addition, the suitability of the data for variance analysis was evaluated by the Levente variance homogeneity test, and it was determined that the variances were homogeneous ($P<0.05$). Comparison of the significance level of the averages obtained from the experimental groups was made using Duncan's Multiple Comparison Test at a significance level of 0.05 (Genç and Soysal, 2018).

RESULTS

A total of 106 ovaries obtained from 53 cattle brought to the slaughterhouse for slaughter were brought to the laboratory to be used in the study. Glutathione peroxidase (GPx) enzyme activities (nmol/minute/ml) at different measurement times and average of bovine oocytes matured in culture media supplemented with fetal calf serum (FBS), bovine serum albumin (SSA) and polyvinyl alcohol (PVA) as protein sources are shown in Figure 3 and Table 1, respectively. In the study, the levels of GPx enzyme activity in the cell extracts isolated from the oocytes in each experimental group did not change over time, but the GPx enzyme activity in the oocytes with PVA added to the culture medium was found to be lower than the oocytes in the other experimental groups ($P>0.05$). In order to examine the effect of different protein sources added to the maturation medium on GPx enzyme activity, oocyte cells were stored in different protein sources and samples were obtained. The data obtained as a result of these activity determinations are shown in Figure 3 below.

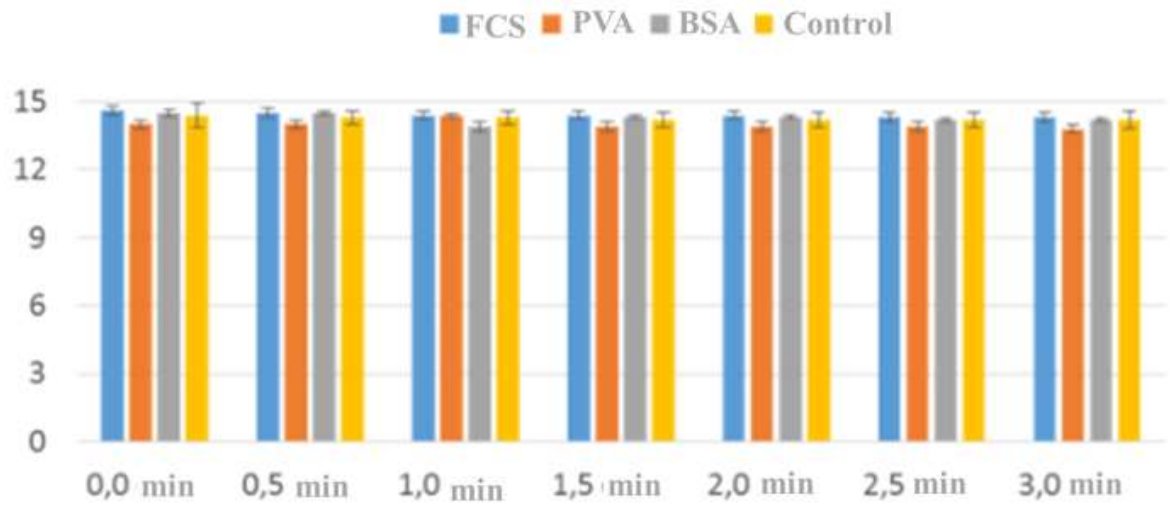

Figure 3. GPx enzyme activity levels (nmol/min/ml) of bovine oocytes added to in vitro maturation medium with different protein sources at different measurement times. FBS = fetal calf serum, SSA = bovine serum albumin, PVA = polyvinyl alcohol.

Table 1. Mean GPx enzyme activity (nmol/min/ml) of bovine oocytes to which different protein sources were added to in vitro maturation medium. a,b (P<0.05).

	SSA	FCS	PVA	Control
GPx EA	14,362±0,18[a]	14,414±0,21[a]	13,932±0,1[b]	14,061±0,2[ab]

[ab]The difference between the means shown with different letters in the same row is statistically significant (P<0.05). GPx EA= glutathione peroxidase enzyme activity, FBS= fetal calf serum, SSA= bovine serum albumin, PVA= polyvinyl alcohol.

DISCUSSION AND CONCLUSION

In vitro maturation of bovine oocytes represents a critical step in embryo production processes, and this process has significant effects on the quality and production efficiency of the embryos obtained. Oocyte maturation under in vitro conditions requires the provision of a suitable environment that takes into account the physiological and biochemical properties of the oocytes (Brackett and Zuelke, 1993; Gordon, 1994). In modern embryo production processes, the composition of the culture medium can directly affect the development of oocytes and embryos. The type of protein sources, nutrient content, and other additives are determining factors in this process. This process can trigger adaptive responses of oocytes and embryos; because in vitro conditions can differ significantly from the natural environment of oocytes, and this can affect cellular responses (Sturmey et al., 2009).

In order to support the biochemical balance of oocytes and embryos and optimize the maturation process, the use of different additives in the in vitro embryo production process is a common approach. In this context, protein sources such as FBS, BSA, and PVA play an important role. These additives can increase the nutrient content of the embryo culture medium and help meet the metabolic needs of the oocytes. Previous studies have shown that such protein supplements have the ability to accelerate critical stages, especially pronucleus formation. In addition, it has been stated that FBS, BSA, and PVA can affect gene transcript levels during in vitro culture, which can create positive effects on embryo development rate and quality (Ali and Sirard, 2002).

The present study investigates the effect of different protein sources on glutathione peroxidase (GPx) enzyme activity in bovine oocytes. GPx is a critical component of

antioxidant defense and provides protection against reactive oxygen species (ROS) in cells. Examination of glutathione peroxidase (GPx) enzyme activity in bovine oocytes aims to evaluate an important parameter in the in vitro embryo production process. The GPx enzyme is a critical component of antioxidant defense and provides protection against reactive oxygen species (ROS) in cells. The antioxidant capacity of oocytes is a determining factor on embryo quality because ROS can negatively affect the health, genetic integrity, and developmental potential of oocytes. The process of oocyte maturation under in vitro conditions ensures that the oocytes are ready for fertilization, and the resistance of the oocytes to environmental stress factors is important in this process. Although oocytes are naturally equipped with antioxidant enzymes, stress and oxidative damage experienced in the in vitro environment can reduce the effectiveness of this antioxidant defense. Therefore, evaluating and optimizing the antioxidant capacity of oocytes is critical for a successful in vitro embryo production process. Examining GPx enzyme activity helps us understand how the culture medium and protein sources used affect the antioxidant defense of oocytes (Cetica et al., 2001). The results of this study provide important insight into how the addition of different protein sources to the medium content can affect the antioxidant capacity of oocytes. The results obtained show that GPx enzyme activity in cell extracts of oocytes obtained from each experimental group did not show a significant change over time. However, it was found that GPx enzyme activity was statistically significantly lower in oocytes grown in media supplemented with PVA compared to other experimental groups. These findings indicate that different protein sources can affect the antioxidant capacity of oocytes during in vitro maturation processes. While lower GPx activity is observed in oocytes supplemented with PVA to the maturation medium, other protein sources may increase the antioxidant defense of oocytes more effectively and support their biochemical balance.

As a result, this study contributes to the development of strategies to optimize in vitro embryo production processes and improve embryo quality by elucidating the effects of different protein sources on antioxidant capacity in in vitro maturation processes of bovine oocytes.

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Investigation of the reproductive effects of vitamin-mineral premix (Bakofix®) application during the late gestational period in chios ewes: preliminary findings

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Abstract

*Mainly hormonal, social, or nutritional supports are provided to increase reproductive efficiency in small ruminants (sheep-goat) breeding in the field. In our project, it is envisaged that supplementing with a premix (Bakofix®) containing vitamins (mainly E) and minerals (mainly Se) via nutrition (injectable form) in late pregnant Chios ewes in a normal flock may increase reproductive efficiency. As part of a TÜBİTAK Student Project (**), a total of 30 (2-5 years old) healthy and pregnant ewes (15 females per group) in late pregnancy (over 3 months) were used herein. Animals were considered by their condition scores (BCS, 1-5 scale), along with considering their body weights (BW), and then divided into two groups (Group I: 2.46 BCS units, and 65.79 kg BW; Group II: 2.48 units, and 60.14 kg BW, resp.). For Group I (Vit-Min, n=15); To the ewe whose gross pregnancy was confirmed (by abdominal palpation), 1 cc Vitamin-Mineral (mainly Vit. E and Se, Bakofix®, DORUVET, Kahramanmaraş-Tuürkiye) per ewe for 40 kg live weight was injected subcutaneously, and repeated 1 week later. The lambs born from these ewes were also injected with vit.-min. at a dose of 0.5 cc/lamb in the first week (mostly on the 2nd or 3rd day postpartum, and the same dose was repeated 1 week later. For Group II (Control, n=15); Pregnant ewes were kept routinely and fed normally (with no premix application). Likewise, no nutritional contribution was made to their lambs. The findings of lambing obtained (also considering possible pregnancy toxemia and enzootic ataxia) were recorded. Statistical analyses were performed using ANOVA and Chi-Square methods. The effects of vitamin and mineral supplementation on pregnant ewes and newborn lambs were compared in the trial and treatment groups. According to the preliminary findings; lambing rates were 80% (12/15) vs 100% (15/15) in Group I and II, respectively. The total number of lambs and litter size (number of lambs per lambing) were 17 and 1.42 (17/12) vs 16 and 1.07 (17/12) in Groups I and II, respectively. The total number of lamb deaths and the death rates were 4 and 23.53% (4/17) vs 1 and 6.25% in Groups I and II, respectively. Further, one of the ewes in Group 2 died at lambing (single). Although the lambing rates were considerably lower in the treatment group (80 vs 100 %), the litter sizes were markedly higher in vit-min-treated ewes (1.42 vs 1.07). However, the rates of lamb deaths were considerably higher in the treatment group (23.53 vs 6.25%). The lower rate of lambing of pregnant ewes in the treatment group might be related to conceptional losses due to heavier physiological upload (greater litter size per pregnancy) and the twinning varieties within the Chios breed in the treatment group. A greater liter size in vit.-min. group might have been supported by premix addition. However, the higher death rates of lambs in the treatment group could have been related to weaker twin lambs compared to single ones in the controls. However, detailed analyses and considerations of the present results in late pregnant Chios ewes and their lambs born following vit.-min. administration are needed to get a more satisfactory explanation of the underlying reasons. **TÜBİTAK Project Number: 1919B012216170/2022.*

Key words: *Sheep, Chios breed, Vitamin-Mineral, Pregnancy, Lambing*

Imaging The Digestive Tract in Long-Legged Buzzard (Buteo Rufınus) with Stereo Microscope

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Abstract

In this study, it was aimed to visualize the microstructures at the mucosal level of the digestive tract of the long-legged buzzard. One long-legged buzzard was used in the study. The digestive tract was removed from the beginning of the esophagus to the cloaca. The organs forming the alimentary canal were viewed individually under a stereo microscope. In the study, the digestive tract of the long-legged buzzard consisted of esophagus, ingluvies, proventriculus, ventriculus, duodenum, jejunum, ileum, rudimente caecum and rectum. Esophagus consisted of longitudinal mucosal folds up to the ingluvies. Ingluvies had an almost flat mucosa. The glandular stomach section was light in color and had mucosal protrusions, the transitional region was yellow-green with relatively mucosal protrusions, and the muscular stomach section had black and smoother mucosa. In the intestinal parts, mucosal color was light in places where absorption was high, and dark in places where it was less. Likewise, there were more dense mucosal protrusions in the duodenum and jejunum compared to the ileum and rectum. Caecum was found to be in the form of two rudimentary protrusions. As a result, in this study, the digestive tract of long-legged buzzard was visualized with a stereo microscope and the mucosal change between the beginning and the end point of the tract was revealed.

Key words: *Long-legged buzzard, Digestive tract, Stereo Microscope*

INTRODUCTION

The long-legged buzzard (Buteo rufinus) is a bird species from the family of Accipitridae with a length of 50–60 cm and a wingspan of 120–150 cm. It is a medium-sized, broad-winged predator. It is easily recognized by the black frame and red color on the wing feathers. Its variegated, light colors are easily distinguishable when flying or perching. Steppes, open spaces and wetlands make up their habitat (Snow and Perrins 1998; Demirsoy 1992).

A bird's gastrointestinal tract morphology, digestive strategy, and metabolic ability have been intertwined during evolution in accordance with the nutrient content and physical characteristics of the foods available in its natural habitat (Klasing 1999). Digestive system organs in poultry can be listed as rostrum, lingua, pharynx (not fully developed), esophagus, ingluvies, ventriculus, intestinum and cloaca (Dursun 2002). In this study, it was aimed to visualize the digestive tract of a carnivorous bird, the long legged buzzard, with a stereo microscope.

MATERIALS AND METHODS

In the study, one long legged buzzard, which was brought to Mehmet Akif Ersoy University Veterinary Faculty Animal Hospital with injuries and could not be saved life despite all the interventions, was used. After the material's feathers and skin were carefully dissected, the visceral organs were reached. To collect the findings, the digestive tract was removed from the beginning of the esophagus to the cloaca. The organs forming the alimentary canal were viewed individually under a stereo microscope (Leica S6D).

RESULTS

In the study, it was observed that the digestive tract of the long legged buzzard consists of esophagus, ingluvies, proventriculus, ventriculus, duodenum, jejunum, ileum, rudimente caecum and rectum. Esophagus consisted of two parts, cervical and thoracal. There were longitudinal mucosal folds in the pars cervicalis and thoracalis of the esophagus. Ingluvies had brown streaks on white. However, it was found to have an almost flat mucosa (Figure 1).

Figure 1. Ingluves inner wall. It has a smooth mucosa and brown stripes on the white color

The mucosa of the pars thoracalis of the esophagus was yellow-green and had few mucosal protrusions (Figure 2).

Figure 2. Thoracic esophagus. It was a gray-cream colored and there were longitudinal mucosal folds.

The transitional part between the esophagus-proventriculus was pale cream in color and there were few mucosal protrusions (Figure 3). The glandular stomach portion was light in color and had numerous protrusions on its mucosa (Figure 4).

Figure 3. Esophagus-proventriculus transition. It was light cream in color, with few mucosal protrusions.

Figure 4. Glandular ventricle. It is light in color, with numerous protruding mucosal membranes.

The mucosa of the transition zone (isthmus gastris) between the proventriculus and ventricle was yellow-green in color and had few mucosal protrusions (Figure 5). The ventriculus (casual stomach part) was black in color and had a flatter mucosa (Figure 6). In the intestinal parts, it was determined that the color of the mucosa was lighter in places where absorption was high, and this color

was darker in places where absorption was low.

Figure 5. Proventriculus ventriculus passage (Isthmus gastris). The mucosa was yellow-green in color with few mucosal protrusions.

Figure 6. Ventriculus (Muscular stomach). It was black in color and relatively smooth mucosa.

Likewise, the duodenum and jejunum had more mucosal protrusions than the ileum and rectum (Figure 7, 8, 9, 10). The caecum was in the form of two rudimentary projections (Figure 11).

Figure 7. Duodenum was gray-white in color and had mucosal protruding.

Figure 8. Jejunum was cream-coloured and had mucosal protrusions

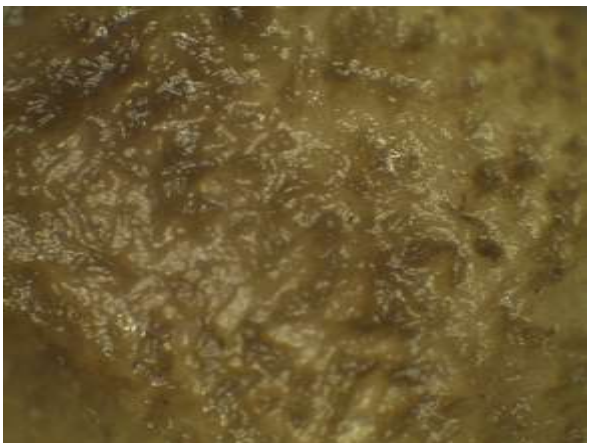

Figure 9. Ileum was dark cream colored and had few mucosal protruding.

Figure 10. Rectum was dark gray, cream colored, and had few mucosal protrusions.

Figure 11. The caecum was dark gray coloured, with two rudimentary projections.

DISCUSSION

In poultry, the esophageus enters the thorax from the neck region and ends in the proventriculus (Kırbaş Doğan 2021). Esophagus has two parts, cervical and thoracal (Preja et al. 2019). Evans (1996) reported that in the budgerigar, the esophagus passes to the right side towards the thorax, dorsal to the trachea in the cranial neck region. Esophagus is fusiform in buteo, and thorasic esophagus has a poorly developed sphincter (Ford 2010). Longitudinal plicas are present in the esophagus to aid in swallowing of large foodstuffs. In the long legged buzzard, it had longitudinal plicae in it's lumen, in accordance with the literature (Scot Ford 2010). The esophagus continues with the proventricle or glandular stomach without clear limitation in the buteo (Preja et al. 2019). In most bird species, with the exception of the owl (Scot Ford 2010), the esophagus expands at the entrance to the thoracic cavity to form ingluvies. In addition, it is mentioned that some grain-fed poultry species do not have an ingluvies in the full sense, but instead the presence of an esophageal sac with a high expansion capacity (Klasing 1999). Ingluvies were present in accordance with literature (Preja et al. 2019) in the long legged buzzard and in this study it was found that its mucosa had brown streaks on white color. However, it was an almost flat mucosa.

Gaster consists of two parts as proventriculus (glandular stomach) and ventriculus (muscular stomach) in poultry (Kırbaş Doğan 2021). The proventriculus is relatively smaller and attaches to the thin wall of the ventriculus (Denbow Micheal 2015). In the study, the glandular stomach section was light in color and had mucosal protrusions, the transition region was yellow-green with relatively mucosal protrusions, and the muscular section was black and had a smoother mucosa.

The intestinal tract is located in the caudal part of the thoraco-abdominal cavity, which is a compact mass surrounded by adipose tissue. The posterior part of the ventricle narrows towards the pyloric region on the right side of the organ (Nigel et al, 1993). The small intestine extends from the level of the pyloric region of the ventricle to the level of the cecum and colon. Duodenal part is relatively long; There is no obvious transition between duodenum and ileum (Murray, 2014; Klaphake and Clancy, 2005). The cecum is expressed as a blunt sac opening to the rectum, found on the right and left in most bird species. While the cecum consists of a single sac in most herons and bitterns (ardeidea), it has been reported to be seen in two parts in secretary birds (Baumel et al, 1993; McLelland, 1989). It has been reported that the cecum in long legged buzzard is rather small and in the form of a hollow sac (Halıgür, 2008). The colon (sometimes called the rectum) is short and extends from the iliosecal juntion to the cloaca (Preja et al. 2019). In the study, it was determined that the mucosal color of the long legged buzzard was light in places where absorption is high in the intestinal

mucosa, and dark in places where it was less. Likewise, there were more dense mucosal protrusions in the duodenum and jejunum compared to the ileum and rectum. Cecum was found in the form of two rudimentary protrusions in accordance with the literature (Halıgür 2008).

CONCLUSIONS

As a result, in the study, the inner wall of the digestive tract of the long legged buzzard was investigated with a stereo microscope, and information about the color and mucosal appearance was recorded. In conclusion, it is thought that this study will contribute to the digestive anatomy in poultry.

ACKNOWLEDGEMENTS

The contribution of the authors is equal. There is no conflict of interest between the authors.

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Use of red cell distribution width, neutrophil-to-lymphocyte count ratio and mean platelet volume-to-platelet count ratio as inflammatory biomarkers in calves with neonatal sepsis

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Abstract

The present study aims to investigate of changes red blood cell distribution width (RDW), neutrophil-lymphocyte count ratio (NLR), and mean platelet volume to-platelet count ratio (MPV/PLT) in calves with neonatal sepsis. To this end, 40 neonatal calves with sepsis (sepsis group, SG) and 20 healthy neonatal calves (control group, CG) were used. Red blood cell (RBC), white blood cell (WBC) and platalet (PLT) indices in anticoagulant blood samples were determined with a hematology analyzer.In serum samples, concentrations of albumin (ALB), globulin (GLOB), total protein (TP), glucose (GLU), blood urea nitrogen (BUN) and creatinine (Cr) were determined using autoanalyzer by commercial kits. These parameters of SG were significantly higher than CG: leukocyte (WBC, NEU, LYM and BAS), red blood cell (RBC, HGB, HCT, MCV, MCH, MCHC and RDW) and platelet indices (PLT, PCT, MPV and PDW), concentrations of ALB, GLOB, TP, GLU, BUN and Cr ($P<0.001$). Also, these parameters of SG were significantly higher than CG: RDW, NLR and MPV/PLT values ($P<0.001$). Sensitivity values for inflammatory process were for RDW: 90%, NLR: 75% and MPV/PLT: 70%. The areas under the ROC curve were 0.95, 0.80, and 0.65 for RDW, NLR and MPV/PLT respectively. In correlation analysis, strong positive correlations between NLR and clinical and biochemical parameters were determined, while negative correlations were observed between clinical and biochemical parameters in RDW and MPV/PLT. In conclusion, sepsis may cause an inflammatory process in neonatal calves. In this study, we have investigated the performances of RDW, NLR and MPV/PLT markers in the diagnosis and prognosis of inflammatory process and found out that RDW and NLR outruns other markers based on both inter-group comparisons and ROC analyses. As a result, inflammatory process may occur in animals with sepsis. Based on the parameters tested in this study, this inflammatory process can be best indicated by RDW. Further studies can be designed with various inflammatory parameters to test the efficiency and sensitivity of them.

Key words: *Red cell distribution width; Neutrophil/lymphocyte ratio; Mean platelet volume/platelet ratio; Neonatal sepsis; Calf, Inflammation*

Use and effects of bentonite in animal nutrition

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Abstract

Natural feed additives have gained even more significance since antibiotics are prohibited. One of these natural feed additives is bentonite, which is a clay mineral belonging to the phyllosilicate group. Bentonite is formed by the weathering of volcanic ash and is rich in aluminum and magnesium. It has a small crystal structure and variable composition. Its primary use in animal nutrition is as a toxin binder, preventing toxins from being absorbed in the digestive tract and allowing them to be excreted. Research on the use and effects of bentonite in animal feeding has shown positive impacts on animal health, welfare, performance, and product quantity and quality. By adding bentonite to animal feed, harmful substances such as toxins in contaminated feed can be adsorbed. However, it is essential to use bentonite within the recommended dosage range. Otherwise, there is a risk of binding and inhibiting nutrient absorption in animals, and individuals handling bentonite additives should also be aware of potential respiratory issues.

Key words: *Bentonite, Animal Nutrition, Ration, Toxin*

INTRODUCTION

Feed additives, also known as feed ingredients, contribute to the preservation of feed and nutrients, enhance the quality of feed and animal products, positively affect the gut microflora, and have no adverse effects on the environment. Since the ban on antibiotic use, natural feed additives have gained importance in the feed industry. One such natural feed additive is bentonite, which is formed by the weathering of volcanic ash. Bentonite belongs to the phyllosilicate group and consists of small crystalline structures with varying compositions, rich in aluminum and magnesium. Due to its highwater absorption capacity, bentonite is preferred for clarifying fruit juices, wines, and treating wastewater. Additionally, it is used as a pellet binder, anti-caking agent, toxin binder, and for controlling radionuclides in animal feed. The recommended maximum usage level for bentonite as a pellet binder is 1-2%. Furthermore, its use as a toxin binder, which is excreted without being absorbed in the digestive tract, is noteworthy in animal nutrition.

Mycotoxins, which are referred to as toxins, are secondary metabolites produced by fungi such as Aspergillus, Fusarium, or Penicillium. Mycotoxins can be widely found in feed raw materials, and they can lead to a decrease in product quality and quantity, adverse effects on animal health, as well as economic losses (Devreese et al., 2013; Mohamed, 2011; Ramos Girona et al., 2020). Therefore, the number of studies on the detoxification and inactivation of mycotoxins is increasing day by day. The general approach to combating mycotoxins can be listed as the separation of contaminated raw materials, the fight against mycotoxins in feed, and the elimination of mycotoxins in the digestive system of animals consuming mycotoxin-contaminated feed (Jard et al. 2010). Phyllosilicates, which are a subgroup of inorganic toxin binders, are divided into six groups: bentonites, montmorillonites, HSCAS, smectites, kaolinites, and illites (Boudergue et al., 2009; Devreese et al., 2013).

BENTONITE IN ANIMAL FEEDING

Bentonite can generally be added to feed in powder and granular form or mixed with water. In addition to being a heavy metal and toxin binder, bentonite also helps regulate the rumen pH, neutralize acidity through its buffering structure, alleviate disorders caused by acidosis, reduce hoof problems, and aid in feed digestion in ruminant animals (Tuğcu and Magglar, 2016).

Table 1. Usage areas and effects of bentonite.

Use of area	Effects	Reference
Feed Additive	Supports digestive system health. Improves performance. Enhances meat and milk quality. Can increase the mineral content of the ration. Increases digestion amount. Regulates pH. Can increase feed utilization. Binds toxins and heavy metals. Positively affects blood and bone parameters. Prevents caking. Keeps barns and litters clean.	Tuğcu and Magglar, 2016 Ghosh and ark., 2015 Gurbuz and Arslan, 2017 Osman, et al. 2021 Kemboi et al. 2023b
Other Industrial Uses	Wine and fruit juice clarification, wastewater treatment, paint industry, fire extinguishers, construction, pharmaceuticals, paper and rubber industry.	Özbey, 2019

Additionally, bentonite is used as an anti-caking agent and pellet binder due to its cationic structure, to reduce the risk of sudden ration changes, to slow down the passage rate of the feed through the digestive tract and to increase the digestibility of the feed due to its water-absorbing property, to positively affect intestinal health and to increase the feed conversion ratio, thereby increasing yield and performance. However, due to its high absorbent property, it retains toxins, ammonium, and heavy metals (Tuğcu and Magglar, 2016). Since bentonite contains natural minerals such as calcium, magnesium, and iron, its use in ruminant animal feeding can increase the mineral content of the ration.

The use of bentonite in poultry and ruminant animal feeding can improve performance, enhance meat and milk quality, positively affect blood and bone parameters, increase digestibility and intestinal health, and strengthen the immune system (Burçak and Yalçın, 2016; Yalçın and Onbaşılar, 2020). Additionally, it keeps the barn and bedding clean by facilitating the absorption of excess water (Tuğcu and Magglar, 2016).

USAGE RATES AND EFFECTS IN ANIMAL NUTRITION

Bentonite is mostly used during fasting periods, mixed with wheat flour to provide a feeling of satiety and facilitate the passage of food substances through the digestive system. Bentonite, which slows down the changes in the digestive system flora and provides time for the animal to adapt to feeding changes, is also used by animal feed producers to prevent the risks associated with sudden or risky feed formula changes (Tuğcu and Magglar, 2016). Depending on the type of feed produced, bentonite is used by the producer companies between 5-10 kg/ton in feed production.

Bentonite is used as a binder, anti-caking agent, and coagulant in animal feeds for all animal species. In addition to this, it is generally used in different proportions on pigs, poultry, and ruminant animal species, depending on the physiological condition of the animal and the ration content (Tuğcu and Magglar, 2016).

Bentonite usage rates and effects in poultry

Bentonite is effective against mycotoxins when added to poultry rations at a rate of 1-2% of the live weight (Ghosh et al., 2015). The addition of bentonite has been observed to have positive effects on health and performance, especially in the case of aflatoxin contamination in feeds (Prasai et al., 2016a; Kemboi et al., 2023a).

Table 2. Bentonite usage rates and effects in poultry.

Animal Type	Usage Rate	Effects	Referance
Broiler	1.5-3%	Increased live weight gain Increased feed intake Increased growth rate Improved performance Decreased litter moisture	Katouli et al., 2010
Broiler	0-5%	Improved feed conversion Improved performance Increased live weight gain Reduced feed cost	Ani et al., 2014
Layer	4%	Reduced pathogen levels	Prasai et al. 2016b

Bentonite used in broiler diet improved animal health and hygiene conditions and increased the growth rate. Additionally, it has been observed that bentonite reduces the microbial load and improves certain blood parameters (Bouderoua et al., 2021). In broilers fed with aflatoxin-contaminated feeds, bentonite has shown positive effects on feed performance, carcass yield, and blood components (Darmawan et al., 2022). The use of 2% sodium bentonite in broiler rations has been reported to increase live weight gain, feed conversion ratio, and feed intake (Mahesh and Lohan, 2008).

Usage rates and effects of bentonite in ruminant animals

In animal nutrition, these substances are primarily important due to their technological advantages. They have binding, fluidizing, and anti-caking properties. They also have absorbent properties that have a positive effect on the digestive system of animals. Particularly, bentonite has a buffering property that is advantageous for the rumen. This helps to regulate the pH value in the rumen or intestines and neutralize the acidity in the digestive system. Bentonite helps to maintain the health and performance of ruminant animals due to its hygienic and digestive regulatory effects (Gürbüz and Alarslan, 2017). It can also improve growth performance, health parameters, and feed use efficiency.

The usage rates of bentonite, which is generally used in sodium bentonite structure in ruminant animals, may vary depending on the animal species, mycotoxin type and dosage. Therefore, it is recommended to test the recommended usage rates beforehand. Additionally, the bentonite used along with the feeds can improve the palatability of the animal feed by modifying the texture, enhancing the taste and odor, reducing the dust, and improving the feed stability. The dosage is important when adding bentonite clay to the ration. Generally, it is recommended to use it at a rate of 1-4% of the dry matter and in the range of 20-45 grams per day.

Table 3. Bentonite usage rates and effects in ruminant animals.

Animal Species	Usage Rate	Effects	Referance
Cattle	60 g/cow/day	Decrease in milk aflatoxin M1 (AFM1) concentration	Kemboi et al. 2023b
Calves	1.5%	Increased feed intake Improved daily live weight gain Improved feed conversion ratio Increased ruminal pH	Stojanović et al. 2009
Calves	1.5%	Increased rumen protozoa count Increased growth rate	Kirovski et al. 2015
Cattle	70 g/cow/day	Increased milk yield Increased milk protein Increased milk fat	Osman et al. 2021
Goat	2.5-5%	Improved daily live weight gain Improved feed conversion ratio Increased digestibility of dry matter (DM), organic matter (OM), and crude protein (CP) Decreased ruminal nitrogen concentration	Mohsen and Tawfik, 2002
Lambs	1-3%	Decreased ruminal nitrogen concentration Increased rumen microbial flora	Al-Neuamy et al. 2020

CONCLUSIONS

Bentonite can be used in the ration in the recommended amount, taking into account the animal's species, gender and physiological condition. It positively affects animal health and feed quality, especially by reducing the effects of toxins such as fumonisin and aflatoxin. In addition, it helps digestion by buffering properties and regulating intestinal pH. Bentonite added to ruminant and poultry rations is also used to increase performance, improve meat and milk quality, positively affect blood and bone parameters, increase digestion level and intestinal health, and strengthen the immune system. Since it contains natural minerals such as calcium, magnesium and iron, bentonite can increase the ration content in terms of mineral substances when used in ruminant animal nutrition.

ACKNOWLEDGEMENTS

The contribution of the authors is equal. There is no conflict of interest between the authors.

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Benefits of Royal Jelly: a comprehensive review

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Abstract

Worker bees naturally produce royal jelly, a chemical compound. It has been extensively researched for its potential health advantages. This review seeks to gather the available information regarding the health benefits and biological effects of royal jelly. This review provides detailed information about the nutritional properties of royal jelly, highlighting its rich protein content, strong antioxidant properties, and potential health benefits. Furthermore, the article thoroughly explores various illnesses and disorders that have demonstrated positive outcomes when treated or prevented with royal jelly. Some of the topics covered are neurological conditions, wound healing, aging, and reproductive health concerns. The study also explores the various mechanisms through which royal jelly provides its advantages, such as its ability to act as an antioxidant, fight against bacteria, and reduce inflammation. In addition, the essay explores the current obstacles and constraints that impede the research on royal jelly. Additional research is necessary to gain a comprehensive understanding of its effects and the possibility of contamination with other substances. This article offers a thorough examination of the advantages of royal jelly and its potential uses in the realm of human health.

Key words: *Royal Jelly, Health Benefits, Biological Actions, Metabolic Diseases, Cancers, Antimicrobial, Anti-Inflammatory, Antioxidant, Reproductive Health, Neurodegenerative Disorders, Wound Healing, Aging.*

INTRODUCTION

What is Royal Jelly

Royal jelly is a fascinating substance created by worker bees, specifically the hypopharyngeal and mandibular glands of young Apis mellifera worker bees. This substance acts as the sole source of nutrition for the queen bee throughout her entire lifespan. This remarkable bee product has been used in traditional and folk medicine for human health care and has garnered interest in modern research because of its therapeutic properties. Royal jelly demonstrates a diverse array of biological and pharmacological potentials, encompassing antibacterial, antioxidant, anti-inflammatory, immunomodulatory, and antitumor activities, among various others (El-Guendouz, Lyoussi, et al., 2020). The intricate makeup of this substance consists of proteins, lipids, carbohydrates, polyphenols, vitamins, and hormones, which play various important roles within the hive and may have potential health benefits for humans(Botezan et al., 2023).

Background and History of Royal Jelly

Royal jelly is a thick, milky substance that is carefully crafted by worker bees and served exclusively to the larvae of queen bees. It is thought to be produced by the pharyngeal glands of young worker bees (Townsend & Lucas, 1940). Throughout history, royal jelly has been highly valued in traditional medicine across different cultures due to its remarkable healing properties and its association with vitality and long life(Pasupuleti et al., 2017).

During the early 20th century, royal jelly became increasingly popular in Europe and North America as a dietary supplement, especially among the upper class. The product was promoted as a holistic solution for a range of health concerns, such as tiredness, anxiety, and skin conditions (Viuda-Martos et al., 2017).

Extensive research has been conducted on the chemical composition of royal jelly,

uncovering its intricate and multifaceted characteristics. The composition of this substance includes proteins, lipids, carbohydrates, vitamins, minerals, and various bioactive compounds, all of which play a role in its potential health benefits(Laskowska et al., 2023; Sonmez et al., 2023).

Currently, royal jelly continues to be utilized as a dietary supplement and in various cosmetic products. The potential health benefits of this substance are currently under investigation, and it is frequently promoted as a natural solution for a range of conditions, including inflammation, oxidative stress, and immune system support (El-Guendouz, Lyoussi, et al., 2020; Ma et al., 2022)(Ma et al., 2022)

The history of royal jelly is characterized by its long-standing presence in traditional medicine and its increasing popularity as a dietary supplement and cosmetic product. However, additional research is necessary to completely understand the precise mechanisms of action and health advantages(Çiçek & Öz Bağcı, 2024; Jităreanu et al., 2022; Sree, 2023).

Importance of Royal Jelly

The significance of royal jelly stems from its complex chemical makeup and wide range of biological effects, rendering it a valuable natural substance with profound implications for human health and overall wellness. Presented below are several key points that emphasize the significance of royal jelly:

Nutritional Value: Royal jelly is widely recognized for its exceptional nutritional content, encompassing proteins, lipids, carbohydrates, vitamins, minerals, enzymes, and hormones. These components collectively contribute to the remarkable health benefits associated with royal jelly(Arfa et al., 2021; Ghadimi-Garjan et al., 2023; Ghosh & Jung, 2024).

Therapeutic Potential: Extensive research has demonstrated the diverse range of biological and pharmacological potentials of royal jelly. These include its ability to combat bacterial infections, neutralize harmful free radicals, reduce inflammation, modulate the immune system, and even potentially inhibit tumor growth, among other beneficial effects(Acar, 2021; Bagameri et al., 2023; El-Guendouz, Lyoussi, et al., 2020).

Metabolic Disorders and Gastrointestinal Diseases: Research has investigated the potential of royal jelly as a treatment option for metabolic disorders such as diabetes mellitus, cardiovascular diseases, and gastrointestinal illnesses. The compound has demonstrated a wide range of therapeutic properties, including antimicrobial, estrogenic, anti-inflammatory, hypotensive, anticancer, and antioxidant effects(Abdelsalam et al., 2023; El-Seedi et al., 2024; Omer et al., 2019).

Anti-Toxoplasma Activity: The therapeutic potential of royal jelly in controlling Toxoplasma gondii infection in mice has been demonstrated. The study demonstrated notable reductions in tissue cysts and oxidative stress markers, along with an increase in pro-inflammatory cytokines (Althobaiti, 2022; Uthaibutra et al., 2023)(Althobaiti, 2022).

Benign Prostatic Hyperplasia (BPH): Research has explored the potential benefits of royal jelly in treating BPH. Studies have shown that it has the potential to lower PSA scores and enhance International Prostate Symptom Score (IPSS) values with minimal adverse effects(Baptista et al., 2023; Pajovic et al., 2016).

Antioxidant Properties: Royal jelly has antioxidant properties thanks to its bioactive components, such as flavonoids, phenolic acids, and terpenoids. Antioxidants are essential in combating diseases caused by oxidative stress(Kocot et al., 2018; Tohamy et al., 2022).

The findings highlight the wide range of therapeutic benefits that royal jelly offers for different health conditions, emphasizing its significance as a natural product that promotes good health. Additional research is necessary to gain a comprehensive understanding of the mechanisms that contribute to its positive effects and to investigate its potential uses in contemporary medicine.

NUTRITIONAL BENEFITS

Abundant in Essential Nutrients: Royal jelly is packed with B-complex vitamins, vitamin C, vitamin E, and vitamin B12. It also offers vital mineral salts that are important

for maintaining electrolyte balance and supporting physiological processes(R. K. Dubey et al., 2023).

High Protein Content: Royal jelly contains a significant amount of protein, which is crucial for maintaining muscle and supporting various bodily functions(Fallah et al., 2021).

Source of Essential Fatty Acids: Royal jelly is a valuable source of essential fatty acids that play a crucial role in maintaining the structure and function of cell membranes(El-Guendouz, Machado, et al., 2020).

Antioxidant Properties: Royal jelly showcases its ability to combat oxidative stress and safeguard cells from harm through its bioactive components such as flavonoids, phenolic acids, and terpenoids, which possess antioxidant properties(Polak-Śliwińska & Tańska, 2021).

The findings presented in this study emphasize the wide range of nutritional advantages that royal jelly offers. This makes it a valuable natural product that has important implications for human health and overall well-being.

HEALTH BENEFITS OVERVIEW

Royal jelly, a natural substance created by diligent bees, has been utilized in traditional medicine for countless years due to its potential health advantages. The health benefits of royal jelly are due to its distinct composition, which consists of proteins, lipids, carbohydrates, vitamins, minerals, and bioactive compounds such as 10-hydroxy-2-decenoic acid (10-HDA)(Karimi, Khorvash, et al., 2023; Laskowska et al., 2023).

Benefits of Antioxidants: Studies have shown that royal jelly has antioxidant properties that can effectively fight against oxidative stress and safeguard cells from potential damage(Bagameri et al., 2023; Tohamy et al., 2022).

Anti-Inflammatory Effects: Royal jelly possesses properties that can reduce inflammation, potentially providing relief from symptoms associated with inflammatory conditions and contributing to overall health(El-Sayed, 2023; Emilija et al., 2022).

Support for the Immune System: Royal jelly has been found to provide support for immune function, potentially bolstering the body's defenses against infections and diseases(H. Zhang et al., 2022).

Skin Health and Wound Healing: Royal jelly has been found to have potential benefits for skin health and the healing of wounds(Darlenski & Fluhr, 2023; Deinsberger et al., 2022).

Exploring Cognitive Function and Neuroprotection: Early studies indicate that royal jelly may potentially enhance cognitive function and provide neuroprotective benefits(Arslan, 2021; Raoufi et al., 2023).

Reproductive Health: Royal jelly has been extensively studied for its positive impact on reproductive health. Research suggests that it may have the ability to enhance fertility and lower the chances of developing reproductive disorders(Chalapathy et al., 2021; Karimi, Khorvash, et al., 2023; Mafruchati & Makuwia, 2022).

Neurodegenerative Disorders: There is evidence to suggest that royal jelly could be beneficial in the treatment and prevention of neurodegenerative disorders like Alzheimer's and Parkinson's diseases(Guardia de Souza E Silva et al., 2020; L. M. Zhang et al., 2022).

Aging: Royal jelly has been found to potentially support healthy aging by mitigating oxidative stress and inflammation(W. Wang et al., 2021a).

Gastrointestinal Health: Royal jelly has been found to have potential benefits for gastrointestinal health. It may help improve gut dysbiosis and lower the risk of non-alcoholic fatty liver disease (NAFLD)(Kobayashi et al., 2023; Mafruchati & Makuwia, 2022).

Cancer Prevention: Recent studies indicate that royal jelly exhibits potential anti-cancer properties and could potentially play a role in inhibiting the growth and advancement of specific forms of cancer(Alu'datt et al., 2018; Mafruchati & Makuwia, 2022).

Mechanisms of Action:

The mechanisms of action of royal jelly are not fully understood, but researchers have proposed several pathways through which it may exert its effects.

Antioxidant and Anti-Inflammatory Effects: The remarkable properties of royal jelly include its ability to act as an antioxidant, protecting cells from harm, and its anti-inflammatory effects, which can help

reduce inflammation(Martiniakova et al., 2023; You et al., 2022).

Immune System Modulation: Royal jelly has been found to have a positive impact on the immune system, improving its function and potentially lowering the chances of developing infections and diseases(Babaei et al., 2016; Harwood et al., 2021).

Protein and Lipid Metabolism: The protein and lipid composition of royal jelly may play a role in regulating metabolic processes and promoting overall health(Inoue et al., 2022; Oreshkova et al., 2023).

Areas for Further Study

Although there is promising evidence regarding the health benefits of royal jelly, more research is required to fully understand how it works and determine the best ways to use it. Future research should prioritize:

Investigating the mechanisms of action of royal jelly and its bioactive compounds through mechanistic studies.

Clinical Trials: Conducting randomized controlled trials to assess the effectiveness and safety of royal jelly in treating specific health conditions.

Exploring new applications: Investigating innovative uses of royal jelly, such as incorporating it into skincare products and developing functional foods.

In general, the health benefits of royal jelly are wide-ranging and show great promise, with potential applications in various areas of medicine and health.

Antioxidant Properties

Royal jelly displays antioxidant properties due to its distinctive composition and bioactive compounds. The antioxidant activity of royal jelly is thought to result from its capacity to counteract free radicals and alleviate oxidative stress(Bagameri et al., 2023; Botezan et al., 2023; Wen et al., 2024).

Table 1. Antioxidant Properties of Royal Jelly

Component	Mechanism of Action	Reference
Flavonoids	Scavenges free radicals	(Botezan et al., 2023)
Phenolic acids	Reduces oxidative stress	(Uthaibutra et al., 2023)
Enzymes	Enhances antioxidant defense	(Tohamy et al., 2022)
10-Hydroxy-2-decenoic acid (10-HDA)	Modulates oxidative stress response	(Ghadimi-Garjan et al., 2023)
Antioxidant peptides	Inhibits lipid peroxidation and oxidative damage	(Bagameri et al., 2023)
Vitamins (e.g., Vitamin C)	Works synergistically with royal jelly to enhance antioxidant capacity	(Anbara et al., 2016)

Table 1 provides a concise overview of the main components found in royal jelly that contribute to its antioxidant properties. It also outlines the specific mechanisms through which these components exert their effects.

Understanding the Mechanisms of Action

There are multiple mechanisms through which the antioxidant properties of royal jelly are believed to operate:

Counteracting Free Radicals: The antioxidant properties of royal jelly, including polyphenols and flavonoids, help counteract the harmful effects of free

radicals and protect against oxidative damage(Diab et al., 2022).

Boosting Endogenous Antioxidant Enzymes: Royal jelly has been found to potentially boost the activity of natural antioxidant enzymes, like superoxide dismutase and glutathione peroxidase. These enzymes play a crucial role in safeguarding cells against oxidative damage(Anbara et al., 2016).

Royal jelly has the potential to inhibit oxidative stress by decreasing the production of reactive oxygen species (ROS) and enhancing the activity of antioxidant enzymes(Anbara et al., 2016; Bagameri et al., 2023; Botezan et al., 2023).

Research Studies:

Numerous academic studies have delved into the antioxidant properties of royal jelly. These studies have explored various aspects of royal jelly's antioxidant capabilities.

In Vitro studies: Royal jelly has demonstrated antioxidant properties, effectively preventing lipid oxidation and DNA damage(R. K. Dubey et al., 2023; Perminaite et al., 2021)(Perminaite et al., 2021) .

In Vivo Studies: Research has shown that royal jelly has antioxidant effects when tested on animals, helping to reduce oxidative stress and improve antioxidant status(Al-Okbi & Al-Siedy, 2022; Lin et al., 2020). The antioxidant properties of royal jelly have been extensively studied and are thought to play a role in its potential health benefits. These benefits may include a reduced risk of chronic diseases like cancer, cardiovascular disease, and neurodegenerative disorders. Additional research is required to gain a comprehensive understanding of how royal jelly's antioxidant properties work and the most effective ways to utilize them.

Figure 1. On Antioxidant Activity in Bee Products(Martinello & Mutinelli, 2021).

Anti-Inflammatory Effects: (Effects of Reducing Inflammation):

Research has shown that royal jelly possesses anti-inflammatory properties, thanks to its distinctive composition and bioactive compounds. The anti-inflammatory effects of royal jelly are thought to operate through various mechanisms:

Suppression of Inflammatory Cytokines: Studies have demonstrated that royal jelly can effectively suppress the production of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-18. These cytokines play a crucial role in triggering the body's inflammatory response (Aslan & Aksoy, 2015; Bagameri et al., 2023; Barkah, 2023).

Altering Inflammatory Signaling Pathways: Studies have shown that royal jelly can modify inflammatory signaling pathways, including the MAPK and NF-κB pathways, which play a role in regulating inflammation(Y.-F. Chen et al., 2016).

Antioxidant Activity: Royal jelly has been found to possess antioxidant activity, which can potentially aid in the reduction of oxidative stress and inflammation. This is achieved by neutralizing free radicals and decreasing the production of reactive oxygen species (ROS)(Bagameri et al., 2023; Y.-F. Chen et al., 2016; El-Sayed, 2023).

Table 2. Anti-Inflammatory Effects and Immune System Support of Royal Jelly

Cell Type	Effect	Mechanism	Reference
Stem Cells	Promotes proliferation	Enhances differentiation pathways	(Okamoto et al., 2023)
Neurons	Neuroprotective effects	Increases synaptic plasticity	(Nazarinia et al., 2023)

Table 2 anti-inflammatory effects and immune system support of royal jelly which is offer a concise overview of the anti-inflammatory effects and immune system support offered by royal jelly.

Research

Numerous academic studies have delved into the potential anti-inflammatory properties of royal jelly. These studies have explored various aspects of its effects.

In laboratory studies: Research has demonstrated that royal jelly can suppress the release of substances that cause inflammation, like nitric oxide and interleukin-10, in macrophages stimulated with lipopolysaccharide(Y.-F. Chen et al., 2016; Clowes et al., 2023).

In Vivo Studies: Research has shown that royal jelly has the ability to decrease inflammation and oxidative stress in different animal models, such as those caused by valproic acid and ethylene glycol (H. Chen et al., 2023).

Clinical Studies: Royal jelly has been utilized in the treatment of different inflammatory conditions such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases(Bagameri et al., 2023; Barkah, 2023; Hegazi et al., 2015; nezhad et al., 2023).

The anti-inflammatory effects of royal jelly have been extensively studied and are thought to play a role in its potential health benefits. These benefits may include a reduced risk of chronic diseases like cancer, cardiovascular disease, and neurodegenerative disorders. Additional research is required to gain a comprehensive understanding of how royal jelly's anti-inflammatory properties work and how they can be best utilized.

Immune System Support:

Research has shown that royal jelly possesses immunomodulatory properties, thanks to its distinct composition and bioactive compounds. The immune system support offered by royal jelly is thought to operate through various mechanisms, including:

Boosting Immune Cell Function: Studies have demonstrated that royal jelly can improve the performance of immune cells, including natural killer cells, T-cells, and B-cells. These cells play a crucial role in identifying and eliminating harmful pathogens(Babaei et al., 2016; Bouamama et al., 2021; Hegazi et al., 2015).

Regulating Inflammatory Response: Studies have shown that royal jelly can regulate the body's inflammatory response by decreasing the production of pro-inflammatory cytokines like TNF-α and IL-1β, while increasing the production of anti-inflammatory cytokines like IL-10(Babaei et al., 2016; Bouamama et al., 2021; Hegazi et al., 2015).

Antioxidant Activity: The antioxidant properties of royal jelly can potentially alleviate oxidative stress and inflammation by counteracting free radicals and minimizing the generation of reactive oxygen species (ROS)(Gunes & Gungormus, 2022; Hegazi et al., 2015; Koçköprü & Daştan, 2023).

Research

Numerous studies have delved into the immune system support offered by royal

jelly. These studies have explored various aspects of this topic.

In laboratory studies: Royal jelly has been found to boost the growth of immune cells, such as T-cells and B-cells, in controlled environments(Babaei et al., 2016; Bouamama et al., 2021; Hegazi et al., 2015).

In Vivo Studies: Royal jelly has been observed to boost the immune response in different animal models, including those caused by infection and inflammation(Babaei et al., 2016; Bouamama et al., 2021; Hegazi et al., 2015).

Clinical Studies: Royal jelly has been utilized in the treatment of several immune-related disorders, such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases(Babaei et al., 2016; Hegazi et al., 2015).

The immune system support offered by royal jelly has been extensively studied and is thought to play a role in its potential health advantages, which include lowering the likelihood of chronic illnesses like cancer, cardiovascular disease, and neurodegenerative disorders. Additional research is required to gain a comprehensive understanding of how royal jelly supports the immune system and its ideal uses.

Skin Health and Wound Healing:

Studies have shown that royal jelly has positive effects on skin health and the healing of wounds. The bioactive compounds found in royal jelly, including hyaluronic acid, glycosaminoglycans, and peptides, play a significant role in promoting skin health(Álvarez et al., 2022; H. Y. Kim et al., 2020; Movahedi et al., 2021).

Understanding the Mechanisms of Action

There are several mechanisms through which royal jelly contributes to skin health and wound healing:

Hydration and Moisturization: The presence of hyaluronic acid in royal jelly aids in maintaining the skin's moisture levels, resulting in improved hydration and elasticity(H. Y. Kim et al., 2020).

Antioxidant Activity: The antioxidant properties of royal jelly, including polyphenols and flavonoids, play a crucial role in combating free radicals and minimizing oxidative stress. This is particularly beneficial for preventing skin damage and slowing down the aging process (Yan et al., 2024).

Inflammation Modulation: The anti-inflammatory properties of royal jelly, including peptides and glycosaminoglycans, play a role in regulating the body's inflammatory response. This can lead to a decrease in inflammation and support the healing process of wounds(Álvarez et al., 2022; Mendolia et al., 2022; Wen et al., 2024).

Cell Proliferation and Differentiation: The bioactive compounds found in royal jelly, such as peptides and glycosaminoglycans, have been shown to support the growth and specialization of cells. This can have positive effects on skin regeneration and the healing of wounds(Cohen et al., 2019; Movahedi et al., 2021; Niu et al., 2013; Wen et al., 2024).

Table 3. Effects of Royal Jelly on Cell Proliferation and Differentiation

Cell Type	Effect	Mechanism	Reference
Stem Cells	Promotes proliferation	Enhances differentiation pathways	(Okamoto et al., 2023)
Neurons	Neuroprotective effects	Increases synaptic plasticity	(Nazarinia et al., 2023)

See Table 3 on effects of Royal Jelly on Cell Proliferation and Differentiation which presents a comprehensive overview of the impact of royal jelly on cell proliferation and differentiation. It provides a detailed account of the various cell types, effects, and underlying mechanisms involved.

Research

Numerous academic studies have delved into the effects of royal jelly on skin health and wound healing. These studies have explored various aspects of this natural substance's potential benefits.

In vitro studies: have demonstrated that royal jelly can enhance the growth and specialization of human keratinocytes, potentially aiding in the rejuvenation of skin and the healing of wounds (Movahedi et al., 2021).

In laboratory studies, royal jelly has shown promising results in speeding up the healing process in animals, by reducing inflammation and aiding in tissue regeneration (Wen et al., 2024; Yan et al., 2024).

Clinical Studies: Royal jelly has been utilized in the treatment of different skin conditions, such as atopic dermatitis and psoriasis, showing encouraging outcomes(H. Y. Kim et al., 2020; Movahedi et al., 2021).

The skin health and wound healing properties of royal jelly have been extensively studied and are thought to play a role in its potential health benefits. Additional research is necessary to gain a comprehensive understanding of how royal jelly affects skin health and promotes wound healing.

Cognitive Function and Neuroprotection:

Royal jelly, a natural substance created by honeybees, has been discovered to have a range of health benefits, such as enhancing cognitive function and providing neuroprotection. The bioactive compounds found in royal jelly, including peptides, glycosaminoglycans, and antioxidants, play a role in promoting cognitive function and protecting the brain(Md Isa et al., 2022; Niu et al., 2013).

Understanding Cognitive Function

Research has indicated that the addition of royal jelly to one's diet may enhance cognitive abilities in people who have experienced an ischemic stroke. A randomized controlled trial revealed that the consumption of royal jelly had a notable positive impact on cognitive function in patients who had experienced an ischemic stroke. This improvement was observed in areas such as memory and attention (Karimi, Arab, et al., 2023; Koc et al., 2024). A recent study discovered that the addition of royal jelly to the diet resulted in higher levels of brain-derived neurotrophic factor (BDNF) in individuals who had experienced an ischemic stroke. BDNF is a protein that is essential for the development and maintenance of neurons(Coakley et al., 2023; Karimi, Arab, et al., 2023).

Neuroprotection is a field of study that focuses on safeguarding the health and function of the nervous system. It involves research and interventions aimed at preventing or minimizing damage to neurons and promoting their survival. This area of study is crucial for understanding and developing strategies to protect against neuro(W. Wang et al., 2021b; L. M. Zhang et al., 2022).

Studies have shown that royal jelly possesses neuroprotective properties, making it potentially beneficial in safeguarding against neurodegenerative conditions like Alzheimer's and Parkinson's disease. A study discovered that the addition of royal jelly had a positive impact on reducing oxidative stress and inflammation in the brain, factors that are known to play a role in the development of neurodegenerative diseases (Hattori et al., 2017). A separate study discovered that the addition of royal jelly boosted the presence of antioxidants in the brain. This can potentially provide protection against oxidative stress and neurodegeneration(Chi et al., 2021).

Understanding the Mechanisms of Action:

The precise mechanisms underlying the effects of royal jelly on cognitive function and neuroprotection remain a subject of ongoing research. However, it is widely hypothesized that these effects are mediated through the modulation of diverse biological pathways. As an academic, it has been discovered that royal jelly can suppress the production of pro-inflammatory cytokines like TNF-α, while promoting the production of anti-inflammatory cytokines like IL-10(Pasupuleti et al., 2017; Snapper et al., 2023). Studies have shown that royal jelly has the potential to enhance the levels of BDNF, a protein that plays a crucial role in supporting the growth and survival of neurons(Karimi, Arab, et al., 2023).

Ultimately, it has been discovered that royal jelly offers a range of health advantages,

such as enhancing cognitive function and providing neuroprotection. The bioactive compounds found in royal jelly, including peptides, glycosaminoglycans, and antioxidants, play a role in enhancing cognitive function and protecting the brain. Additional research is required to gain a comprehensive understanding of how royal jelly affects cognitive function and neuroprotection, as well as to investigate its potential therapeutic uses.

Reproductive Health

Royal jelly, a natural substance produced by honeybees, has been discovered to possess numerous health benefits, including advantages for reproductive health. The bioactive compounds found in royal jelly, including proteins, peptides, and antioxidants, play a significant role in promoting reproductive health.

Reproductive Health Benefits

Research has indicated that the addition of royal jelly to one's diet can have positive effects on reproductive health for individuals of all genders. Research on male rabbits indicated that supplementing with royal jelly increased sperm quality and fertility(Khadr et al., 2015). In a separate study, it was discovered that the addition of royal jelly had a positive impact on the reproductive health of female hamsters. Specifically, it helped to mitigate the harmful side effects caused by the Adriamycin drug(Mahmoud & Anas, 2015).

Understanding the Mechanisms of Action

The precise mechanisms by which royal jelly affects reproductive health remain a subject of ongoing research. However, it is thought to exert its effects by influencing a range of biological pathways. For instance, research has shown that royal jelly can enhance sperm quality by lowering oxidative stress and raising antioxidant levels(Ibrahim et al., 2023). In addition to its potential benefits for reproductive health in females, royal jelly has been shown to mitigate the toxic side effects of the Adriamycin drug and promote hormonal balance(Mahmoud & Anas, 2015; Md Isa et al., 2022).

Ultimately, royal jelly has been discovered to possess a range of health advantages, such as promoting reproductive well-being. The bioactive compounds found in royal jelly, including proteins, peptides, and antioxidants, play a significant role in promoting reproductive health. Additional research is required to gain a comprehensive understanding of how royal jelly affects reproductive health and to investigate its potential uses in therapy.

Neurodegenerative Disorders:

Research has shown that royal jelly, a natural substance created by honeybees, offers numerous health benefits. One area of interest is its potential to provide therapeutic effects in the prevention and management of neurodegenerative disorders like Alzheimer's and Parkinson's diseases.

Alzheimer's Disease

Research has indicated that incorporating royal jelly into one's diet could potentially have a positive impact on the prevention or progression of Alzheimer's disease. As an example, one study indicated that rats with Alzheimer's disease showed an improvement in cognitive performance and a reduction in oxidative stress when given royal jelly supplements(X. Wang et al., 2016). A recent study discovered that the addition of royal jelly to the diet resulted in elevated levels of brain-derived neurotrophic factor (BDNF) in mice afflicted with Alzheimer's disease. BDNF is a vital protein that supports the growth and survival of neurons(R. Dubey et al., 2024).

Parkinson's Disease

Studies have shown that royal jelly may offer promising therapeutic benefits in the prevention and management of Parkinson's disease. As an academic, it is worth noting that a study discovered the positive effects of royal jelly supplementation on motor function and oxidative stress in mice with Parkinson's disease. In a recent study, it was discovered that the addition of royal jelly to the diet resulted in elevated dopamine levels in mice with Parkinson's disease. Dopamine is a vital neurotransmitter involved in motor function (Ali & Kunugi, 2020a; X. Wang et al., 2016).

Understanding the Mechanisms of Action

The precise mechanisms by which royal jelly affects neurodegenerative disorders remain a subject of ongoing research. However, it is thought to exert its effects by influencing a range of biological pathways. For instance, it has been discovered that royal jelly

enhances cognitive performance by lowering oxidative stress and raising BDNF levels. Studies have shown that royal jelly can enhance motor function by boosting dopamine levels and reducing oxidative stress (Azimpour et al., 2022; Khalil et al., 2023; Snapper et al., 2023).

Ultimately, studies have shown that royal jelly offers a range of health advantages, potentially serving as a valuable treatment option for neurodegenerative conditions like Alzheimer's and Parkinson's diseases. The bioactive compounds found in royal jelly, including proteins, peptides, and antioxidants, play a role in its ability to protect the nervous system. Additional research is necessary to gain a comprehensive understanding of how royal jelly affects neurodegenerative disorders and to investigate its potential as a therapeutic treatment.

Aging:

Research has been conducted on royal jelly, a natural substance created by honeybees, to explore its potential health advantages in relation to the aging process. Although there have been studies conducted on the potential anti-aging effects of royal jelly, the available evidence is currently limited. Further research is required to definitively establish the effectiveness of royal jelly in slowing down or preventing the aging process.

Potential Anti-Aging Effects:

An investigation conducted on the nematode worm Caenorhabditis elegans, which is commonly used in aging research, revealed that the addition of royal jelly and enzyme-treated royal jelly had a notable impact on delaying age-related paralysis and enhancing proteostasis (protein homeostasis). These effects were found to be dependent on insulin/IGF signaling. This implies that royal jelly might have positive impacts on the aging process by supporting the maintenance of healthy proteostasis(Honda et al., 2011; Natarajan et al., 2021).

Nevertheless, the search results do not offer substantial evidence regarding the impact of royal jelly on animal growth, longevity, or fertility(Dayan, 1960). There is ongoing debate surrounding the biological effects of royal jelly, and further research is required to fully understand its potential anti-aging properties in humans.

Restrictions and Factors to Keep in Mind:

Notably absent from the search results are any human clinical trials investigating the anti-aging properties of royal jelly. It is important to note that the anti-aging effects seen in animal studies may not necessarily apply to humans.

In addition, the search results emphasize the importance of further research in order to gain a comprehensive understanding of the composition and biological impacts of royal jelly(Z. Yang et al., 2022). We still have much to learn about the composition of royal jelly and its impact on different health outcomes, such as aging. Ongoing research is dedicated to uncovering more information in this area.

Although there have been some promising findings from animal studies, further research is required to fully understand the effectiveness and safety of royal jelly as an anti-aging supplement for humans. There is limited evidence available regarding the effects of royal jelly on growth, longevity, or fertility in animals. Furthermore, there is ongoing debate surrounding the biological effects of royal jelly(Ali & Kunugi, 2020b; Alnomasy & Shehri, 2022).

Prior to incorporating royal jelly or any dietary supplements into your routine, it is crucial to seek guidance from healthcare professionals. The effectiveness and safety of these supplements can vary, so it's best to consult with experts.

Gastrointestinal health:

Royal jelly (RJ) is a natural substance produced by honeybees that has been extensively researched due to its potential health benefits, particularly its impact on gastrointestinal health. Here are a few of the main discoveries:

Gastrointestinal Disorders: Royal jelly has shown promise in treating gastrointestinal conditions like irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and gastroesophageal reflux disease (GERD). A study published in an academic journal revealed that RJ supplementation showed promising results in alleviating the symptoms of IBS in patients with mild to moderate cases(Miyauchi-Wakuda et al., 2019; Pasupuleti et al., 2017).

Anti-Inflammatory Activity: Royal jelly has demonstrated its ability to reduce inflammation, providing relief for individuals with gastrointestinal disorders. A study published in the Journal of Ethnopharmacology discovered that RJ extract demonstrated anti-inflammatory properties in a rat model of colitis(Alamdari et al., 2024; Md Isa et al., 2022; Y.-C. Yang et al., 2018).

Wound Healing: Royal jelly has been discovered to possess remarkable wound healing properties, making it a valuable asset in the treatment of gastrointestinal disorders like ulcers and wounds. A study published in an academic journal demonstrated the effectiveness of RJ cream in promoting wound healing in patients with chronic wounds(L. Chen et al., 2024; Nega & Eshete, 2018).

Antioxidant Activity: Royal jelly has been discovered to possess antioxidant activity, offering protection against oxidative stress and inflammation in the gastrointestinal tract. A recent study published in the Journal of Agricultural and Food Chemistry revealed the remarkable antioxidant properties of RJ extract in a model of oxidative stress(Mohammad et al., 2022).

Gut Health: Royal jelly has been discovered to have positive impacts on gut health, such as supporting the growth of beneficial gut bacteria and reducing inflammation. A study published in an academic journal revealed that RJ supplementation had a positive impact on the gut bacteria of patients with IBS, leading to increased abundance of beneficial microorganisms(Chi et al., 2021; Kobayashi et al., 2023).

Ultimately, royal jelly has been discovered to possess numerous health advantages pertaining to gastrointestinal well-being. These include its ability to reduce inflammation, act as an antioxidant, and promote wound healing. Additional research is required to gain a comprehensive understanding of the mechanisms of action of royal jelly and to validate its effectiveness in treating gastrointestinal disorders. **See Figure 2.** On potential activity of royal jelly in the most common gastrointestinal diseases(Barkah, 2023).

Cancer Prevention:

Studying cancer prevention holds great importance, and royal jelly has shown potential in its ability to combat cancer. Here are a few important discoveries:

Anticancer Effects: Royal jelly has been found to demonstrate anticancer effects in several studies. As an illustration, a study published in the Journal of Medicinal Food discovered that the addition of royal jelly to the diet had a positive impact on alleviating symptoms of irritable bowel syndrome (IBS) in patients with mild to moderate IBS. This condition is recognized as a risk factor for colorectal cancer (Miyata et al., 2020; Salama et al., 2022).

Antioxidant Activity: Royal jelly has been discovered to possess antioxidant activity, providing protection against oxidative stress and inflammation, both of which are recognized as risk factors for cancer. A study published in the Journal of Agricultural and Food Chemistry revealed that royal jelly extract demonstrated antioxidant activity in a model of oxidative stress(Botezan et al., 2023; Hashemi et al., 2023; Mohammad et al., 2022).

Anti-Inflammatory Activity: Royal jelly has been discovered to possess anti-inflammatory properties, making it beneficial in reducing inflammation, a recognized risk factor for cancer. A research paper published in the Journal of Ethnopharmacology discovered that royal jelly extract demonstrated anti-inflammatory properties in a rat model of colitis(Cho et al., 2024; Jovanović et al., 2022).

Enhanced Effects with Chemotherapeutic Drugs: Royal jelly has been discovered to work in harmony with chemotherapeutic drugs, amplifying their efficacy and minimizing any potential adverse effects. A recent study published in the Journal of Medicinal Food revealed a fascinating discovery regarding the potential enhancement of chemotherapy's effectiveness in cancer treatment. The study explored the effects of combining royal jelly with chemotherapy drugs, yielding promising results. This finding opens up new possibilities for improving the outcomes of chemotherapy treatment(Abu-Serie & Habashy, 2022; Münstedt & Männle, 2019).

Inhibition of Cancer Cell Growth: Royal jelly has been discovered to effectively

hinder the growth of cancer cells. As an illustration, a study published in the Journal of Functional Foods discovered that royal jelly effectively hindered the growth of breast cancer cells (MCF-7) and liver cancer cells (Huh-7) in laboratory experiments(Ayna & Darendelioglu, 2022; M. Kim et al., 2022).

Anticancer Activity in Various Cell Lines: Royal jelly has demonstrated its ability to inhibit the growth of cancer cells in different cell lines, such as cervical cancer cells (HeLa), colon cancer cells (WiDr), and breast cancer cells (MCF-7) (Al-Okbi & Al-Siedy, 2022; Shirzad et al., 2013).

Mechanisms of Action: The exact mechanisms by which royal jelly prevents cancer are not fully understood, but it is believed to have antioxidant, anti-inflammatory, and anti-proliferative properties that contribute to its potential in cancer prevention. A study published in an academic journal discovered that royal jelly has the ability to hinder the growth of cancer cells. It achieves this by altering the expression of specific genes related to cell proliferation and cell death. This research provides valuable insights into the potential benefits of royal jelly in combating cancer(Ahmad et al., 2020; Uthaibutra et al., 2023).

Ultimately, researchers have discovered that royal jelly possesses promising qualities that could potentially combat cancer. These qualities encompass its ability to act as an antioxidant, reduce inflammation, and inhibit cell growth. Additional research is required to gain a comprehensive understanding of the mechanisms of action of royal jelly and to validate its effectiveness in preventing cancer.

ROYAL JELLY IN DISEASE PREVENTION AND TREATMENT

Metabolic Disorders:

Researchers have extensively examined royal jelly, a natural substance created by honeybees, to explore its potential health benefits, particularly its impact on metabolic disorders. Here are a few important discoveries:

Therapeutic Potential: Royal jelly (RJ) has shown promise in managing metabolic disorders like diabetes mellitus, cardiovascular disease, and gastrointestinal disorders. This substance demonstrates a wide range of therapeutic properties, such as antimicrobial, estrogen-like, anti-inflammatory, hypotensive, anticancer, and antioxidant effects(Ait Eldjoudi et al., 2022; El-Seedi et al., 2024).

Cholesterol and Glucose Lowering: Research has indicated that RJ supplementation may have the ability to reduce cholesterol and glucose levels in rats with diabetes, indicating potential advantages for individuals with metabolic disorders(El-Sayed, 2023; Macadangdang et al., 2021).

Immune Modulation: RJ has been discovered to have an impact on immune responses, boosting anti-inflammatory cytokines while suppressing important inflammatory mediators. This may have potential therapeutic benefits for metabolic disorders(良也 et al., 2009).

Antioxidant and Anti-Inflammatory Effects: RJ has been discovered to possess properties that can combat oxidative stress and inflammation, potentially providing relief for individuals with metabolic disorders(Al-Okbi & Al-Siedy, 2022; El-Sayed, 2023; Macadangdang et al., 2021; Tohamy et al., 2022).

Research has shown that royal jelly has promising therapeutic properties for managing metabolic disorders. It has been found to help lower cholesterol and glucose levels, modulate the immune system, and provide antioxidant and anti-inflammatory benefits. Additional research is necessary to gain a comprehensive understanding of the mechanisms behind RJ's therapeutic effects and to validate its effectiveness in studies involving humans.

Reproductive Health:

Researchers have extensively studied royal jelly, a natural substance created by honeybees, due to its potential advantages in the realm of reproductive health. Here are a few important discoveries:

Effects on Fertility and Hormonal Balance

Fertility and Infertility: Traditionally, royal jelly (RJ) has been utilized in Indian traditional medicine as a treatment for infertility. Recent research has indicated that the addition of RJ supplementation may have positive effects on egg cell physiology and fertility in women. While there is a lack

of clinical research studies on RJ, its potential therapeutic effects on infertility show promise(Chalapathy et al., 2021; Widjiati et al., 2011).

Hormonal Balance: RJ has been discovered to have effects similar to estrogen, which may impact reproductive health. For instance, studies have shown that RJ can have estrogen-like effects in adult female rats, which may have positive effects on hormonal balance(Abdelnour et al., 2020; Ishida et al., 2022).

Sperm Traits and Fertility Rate: RJ has been shown to enhance sperm traits and increase fertility rates in animals. As an example, the study found that administering RJ orally improved sexual behavior and semen quality in male rabbits. Additionally, it was observed that RJ helped to alleviate "summer infertility" in male rabbits that were exposed to heat stress(Abdelnour et al., 2020; Cetin et al., 2023).

Studies have shown that royal jelly may offer promising benefits for fertility and hormonal balance, especially in women. With its remarkable ability to enhance egg cell physiology, this natural supplement shows great promise as a potential treatment for infertility. Additional research is necessary to gain a comprehensive understanding of the mechanisms behind RJ's therapeutic effects and to validate its effectiveness in studies involving humans.

Digestive Health:

Benefits for gastrointestinal function and microbiota.

Royal jelly (RJ) has been shown to have positive effects on gut dysbiosis and non-alcoholic fatty liver disease (NAFLD) in db/db mice. RJ effectively modulated the body's natural immune response in the small intestine, leading to a reduction in the expression of genes linked to inflammation and nutrient absorption transporters. RJ significantly expanded the number of operational taxonomic units, the abundance of bacteria, and seven taxa, which included bacteria capable of producing short-chain fatty acids(Kobayashi et al., 2023; Tashchilova et al., 2022).

Exploring the Influence of Gut Microbiota:

RJ has been discovered to influence the gut microbiota by enhancing the presence of beneficial bacteria like Bacteroides and supporting the growth of bacteria that produce short-chain fatty acids, which are beneficial for health. This modulation has been shown to have positive effects on gastrointestinal function and overall health(Kobayashi et al., 2023; Kowalska et al., 2024; W. Wang et al., 2023).

Benefits of Antioxidants and Anti-Inflammatory Effects:

RJ has been discovered to possess antioxidant and anti-inflammatory properties, which may aid in reducing oxidative stress and inflammation linked to gastrointestinal disorders. These effects may contribute to its therapeutic potential in the management of gastrointestinal diseases(El-Seedi et al., 2024; Md Isa et al., 2022).

Studies have shown that royal jelly may have beneficial effects on gastrointestinal function and the balance of gut bacteria. Its antioxidant and anti-inflammatory effects can provide relief for the oxidative stress and inflammation commonly found in gastrointestinal disorders. Additional research is necessary to gain a comprehensive understanding of the mechanisms behind RJ's therapeutic effects and to validate its effectiveness in studies involving humans.

Bone Health:

Effects on bone density and osteoporosis prevention.

Royal jelly (RJ) has been discovered to possess advantageous properties for promoting bone health and preventing osteoporosis.

Preventing Bone Loss: RJ and protease-treated RJ (pRJ) were nearly as effective as estradiol in preventing bone loss caused by ovariectomy in rats. The administration of 2.0% (w/w) RJ and 0.5-2.0% (w/w) pRJ effectively reversed the decrease in tibial bone mineral density (BMD) by 85% or more(Deyhim et al., 2005; Hidaka et al., 2006).

Improving Bone Metabolism: RJ and pRJ were found to increase the calcium content in tissue cultures taken from normal male rats, specifically in the femoral-diaphyseal and femoral-metaphyseal regions. This suggests that they could potentially play a role in preventing osteoporosis by enhancing the absorption of calcium in the

intestines(Hidaka et al., 2006; Machado et al., 2014).

Exploring the Inhibition of Osteoclastogenesis: The crucial component 10-hydroxy-2-decenoic acid (10H2DA) safeguards against bone loss by blocking NF-κB signaling after free fatty acid receptor 4 (FFAR4) activation in osteoclasts. This, in turn, reduces the activation of the main transcription factor for osteoclastogenesis, NFATc1(Sato et al., 2022; Tsuchiya et al., 2020).

Enhancing Multilineage Differentiation: RJ improves the capacity of myoblast C2C12 cells to differentiate into various lineages, such as osteoblasts. This indicates that it might support osteogenesis by leveraging the antioxidant properties of glutathione(Ito et al., 2024).

Nevertheless, a pilot study discovered that the introduction of 10H2DA or 10-hydroxydecanoic acid (10HDAA), which are two specific fatty acids found in RJ, did not improve bone loss in rats after ovariectomy. This suggests that the beneficial effects of RJ may not solely depend on these two components(Hanai et al., 2023).

Ultimately, RJ has demonstrated encouraging results in mitigating bone loss and osteoporosis in animal studies. However, additional investigation is necessary to comprehensively grasp the underlying processes and validate its effectiveness in human subjects.

POTENTIAL AREAS FOR FUTURE RESEARCH.

Here are some possible areas for further investigation into the health advantages of royal jelly:

8.1. Mechanisms of Action: Although the health benefits of royal jelly have been extensively studied, the precise mechanisms behind its anti-inflammatory, antioxidant, antitumor, and antibacterial properties remain a subject of ongoing research(Bagameri et al., 2023). Additional investigation is required to better understand the biological mechanisms underlying its therapeutic effects, potentially resulting in the creation of more precise and efficient treatments(Pandeya et al., 2019; Severo et al., 2022).

Synergistic Effects: The research on effervescent tablets containing royal jelly indicates that royal jelly can work together with other functional food components(Menkovska, 2013). Exploring the possible combined benefits of royal jelly with other natural compounds or conventional therapies could pave the way for improved interventions in treating a range of health conditions. Further investigation is necessary to determine the most effective combinations and dosages of royal jelly with other compounds in order to fully harness its potential health benefits(Ghorbanpour et al., 2022; Mochizuki et al., 2021, 2023).

Targeted Applications: Studies have shown that royal jelly may offer potential health benefits to different groups of people, including children, pregnant women, diabetics, and athletes(Menkovska, 2013; Viuda-Martos et al., 2017). Nevertheless, there is a requirement for focused implementations of royal jelly in these particular groups, which can amplify its health advantages and enhance quality of life. Further research should prioritize the development of royal jelly products that cater to the specific requirements of these groups, considering factors like age, gender, and overall well-being(Carpenter et al., 2023; Md Isa et al., 2022).

Ensuring the long-term safety and efficacy: Although short-term studies have provided encouraging findings, it is imperative to conduct long-term studies in order to establish the safety and effectiveness of royal jelly supplementation(L. Chen et al., 2024). Studying the effects of royal jelly supplementation on age-related disorders, gut function, and overall health can be greatly enhanced by conducting longitudinal studies on both naturally aging animal models and human populations. These studies offer valuable insights into the long-term impacts of this supplementation. It is important for these studies to consider any factors that may affect the results and to make sure that royal jelly supplementation is safe and well-tolerated for long-term use(Cohen et al., 2019; Lovell et al., 2015; Majeed et al., 2021).

Exploring the Standardization of Royal Jelly Products: Ensuring the efficacy and safety of royal jelly products is of utmost importance(Barkah, 2023; Menkovska, 2013). Further investigation is needed to establish uniform procedures for the creation and assessment of royal jelly products, considering elements like composition, bioavailability, and stability. This will assist in guaranteeing the consistency and dependability of royal jelly products, facilitating healthcare professionals and consumers in making well-informed decisions regarding their usage(\vSimúth et al., 2004; Darwish et al., 2022; Demirta, 2020; Rzetecka et al., 2023).

Ultimately, there are numerous promising avenues for further exploration into the health advantages of royal jelly. These include gaining a deeper understanding of how it works, exploring its potential in combination with other substances, creating specific uses for it, ensuring its safety and effectiveness over extended periods, and establishing consistent standards for royal jelly products. By addressing these research gaps, we can fully explore the potential of royal jelly in disease prevention and treatment.

CONCLUSIONS:

Honeybees create royal jelly, a natural substance that has attracted considerable interest due to its wide range of therapeutic properties. Research has demonstrated the potential of this substance to decrease inflammation, counteract oxidative stress, inhibit tumor growth, and delay the aging process. Researchers have also discovered that it possesses protective properties against bacterial infections. This review highlights the wide range of advantages that royal jelly provides in the field of health and medicine. The anti-inflammatory properties of this substance are especially useful in the treatment of conditions such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel diseases. Moreover, the antioxidant and antitumor properties of royal jelly play a vital role in safeguarding against oxidative stress and cancer. The substance's properties that combat aging and promote regeneration have a positive impact on one's overall health and well-being. Its composition is rich in nutrients and bioactive compounds such as lipids, phenolic compounds, flavonoids, organic acids, minerals, vitamins, enzymes, and hormones, which further enhance its health benefits. Royal jelly has demonstrated its ability to promote gut health, enhance immune function, and improve cognitive abilities, making it a valuable functional food. Ultimately, royal jelly emerges as a potent natural substance that holds considerable promise for enhancing overall health and well-being. With its wide range of health benefits, this versatile substance is a valuable addition to any health regimen. Further research is required to gain a comprehensive understanding of the mechanisms underlying these effects and delve deeper into the potential therapeutic uses of royal jelly.

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Linear Programming model for ration preparation of dairy cattle

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Abstract

The livestock sector is predominantly affected by feed costs, constituting approximately 70% of the total expenses. Reducing these costs poses a significant challenge for farms. Feed expenses typically account for a large portion of the total operating costs for enterprises in this sector, adversely affecting profitability. To address this issue, we employed a linear programming model to minimise feed costs while optimising nutrition levels to enhance productivity, mainly focusing on dairy cattle operations. We aimed to develop ration formulations tailored to the needs of individual farms, utilising widely accessible office software such as Microsoft Excel. Excel's computational capabilities and user-friendly interface make it an ideal platform for creating customised rations and ensuring continuous updates. With our program, raw materials for farm use are formulated based on the nutritional requirements of animals, resulting in economically viable rations.
Moreover, the feed ingredient database can be updated according to farm requirements, accommodating new ingredients and automatically adjusting rations based on periodic nutritional needs and optimisation criteria. This approach offers significant advantages, including avoiding costly commercial ration programs and minimising external dependency issues. By providing an accessible Excel-based linear programming model for dairy ration formulation, our solution aims to reduce costs, enhance productivity, and decrease reliance on external sources for dairy cattle operations.

Key words: *Ration formulation, Linear programming, Dairy cattle nutrition, Nutrients, Requirements, Feed Database*

INTRODUCTION

Since the existence of humanity, livestock farming has been the most important economic activity (Doğruyol, 2021). Livestock farming is crucial in ensuring the ever-increasing global population's adequate, balanced, and healthy nutrition (Ergün and Bayram, 2021). Today, in livestock farming, animal-derived products obtained primarily from cattle are widely used. It is apt to say that cattle function like a factory in producing consumable and usable animal products such as meat, milk, leather, etc. For this reason, cattle farming is widely practised almost everywhere globally, particularly in regions with advanced plant production (Canbolat, 2015; Ergün and Bayram, 2021). The rapidly growing global population is increasing the demand for food, and the majority of the required protein sources are derived from animal products. Balanced nutrition depends on the consumption of sufficient levels and quality of animal protein (Yaylak and Alçiçek, 2003). Therefore, the importance of milk and dairy products as a protein source is also growing daily (Turan, Şanver, and Öztürk, 2017). The digestive system of dairy cattle is significantly different from monogastric animals. Cellulose, which humans cannot utilise, can be considered a high-quality feed material for cattle. Consequently, the ability of cattle to produce meat and milk from such feed highlights the importance of dairy farming (Kızılaslan and Demirbük, 2018). In dairy cattle farming, which provides the highest milk yield, feed inputs constitute the most significant portion of production costs. Feed expenses account for approximately 60-70% of the total operating costs. Any reduction in these costs directly increases the profitability of the operation. Therefore, every improvement in feed efficiency within the

operation leads to an increase in the operation's revenue (Boğa and Çevik, 2012).
Numerous factors influence the nutrient requirements of animals. These include weather conditions (temperature, humidity), the age of the animal, genetic factors, production type (meat, milk, wool, reproduction, etc.), number of lactations, type and number of births, days in lactation, and feeding practices, among others (Boğa and Çevik, 2012). In this context, without neglecting the nutrient requirements of the animals, their maintenance and production needs should be carefully calculated, and their daily rations should be formulated in the most economical way possible (Doğan, İ., Doğan, N., and Akcan, 2000). Thus, by meeting the animal's nutrient requirements, the operation can effectively address issues arising from nutrient deficiencies. Additionally, the risk of diseases caused by inadequate feeding can be mitigated through proper ration formulation (Boğa and Çevik, 2012). In this context, the dairy cattle ration program prepared using Excel, which includes both roughage and concentrate feeds mixed in a balanced manner, will create a feeding strategy known as TMR (Total Mixed Ration) used in dairy cattle nutrition. Using TMR, the combined use of roughage and concentrate feed will prevent animal nutritional diseases and significantly increase milk yield by maximising nutrient intake (Kılıç and Polat, 2002; Parlar and Fisun, 2020). The ration program's primary goal, prepared using Microsoft Excel, is to create a low-cost feed while ensuring proper and balanced nutrition. In this context, the program calculates the feed database and the nutrient requirements of the animals. After the criteria are established, the ration can be formulated based on the limitations specified by the producer. The prepared ration program operates on the principles of a linear programming model, similar to commercial ration programs available on the market. It is also important to note that commercial ration programs often come with annual maintenance and repair costs, which can increase the dependency of operations on these programs and elevate economic expenses. The prepared ration program calculates the individual needs of each animal. It determines the lowest-cost ration using the Excel solver, thereby reducing dependency on external resources and eliminating the high costs associated with annual maintenance and repair of commercial software. In an era where feed costs are steadily rising, reducing expenses and achieving efficient production are priorities for dairy cattle operations. It is anticipated that formulating the most cost-effective roughage and concentrate feed mixture in line with the program will lead to increased profitability for the operation.

NUTRITION OF DAIRY CATTLE

The nutrition of dairy cattle is crucial for efficient and high-quality milk production, good animal health, and sustainable reproductive performance, which is essential for the continuity of herds. This section focuses on the digestive system of dairy cattle and their nutrition during lactation and dry periods.

Digestive System of Dairy Cattle

The digestive system of dairy cattle differs from that of monogastric animals as they possess a four-chambered stomach. Due to this characteristic, they are classified as ruminant animals. The four main compartments of their stomach are the Rumen, Reticulum, Omasum, and Abomasum. The primary function of this complex digestive system in ruminants is to digest complex plant materials, such as cellulose, which other animals cannot digest, and convert them into forms of energy and nutrients that the animal can utilise. The largest and most important part of the digestive system, the rumen, ferments complex plant materials such as cellulose and other carbohydrates into Volatile Fatty Acids (VFAs) with the help of microorganisms (Smith and Jones, 2015). This process meets the animal's energy requirements. The Rumen also functions as a storage site for food and facilitates the regurgitation of food back to the mouth for rumination. The rumen plays a central role in enabling ruminants to digest plant-based nutrients and effectively utilize energy efficiently. The reticulum, which resembles a honeycomb structure, is responsible for mixing ingested feed, breaking it down into smaller particles, supporting microbial

fermentation, and regulating feed passage from the rumen to the omasum. Additionally, the reticulum has a protective function, trapping foreign objects to safeguard the digestive system.

The omasum is the third stomach compartment in the digestive system of ruminants. Its primary function is to absorb water and nutrients from the feed, breaking it into smaller particles to facilitate digestion. During this process, the omasum aids in water absorption and the concentration of nutrients, regulating feed passage to the abomasum. These functions of the omasum allow ruminants to maximise the nutritional benefits of their feed. The abomasum is the ruminant stomach's final compartment called the "true stomach." It functions similarly to the stomach of monogastric animals. The primary role of the abomasum is to break down food using gastric acids and digestive enzymes, initiating the digestion of proteins. Here, the partially digested food from the rumen and omasum is processed in an acidic environment, preparing it for passage into the small intestine. The abomasum is critical for the effective digestion of nutrients and optimising nutrient absorption in ruminants.

Nutrition During Lactation Period

In dairy cattle, the lactation period refers to the milk production phase that begins after calving. During this period of milk yield, it is crucial to pay attention to nutrition and management practices. The lactation period is generally divided into three stages: early, mid-lactation, and late.

Early lactation period (0 – 70 Days)

The early lactation period encompasses the first 70 days following the calf's birth. Milk yield reaches its highest level during this time, typically peaking within 4 to 10 weeks. This period is characterised by a high energy demand in dairy cattle, and to address the negative energy balance that occurs, any sudden changes in the amount of concentrate feed in the ration can lead to reduced appetite and metabolic issues such as acidosis in the animals. Maintaining a proper balance between roughage and concentrated feed is crucial during this initial stage of lactation. If the proportion of concentrate feed exceeds 60%, there is an observed increase in the production of Volatile Fatty Acids (VFAs) in the rumen. As a result, the level of propionic acid within VFAs rises while the level of acetic acid decreases. This imbalance rapidly declines milk yield and reduces milk fat content (Canbolat, 2015).

The most significant challenge during this period is that, despite reaching their peak milk production, cows cannot consume enough dry matter to meet the energy demands of their high milk yield. To compensate for the energy deficit, cows attempt to utilise their body fat reserves, leading to a loss of body condition (Bauman and Currie, 1980). During the early lactation period, it is crucial to feed cows with high-energy feeds that meet their energy requirements. Since protein requirements are also elevated during this period, an optimal ration should provide sufficient amounts of high-quality protein and energy (Bauman and Currie, 1980).

Mid-lactation period (70-140 Days)

The mid-lactation period encompasses the 70th day to the 140th day after calving. During this period, milk yield begins to decline. However, dry matter intake in dairy cows reaches its peak, allowing for more effortless fulfilment of the animal's nutritional requirements. Due to the positive energy balance observed during this period, there is an increase in body weight and improvement in body condition scores. Mid-lactation is critical for restoring body condition and preparing cows for the next reproductive cycle (Bauman and Currie, 1980). As milk production begins to decrease, the ratio of roughage to concentrate in the ration should be adjusted to 50% roughage and 50% concentrate. This period is relatively more straightforward regarding feeding, and nutritional diseases are generally less common than other stages (Canbolat, 2015).

Late lactation period

The late lactation period spans from the 140th day of lactation until calving. During this time, when most cows are pregnant, milk yield continues to decline, and the

energy requirements of the cows decrease. The primary focus during the late lactation period is maintaining the cows' body condition and preparing them for the dry period. The energy content of the ration can be reduced during this stage, but it is crucial to ensure that the cows' mineral and vitamin needs are met. The total ration in this period should consist of 35-40% concentrate feed and 60-65% roughage. Excessive use of concentrate feed during this period can lead to excessive fat accumulation in cows, which can cause problems such as dystocia (difficult calving), ketosis after calving, a decrease in milk production, and fertility issues. The late lactation period allows cows to recover after lactation and helps ensure a healthy dry period (Grummer, 1995).

Nutrition During the Dry Period

The dry period is a phase that begins when lactation ends and lasts for approximately 60 days before calving, during which milk production ceases. Nutrition during this period is critical as it influences the postpartum lactation performance and the birthing process. Additionally, this period significantly affects metabolic diseases that can arise before and after calving. A nutrition plan that maintains the cow's body condition while preventing excessive weight gain should be implemented early in the dry period. Excessive body fat increases the risk of postpartum metabolic diseases (Drackley, 1999).

During this period, the calcium and phosphorus requirements should be met in a balanced manner. Studies have shown that including 0.40% calcium and 0.24% phosphorus in the rations of dry cows is sufficient. This equates to a daily intake of 60-80 grams of calcium and 30-40 grams of phosphorus per animal. Exceeding these recommended amounts significantly increases the risk of milk fever (Canbolat, 2015).

During the dry period, cows' nutrition should be supported with roughage with a high cellulose content and feed with low energy density. However, the energy density should be increased as the cows approach the calving period (Grummer, 1995).

Nutrition During the Transition Period

The transition period, the second half of the dry period, encompasses the 15 days leading up to calving. During this time, dairy cows' metabolic and physiological requirements change rapidly. Their nutritional needs increase significantly in this period. Following a proper nutritional strategy during the transition period directly impacts postpartum milk yield, health status, and reproductive performance (Drackley, 1999).

During the transition period, critical for ensuring a healthy lactation period in dairy cows, it is essential to use high-energy density concentrates to prevent the risk of negative energy balance (Grummer, 1995). Alongside energy, the protein requirements also increase during this period. It has been reported that adding feeds with a high rumen-undegradable protein (RUP) to the ration can enhance milk yield during the lactation period (Broderick, 2003). Additionally, calcium, magnesium, and vitamin supplementation is critically important during this period. To reduce the risk of postpartum hypocalcemia, a low-calcium ration should be provided during the dry period, and calcium supplementation should be increased as calving approaches (Goff and Horst, 1997).

The balance between roughage and concentrate in the ration must be carefully adjusted, and sufficient cellulose content must be provided to maintain rumen health (Mertens, 1997). In conclusion, transition period nutrition requires excellent attention, impacting postpartum milk yield, health, and reproductive performance. By implementing an appropriate feeding strategy during this period, cows can be ensured a healthy lactation period (Bell, 1995).

NUTRIENT REQUIREMENTS OF DAIRY CATTLE

The nutrition of dairy cattle is of great importance for increasing milk yield and maintaining animal health. The nutrient requirements of dairy cattle vary based on their body weight, daily milk production, milk fat content, and the stage of lactation they are in. Therefore, accurately meeting the nutrient requirements of cattle is crucial

for ensuring efficiency in milk production and maintaining milk quality.

Water Requirements

Approximately 60-70% of a dairy cow's body is composed of water, and about 87% of the milk produced is water. Therefore, water is one of the most critical nutrients for dairy cattle. The amount of water required for milk production directly impacts the daily water intake of the animals. Practically speaking, a lactating dairy cow typically consumes 60-70 litres of water for maintenance and an additional 4-5 litres of water per litre of milk produced (Saçaklı, 2019).

Several factors influence water intake in dairy cows, including body weight, milk yield, dry matter intake, ambient temperature, moisture content in the ration, and the quality and temperature of the water. Adequate water intake positively affects milk yield and overall health (Murphy, 1992).

Energy Requirements

The energy requirements of dairy cows directly affect milk yield, milk composition, body condition, and overall health. The energy needs of dairy cows are essential for sustaining maintenance and productivity processes. These energy requirements vary depending on factors such as the stage of lactation, milk production level, age, body weight, environmental conditions, and physical activity (NRC, 2001).

When energy requirements are not adequately met, it can lead to a decrease in milk yield, deterioration in milk quality, and a decline in the animal's health. Insufficient energy intake triggers the mobilisation of body fat, leading to weight loss and metabolic disorders in animals (Van Soest, 1994). Net Energy for Lactation (NEL) is the energy system used to calculate lactating and dry cows' energy requirements. The formulas used to calculate the net energy requirements for maintenance and production in dairy cows are as follows (equation 1,2):

Maintenance Energy Requirement (equation 1):

$$NEL(Mcal/day) = 0.08 \times (BodyWeight\ 0.75\ kg)$$

Production Energy Requirement (equation 2):

$$NEL(Mcal/kg) = 0.0929 \times \%Fat + 0.0563 \times \%Protein + 0.192$$

These formulas are essential for determining the energy needs of dairy cows to ensure proper maintenance and optimise milk production (equation 1,2)

Protein Requirements

In dairy animals, protein needs must be met for the continuity of life, growth, development, pregnancy, and lactation. The protein requirement in dairy cattle varies depending on factors such as age, milk fat content, stage of lactation, milk yield, genotype, and the composition of the ration. Amino acids, the building blocks of proteins, are crucial in milk production and composition (Schwab, 1996). In cows where protein requirements are not adequately met, there is a decline in milk yield and quality, along with a weakened immune system, which can lead to weight loss and negative impacts on reproductive performance (Eastridge, 2006). The protein needs of dairy cows should be met with digestible and nutritionally valuable protein sources. The amount of protein the animals require varies depending on the form of protein. These include (equation 3):

- Crude Protein (CP): The rude protein (CP) content in the diet, expressed as a percentage of dry matter (DM), is the sum of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) (equation 3):

$$CP\ (\%\ of\ DM) = \%RDP + \%RUP$$

This equation highlights that the total protein content in a diet consists of the fraction that is degradable in the rumen and the fraction that bypasses the rumen for digestion in the small intestine (equation 3).

- Rumen-Degradable Protein (RDP): This is the portion of protein digestible in the rumen and can be utilised by rumen microorganisms.
- Rumen-Undegradable Protein (RUP): Also known as bypass protein, this portion of protein bypasses the rumen and is directly digested in the small intestine. RUP is vital for lactation efficiency (NRC, 2001; Schwab, 1996).

Recommendations for Crude Protein (CP), Rumen-Degradable Protein (RDP), and Rumen-Undegradable Protein (RUP) in

rations for lactating and dry cows are provided in Table 1.

Table 1. Dietary HP, RDP and RUP recommendations for dairy cows in lactation and dry period

Protein Forms	Lactation Periods			Dry Periods	
	Early	Mid	Late	First 3 Weeks	Last 3 Weeks
CP, % DM	17-20	16-17	15-16	12-13	13-15
RDP, % CP	60-65	64-68	64-68	65-68	62-66
RUP, % CP	35-40	32-36	32-36	32-35	34-38

Carbohydrate Requirements

Carbohydrates, a significant portion of dairy cow rations, are crucial in maintaining rumen health and supporting digestive functions. Carbohydrates provide the energy rumen microorganisms require and are essential for synthesising fat, protein, and lactose found in milk. Carbohydrates in animal nutrition are generally divided into two categories: structural carbohydrates and non-structural carbohydrates.

1. Structural carbohydrates include Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF). NDF comprises cellulose, hemicellulose, and lignin, while ADF consists of cellulose and lignin.
2. Non-structural carbohydrates (NFC) are considered to be starches and sugars.

When determining the carbohydrate requirements of dairy cows, it is essential to consider both the quantity and the form in which the feed is provided. Alongside the preparation of feeds, the interaction between different nutrients in the ration must also be considered. Meeting the high energy needs of high-producing dairy cows is possible with rations containing a large amount of concentrate feed and high-quality roughage. In such feeding programs, the amount of cellulose (fiber) in the ration may decrease. However, adequate cellulose intake is necessary for normal rumen function and milk fat synthesis. Maintaining an optimal balance between structural and non-structural carbohydrates in dairy cow rations is a critical challenge (Saçaklı, 2019).

Below is Table 2, which outlines the recommendations for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Non-Structural Carbohydrates (NFC) as a percentage of dry matter (DM) for lactating and dry cows (550 kg body weight):

Table 2. Dietary NDF, ADF and NFC recommendations for dairy cows in lactation and dry period

Lactation Period	Total NDF	Roughage NDF	ADF	NFC	Starch	Sugar
Early	>28	>19	>18	37-44	24-26	5-6
Mid	29-32	20-22	>19	35-42	23-25	5
Late	>32	21-24	>19	35-42	22-25	5
Dry Period	>25	>35	>25	<35	<20	5

This table provides specific recommendations for different lactation periods and the dry period, focusing on total Neutral Detergent Fiber (NDF), roughage NDF, Acid Detergent Fiber (ADF), Non-Fibrous Carbohydrates (NFC), starch, and sugar content as a percentage of dry matter (DM).

Mineral and Vitamin Requirements

For dairy cows to remain healthy and produce milk at high levels of efficiency, the minerals and vitamins required must be carefully balanced in the ration. Macro minerals such as calcium, phosphorus, magnesium, potassium, sodium, chlorine, and sulfur are vital for milk production and bone health. Additionally, trace minerals (such as zinc, copper, iodine, selenium, etc.) and vitamins (A, D, E, and K) should also be included in adequate amounts in the rations (Underwood and Suttle, 1999).

Mineral	Early Lactation	Mid Lactation	Late Lactation	Dry Period - First 5 Weeks (Far Off)	Dry Period - Last 3 Weeks (Close Up)
Calcium (Ca)	0.8-1.0% DM	0.7-0.9% DM	0.7-0.9% DM	0.6-0.8% DM	0.8-1.0% DM
Phosphorus (P)	0.4-0.45% DM	0.35-0.4% DM	0.35-0.4% DM	0.3-0.35% DM	0.4-0.45% DM
Magnesium (Mg)	0.25-0.35% DM	0.25-0.35% DM	0.25-0.35% DM	0.2-0.3% DM	0.3-0.35% DM
Potassium (K)	1.2-1.5% DM	1.0-1.3% DM	0.9-1.1% DM	0.8-1.0% DM	1.2-1.5% DM
Sodium (Na)	0.1-0.15% DM	0.1-0.15% DM	0.1-0.15% DM	0.08-0.12% DM	0.1-0.15% DM
Chlorine (Cl)	0.2-0.3% DM	0.2-0.3% DM	0.2-0.3% DM	0.15-0.2% DM	0.2-0.3% DM
Sulfur (S)	0.2-0.3% DM	0.2-0.3% DM	0.2-0.3% DM	0.2-0.25% DM	0.2-0.3% DM
Zinc (Zn)	40-60 ppm	40-60 ppm	40-60 ppm	40-50 ppm	50-60 ppm
Copper (Cu)	10-20 ppm	10-20 ppm	10-20 ppm	8-10 ppm	10-15 ppm
Iodine (I)	0.5-1.0 ppm	0.5-1.0 ppm	0.5-1.0 ppm	0.3-0.5 ppm	0.5-0.7 ppm
Selenium (Se)	0.3 ppm	0.3 ppm	0.3 ppm	0.25-0.3 ppm	0.3 ppm
Manganese (Mn)	40-60 ppm	40-60 ppm	40-60 ppm	40-50 ppm	50-60 ppm
Iron (Fe)	50-100 ppm	50-100 ppm	50-100 ppm	50-80 ppm	50-100 ppm

DM = Dry Matter; ppm = parts per million

Figure 1. Recommended Mineral Requirements for Dairy Cows (NRC 2001).

Table 3. Recommended Vitamin Requirements for Dairy Cows

Vitamins	Unit	Lactation Periods	Dry Periods
A	IU / day	85.000 – 100.000	85.000 – 100.000
D	IU / day	20.000 – 30.000	20.000 – 30.000
E	IU / day	500	1000

Dry Matter Intake (DMI)

Dry Matter Intake (DMI) is one of the fundamental parameters that determine the capacity of dairy cows to meet their nutrient requirements. Dry Matter (DM) includes all the substances in the feed that the animal consumes, excluding water. Dairy cows' daily dry matter intake is typically about 3-4% of their body weight. DMI is directly related to milk yield and the stage of lactation (NRC, 2001).

Adequate dry matter intake ensures that necessary nutrients, such as energy, protein, minerals, and vitamins, are consumed in sufficient quantities. DMI, which affects milk yield, is higher in high-producing cows compared to low-producing cows. Optimising DMI should be supported by adjusting the energy density of the rations to enhance milk production efficiency (Allen, 2000).

To calculate the DMI required by lactating dairy cows, the following formula developed by NRC 2021 is used (equation 4):

DMI (*Kg/Day*): [3.7 + (*LacNo* x 5.7) + (0.305 x *MilkE*) + (0.22 x *BW*) + (-0.689-1.87 x *LacNo*) x *BCS*] x [1 – (0.212 + *LakNo* x 0.136) x (−0.053× *DIM*)]

LacNo: Number of Lactations
MilkE: Energy Content of Milk (Mcal/Day)
BW: Body Weight (Kg)
BCS: Body Condition Score
DIM: Days in Milk

LINEAR PROGRAMMING IN FORMULATING ECONOMICAL RATIONS

Linear programming is a mathematical optimisation method that aims to make the most efficient use of defined resources. Conceptually, it helps find the optimal solution to achieve a specific goal (minimising costs or maximising profits) under constraints defined by linear relationships. Linear programming is widely used in various fields, including agriculture, engineering, and economics. This technique is vital for solving problems related to resource management, production planning, and ration formulation (Hillier & Lieberman, 2010).

Using linear programming in ration formulation allows for the simultaneous evaluation of the nutritional values and costs of different feed ingredients, enabling the determination of the most optimal combination. This method contributes to creating rations that meet the animal's nutritional requirements and are economical and balanced.

To formulate an optimal ration, the first step is to determine the dairy cows' nutrient requirements, the nutritional values of the available feed ingredients, and their current prices. The second step involves defining the objective of the linear programming model, which is typically to minimise the ration cost. The third step is to establish the linear programming model. This model incorporates constraints such as meeting the nutritional requirements and minimising the total cost of the feed combinations. Finally, the model is solved using various optimisation techniques to find the least-cost ration (Stigler, 1945). The goal of this analysis is to ensure that the nutritional

needs of the dairy cows are fully met while minimising feed costs, thereby increasing the operation's profitability. Rations formulated using linear programming are crucial for making the most efficient use of limited resources (Charnes & Cooper, 1961).

RATION FORMULATION PROGRAMS FOR DAIRY COWS

There are numerous ration formulation programs available for ruminant animals. Some of these are developed as applications or Windows-based software. The first ration formulation program in the literature is the Ohio Dairy Ration Program (Version 5.2), created by Eastridge and Weiss (1999). This program is designed to formulate rations for lactating and dry cows and heifers to achieve the least cost formulation. It also provides a nutrition library, allows the formation of feeding groups, and summarises feeding costs at the individual animal, group, or herd level. A unique feature of this program is its ability to calculate the nitrogen and phosphorus output in milk and manure for a group of animals or an entire herd. This program aids in minimising feed costs while meeting the nutritional needs of the animals. It helps monitor nitrogen and phosphorus balance on dairy farms, thus contributing to developing feeding strategies.

Another prominent program is the Cornell Net Carbohydrate and Protein System (CNCPS), which uses models of carbohydrate and protein systems to estimate the requirements of beef and dairy cattle under specific conditions, such as the physicochemical composition of available feeds, animal management practices, and environmental factors (climate). This program was initially developed using Microsoft Visual Basic 6.0 and is designed for farm-specific management, environmental factors, and predicting dairy cattle's nutritional needs and production stages (Fox et al., 2004).

OptiTMR, designed by İlikSoft, uses the formulas from NRC and INRA to accurately determine the daily needs of animals and formulate the least-cost ration. However, NRC values may not be sufficient to meet the needs of breeds in Turkey, so OptiTMR has adjusted NRC formulas to address this issue. OptiTMR allows users to solve their daily rations based on NRC Net Energy (NEL, NEM, NEG), NRC Metabolizable Energy (ME), and INRA (UFL, UFV) at their discretion. Additionally, the program requests specific information about the animal to determine its daily needs accurately. Users can then input the available feeds into the program to reach the optimal solution.

Omix, developed by Yetişir (2015) for Windows XP/Vista/7/10/11 operating systems, is aimed at feed mills, organisations that wish to prepare their own mixed feed, dairy cattle, beef cattle, and ruminant animal breeders, as well as research and educational institutions related to livestock. The program benefits feed mills and livestock enterprises that want to produce their own feed. With Omix, it is possible to formulate both mixed feed and rations. The program also includes relevant scientific articles, an online help system, tip routines, and a catalogue, providing sufficient resources for learning the software.

RATION FORMULATION AND LINEAR PROGRAMMING APPLICATION USING MICROSOFT EXCEL

A ration formulation program for dairy cows has been developed using Microsoft Excel. The Excel-based ration program consists of several key components: a feed database, a nutrient requirement, ration formulation processes, and a ration report section.

Feed Database

The feed ingredients used in the nutrition of dairy cows are categorised into three classes in the created database: roughages, concentrates, and feed additives.

- Roughages provide essential nutrients and play a crucial role in ensuring mechanical fullness in the rumen, facilitating effective microbial fermentation, and promoting the balanced and adequate secretion of volatile fatty acids. These functions are critical for maintaining rumen health and optimising milk fat synthesis.
- Concentrates are rich in energy and protein and complement roughages by providing the nutrients that roughages alone may not adequately supply.

- Feed additives are used in dairy cow nutrition to support animal health, enhance productivity, and optimise rumen functionality. These additives improve the digestive system, increase disease resistance, and strategically boost milk yield.

In the Excel-based ration formulation program, the nutrient values of the feed ingredients, or the feed ingredients database, were created using sources such as "Tables of Composition and Nutritional Value of Feed Materials" (Sauvant et al., 2004) and the National Research Council (NRC). It is well-known that the nutrient values of feed ingredients can vary depending on the region, country, and soil management practices. Therefore, the user can update the nutrient content of these ingredients based on their own analyses (Boğa & Çevik, 2012).

The design includes information related to the feed (e.g., Feed Name, Feed Class, Ingredient Price, Dry Matter (DM), Metabolizable Energy (ME), Crude Protein (CP), etc.). The rationing program allows easy access to the database, enabling users to easily modify feed information, add new feeds, copy and modify existing feeds, or delete unwanted feed ingredients—all facilitated by the user-friendly features of Excel.

As shown in Figure 2, the feed classes—roughages, concentrates, and feed additives—are displayed in separate Excel columns, with each feed ingredient's nutrient content listed. A nutrient value for a specific feed ingredient can be added to the database by creating a new column based on analysis results. Additionally, as shown in Figure 2, if a feed ingredient lacks a nutrient value, the ration program highlights the relevant cell in a different colour using Excel's conditional formatting, drawing attention to it. This feature allows the user to easily view and modify the content of the feed ingredient based on their analysis results and save the updated information in the database.

FEED CLASS	RAW MATERIALS	PRICE	FEED GROUP	DM	NEL	NEY	NEK	ME-RU	TDN	CP	DP	RDP	RUP	CS	ADF	NDF
		TL / TON		%	Mcal / Kg	Mcal / Kg	Mcal / Kg	Mcal / Kg	Kg/Kg	gram/kg	gram/kg	gram/kg	gram/kg	kg/kg	kg/kg	kg/kg
Roughage	*Corn Silage - 26% DM*	₺ 5.500,00	Kaba Yem	25,000	1,457	1,457	0,817	2,397	0,650	80,000	44,000	76,256	3,744	0,260	0,320	0,540
Roughage	*Wheat Silage - 35% DM*	₺ 3.300,00	Kaba Yem	40,000	1,330	1,280	0,710	2,130	0,580	125,000	56,250	101,250	23,750	0,290	0,380	0,607
Roughage	*Barley Straw*	₺ 4.000,00	Kaba Yem	90,000	0,927	0,971	0,000	1,510	0,430	40,000	7,000	14,800	25,200	0,420	0,520	0,780
Concentrated Feed	*Barley*	₺ 7.200,00	Kesif Yem	89,000	2,030	1,850	1,340	2,820	0,800	106,800	22,000	80,190	26,610	0,045	0,062	0,169
Concentrated Feed	*Sunflower Seed Meal - % 25.9 CP*	₺10.000,00	Kesif Yem	90,000	1,470	1,470	0,880	2,200	0,650	259,000	77,700	194,250	64,750	0,210	0,330	0,400
Concentrated Feed	*Soybean Meal - % 44 CP*	₺19.500,00	Kesif Yem	90,000	1,940	2,060	1,400	3,290	0,840	440,000	161,700	308,000	132,000	0,096	0,100	0,140
Concentrated Feed	*DDGS, Corn - % 29 CP*	₺11.500,00	Kesif Yem	91,000	2,040	2,180	1,500	3,470	0,880	290,000	18,240	79,040	224,960	0,120	0,180	0,460
Feed Additives	*Sodium bicarbonate*	₺ 4.800,00	Kesif Yem	98,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Feed Additives	*Magnesium Oxide*	₺55.000,00	Kesif Yem	98,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Feed Additives	*Mineral / Vitamin Premix*	₺60.000,00	Kesif Yem	99,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000

Figure 2. Feed Ingredients Database Table.

Nutrient Requirements

The nutrient requirements of dairy cows vary depending on factors such as breed, age, body weight, and production stages. These requirements are categorised into two main groups: "maintenance nutrient requirements" and "production nutrient requirements" (e.g., milk production, pregnancy). After accurately determining the nutrient requirements of the animals within these two groups, appropriate and economical rations can be formulated (Canbolat, 2015). To achieve optimal feeding, it is essential to identify the needs and formulate rations accordingly correctly.

In this context, the ration program designed for dairy cows utilises formulas from the National Research Council (NRC) publications, specifically the National Academies of Sciences, Engineering, and Medicine (NASEM), to calculate the nutrient needs of dairy cows. As shown in Figure 3, the design allows users to input relevant animal data (such as body weight, milk yield, milk fat content, etc.). Based on these inputs,

the nutrient requirements like Dry Matter Intake (DMI), Crude Protein (CP), Net Energy for Lactation (NEL), Calcium (Ca), and others are calculated accurately, as shown in Figure 4.

By correctly defining these parameters, the necessary nutrient requirements for dairy cows can be calculated using the formulas, completing the second step of formulating an optimal ration through linear programming.

ANIMAL DESCRIPTION	
Age (Months)	**48**
Body Weight (Kg)	**650**
Genotypes	**Holstein**
Number of lactations	**2**
Number of Days in Pregnancy	**190**
Milk Yield (Kg/day)	**25**
Milk Fat (%)	**3,5**
Body Condition Score	**3**
Temperature (^{o} C)	**20**

Figure 3. Animal description section.

Nutrient Requirements			
DMI, kg/day	20,74		
NEL, Mcal/day	30,24	***Na, g/gün***	40,45
NDF, Kg/day	6,02	***S, g/day***	41,49
ADF, Kg/day	3,94	***Co, mg/day***	2,28
NFC, Kg/day	7,26	***Cu, mg/day***	9,87
CP, g/day	3394	***I, mg/day***	9,75
MP, g/day	2333	***Fe, mg/day***	25
RDP, g/day	1876	***Mn, mg/day***	2,05
RUP, g/day	1518	***Se, mg/day***	6,22
Ca, g/gün	51,14	***Zn, mg/day***	129,25
P, g/gün	46,31	***Vitamin A, 1000 IU/kg***	71,5
Mg, g/gün	5,7	***Vitamin D, 1000 IU/kg***	19,5
Cl, g/gün	43,38	***Vitamin E, IU/kg***	520

Figure 4. The nutrient requirements are calculated according to the animal descriptions.

Ration Formulation and Solution

In the ration program developed using Microsoft Excel, a linear algorithm running in the background ensures that the nutrient requirements are determined according to the specified values. The feed ingredients included in the ration are selected based on the availability of feeds on the farm or those that can be purchased, considering the person's experience formulating the ration and the animal's needs. The feed ingredients in the ration are classified as roughages, concentrates, and feed additives. In the prepared ration program, the feed ingredients will be selected using "checkboxes."

On the "Select Ingredient" page, the feed class desired for use in the ration must be selected, and the defined ingredients for the chosen class will be displayed accordingly, allowing the desired ingredient to be selected from the desired class. After the ingredient selection process is completed, the nutrient values of the chosen ingredients should be reviewed. Any missing data or changes need to be made before solving the ration.

After completing the ingredient selection, the values for the use of ingredients in the individually prepared ration (minimum and maximum) should be specified in the relevant column on the "RATION" page based on the user's experience, the stock status of the ingredient, and the price of the ingredient.

Thanks to Excel's "Solver add-in," users can define the necessary parameters, as shown in Figure 5, thereby setting up the linear algorithm. The Solver will then solve the ration based on the specified criteria, providing the optimum ration that meets the nutritional needs of the animals. In Figure 6, the result provided by the Solver when the "SOLVE" command is executed shows the optimal solution based on the desired criteria. Thus, Excel will deliver the most economical and suitable ration, ensuring the nutritional needs of dairy cows are met using the selected ingredients.

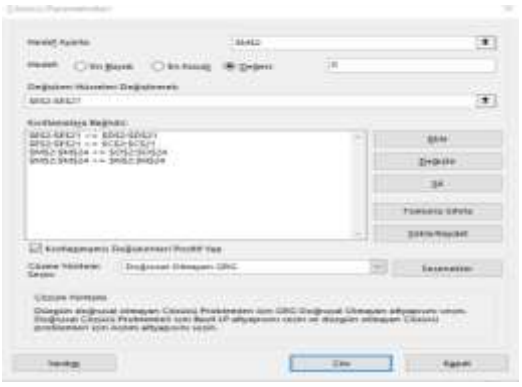

Figure 5. Defining the Solver in Excel.

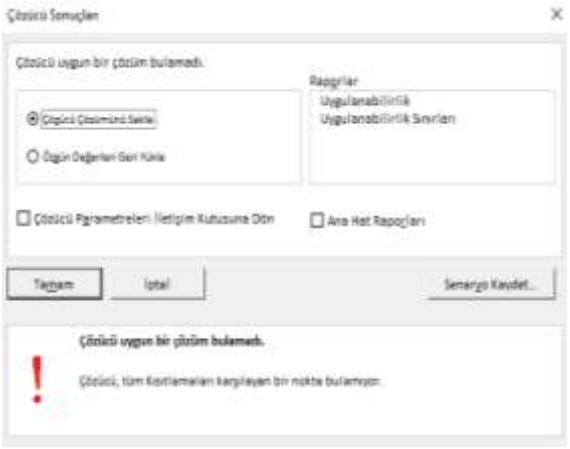

Figure 6. Results Obtained After Solving with Excel Solver.

Ration Report

As in any industry, reading and interpreting reports is crucial for reaching accurate, reliable, and definitive results in the agricultural sector. In this context, the reporting feature in the Microsoft Excel-based ration program plays a pivotal role in ensuring accurate outcomes. As shown in Figure 7, the report section includes the names of the feed ingredients used in the ration, the nutrient values derived from these feeds, their usage proportions in the ration, and the overall cost of the ration.

If desired, the user can print the report for archiving purposes. Additionally, the ration program allows for electronically storing the report in PDF format. This feature enables comparisons with previous rations on a farm-by-farm basis, providing valuable insights for future ration formulations.

RATION RESULT REPORT — 21.08.2024 19:48 — Page 1

RATION NAME: *DAIRY COW TMR RATION*

FEED RAW MATERIALS	UNIT	AMOUNT	%
Corn Silage - 26% DM	Kg	7,000	17,75%
Corn - 8.8% CP	Kg	3,800	9,63%
Wheat Silage - 35% DM	Kg	10,000	25,36%
Barley Straw	Kg	2,800	7,10%
Barley - 10.68% CP	Kg	2,500	6,34%
Sunflower Seed Meal - 25.9% CP	Kg	1,800	4,56%
Soybean Meal - 44% CP	Kg	2,000	5,07%
DDGS Corn - 29% CP	Kg	1,383	3,51%
Sodium Bicarbonate	Kg	0,200	0,51%
Magnesium Oxide	Kg	0,040	0,10%
Vitamin Premix	Kg	0,020	0,05%
Wheat Bran - 14% CP	Kg	0,800	2,03%
Limestone - Powder	Kg	0,251	0,64%
Salt	Kg	0,080	0,20%
Toxin Binder	Kg	0,060	0,15%
Yeast	Kg	0,050	0,13%
Water	Kg	1,956	4,96%
Bypass Fat - 99%	Kg	0,200	0,51%
Cattle Milk Feed 18% CP	Kg	4,500	11,41%
TOPLAM		39,440	100%

BESİN MADDELERİ	RATION	UNIT	DM	UNIT
Price	274,58	TL / Kg	274,58	TL / Kg
Dry Matter Intake (DMI)	24,12	Kg/Kg	24,12	Kg/Kg
Water	40,00	%	40,00	%
Net Energy for Lactation (NEL)	38,72	Mcal / Kg	1605,32	Kcal / Kg
Total Digestible Nutrient - Ruminant (TDN-R)	0,68	Kg/Kg	67,84	%
Metabolizable Energy - Ruminant (ME-R)	60,81	Mcal / Kg	2521,13	Kcal / Kg
Crude Protein (CP)	3669,82	gram/kg	15,22	%
Rumen Degradable Protein (RDP)	2427,42	gram/kg	10,06	%
Rumen Undegradable Protein (RUP)	1291,00	gram/kg	5,35	%
Crude Fiber (CF)	4,12	kg/kg	17,07	%
Acid Detergent Fiber (ADF)	5,26	kg/kg	21,82	%
Neutral Detergent Fiber (NDF)	8,81	kg/kg	36,55	%
Starch	4849,99	gram/kg	20,11	%
Sugar	754,32	gram/kg	3,13	%
Ether Extract (EE)	896,91	gram/kg	3,72	%
Calcium	180,00	gram/kg	0,75	%
Phosphorus	111,55	gram/kg	0,46	%
Salt	0,33	%	0,33	%

Figure 7. Ration Calculation Results Report.

CONCLUSIONS

The ration program developed using Excel aims to create a ration formulation tool for dairy farms that minimises feed costs while optimally meeting the nutritional requirements of the animals by employing a linear programming model. This model, created with Microsoft Excel, offers a readily accessible and user-friendly solution for preparing economical and balanced rations for farms. Due to the flexibility of Excel, farms can customise rations according to their needs and quickly adapt to changing conditions.

Rations formulated using the linear programming method have been shown to meet the nutritional needs of dairy cows most cost-effectively and can significantly reduce farm feed costs. Additionally, one of Excel's most significant advantages is the ease with which users can regularly update and manage rations. For the program to be used effectively, the user must be trained in linear programming, Excel usage, and animal nutrition. This ensures that the ration program can be fully utilised, yielding more accurate results. Furthermore, regularly updating the feed ingredients and nutrient requirements data will ensure that the

program consistently provides up-to-date and accurate results.

In conclusion, this ration program offers a practical solution for dairy farms to reduce feed costs, increase efficiency, and decrease reliance on external resources. The Excel-based linear programming model will help farms control costs while boosting productivity.

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Postnatal (neonatal period) lamb deaths

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Abstract

The losses of lambs are significantly contributing to the decrease in profitability on farms. High mortality rates before weaning lambs from milk are limiting sheep production worldwide. It is stated that lamb mortality rates have varied between 15-20% over the last 40 years in most countries. Although the mortality rate varies according to enterprises, in well-managed sheep enterprises in our country, the postnatal lamb death rate is determined to be around 10%, while in some enterprises, it is around 5%. Postnatal lamb deaths in our country have been found to be between an average of 10-25%. Although there are many factors affecting lamb deaths in the postnatal period, factors such as the type and management of the enterprise, birth weight of the lamb, age of the mother, care-feeding method, housing conditions, and lambing season play a significant role in lamb deaths. Another common cause of postnatal lamb deaths is hypothermia, which occurs due to heat loss and inability to feed. In sheep farming, minimizing lamb deaths is crucial for breeders to make a profit. With preventive measures taken during the postnatal period, mortality rates of neonatal lambs can be reduced. Among the solutions to reduce lamb deaths, it is reported that providing a sufficient amount and quality of colostrum, ensuring umbilical cord disinfection, and placing lambs in separate pens according to the same age group and disease detection or suspicion are essential. The necessity of developing high mortality rates that can be reduced with changeable factors and solution methods for this purpose constitutes the aim of this study. In this study, detailed examination of factors such as lamb deaths observed in the postnatal period, practices required to reduce these deaths, errors made in care and feeding, reasons related to the mother or lamb, diseases, enterprise type, and management will be conducted. Solutions to be used in the field and the development of different feeding-raising strategies based on the cause will be compiled.

Key words: *Key words: Lamb, postnatal period, lamb deaths, neonatal mortality*

Probiotic use in lamb and calf diarrhea

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Abstract

Probiotics can be widely used as live microbial feed additives in animal nutrition. It has positive effects on the improvement of the digestive system and health by positively improving the intestinal microbial balance in sufficient amounts with feed. Such advantages stimulate growth and reduce gastrointestinal upset. The most commonly used species are Gram (+) bacterial strains such as Lactobacillus, Bacteriodes, Enterococcus, Streptococcus, Pediococcus, Bacillus and Bifidobacterium spp., fungi Aspergillus spp. and yeasts Saccharomyces cerevisiae and Kluyveromyces. Calf mortality rate in Turkey is approximately 15%. This means approximately 500000 calves. In lambs, this rate varies between 9.50-14.43%. It is suggested that it is possible to use probiotics to minimize financial losses and thus contribute to increasing the number of animal population in our country. Therefore, in our study, the use, duration and amount of probiotics regulating the digestive system in both calves and lambs were compiled with the aim of reducing these rates in order to reduce lamb-calf mortality rates.

Key words: *Calf mortality, Lamb mortality, Probiotics, Probiotic use, Duration of probiotic use*

Investigation of animal welfare by applying TOPSIS method

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Abstract

The aim of this research is the applicability of TOPSIS method, in order to examine animal welfare of small-scale cattle holdings registered in Şırnak-İdil District Directorate of Agriculture and Forestry. In this study, 7 different criteria were evaluated for the evaluation of small-scale cattle holdings according to animal welfare. These criteria were evaluated as area per animal, social relationship, barn floor type, ventilation, lighting, care and nutrition. The decision maker in the evaluation was determined by the Veterinarian working in the Şırnak-İdil District Directorate of Agriculture and Forestry.
As a result of all the calculations, it has been concluded that the TOPSIS method will be efficient and effective in solving the problem of evaluating animal welfare in cattle enterprises.

Key words: *Animal welfare, Multi-Criteria, TOPSIS,*

INTRODUCTION

Animal welfare is defined as the animal's being in harmony with its environment, its ability to adapt to its environment without suffering, and its ability to lead a healthy life both psychologically and physically (Krohling and Pacheco, 2015).

While animal welfare positively affects the productivity of animals, it constitutes an important role of sustainable livestock practices. The implementation of animal welfare practices will positively affect the increase in production economically and ecologically, as well as the increase in sustainability animal husbandry (Küçükönder et al., 2013; Tuğrul and Çitil, 2022).

It is often difficult to explain cattle holdings with statistical methods in order to interpret and evaluate them in terms of animal welfare. Thus, there is a need to scale the animal welfare characteristics, which is an abstract concept, with concrete criteria. For this purpose, animal welfare evaluation criteria have been applied in order to measure cattle holdings according to animal welfare. In the application, a total of 7 criteria were evaluated, including area per animal, social relationship, barn floor type, ventilation, lighting, care and nutrition. Technique for Order Preference by Similarity to Ideal Solution (TOPSIS), which is the most preferred among the Multi-Criteria Decision Making (MCDM) methods, was used to evaluate the 7 criteria discussed in the application. MCDM method provides to reach the most appropriate compromise solution by evaluating different approaches and alternatives according to their decision-making values (Wang and Lee, 2007).

The aim of this research is the applicability of TOPSIS method, in order to examine animal welfare of small-scale cattle holdings registered in Şırnak-İdil District Directorate of Agriculture and Forestry. In this study, 7 different criteria were evaluated for the evaluation of small-scale cattle holdings according to animal welfare. These criteria were evaluated as area per animal, social relationship, barn floor type, ventilation, lighting, care and nutrition (Yong, 2006).

In this study, after the introduction part, the theory of the MCDM method is explained in

detail. In this study, the data on which the method is applied, the purpose of the study, the solutions of the methods and its application in the excel program are shown in detail. Finally, the aim of the study and its general evaluation were made.

MATERIALS AND METHODS

The data used in the application part of the research were taken from 7 small-scale enterprises registered to the Şırnak-İdil District Directorate of Agriculture and Forestry. These enterprises, which are considered in practice, are obtained from the center of the İdil district or from its villages. The names of the villages where the small-scale enterprises are located and the number of animals are shown in Table 1.

Table 1. Village Names and Number of Animals of Small-Scale Businesses

Rank No	Village	Number of Animals
B1	Bereketli	102
B2	Bozburun	107
B3	Çukurlu	196
B4	Dirsekli	197
B5	Özbek	170
B6	Yörük	114
B7	Kayalı	101

RESULTS

In the study, small-scale cattle holdings primarily dealt with the evaluation of scales according to animal welfare. The decision maker in the evaluation was determined by the Veterinarian working in the Şırnak-İdil District Directorate of Agriculture and Forestry. The evaluation features and animal welfare evaluation criteria are given in Table 1. These criteria were 7 criteria as Space per Animal (C1), Social Relationship (C2), Barn Floor Type (C3), Ventilation (C4), Lighting (C5), Care (C6) and Nutrition (C7). Considering the qualitative and quantitative evaluation features of these criteria, the veterinarian has scored between 1-5 values.

Determining the method objectives and evaluation criteria: First of all, the criteria created in small-scale cattle enterprises for the purpose of the study were selected as animal welfare criteria and were weighted according to the degree of importance by the Specialist Veterinarian. The weights of these criteria are given in Table 3.

Table 2. Animal Welfare Evaluation Criteria

	Evaluation Features				
C1: Space per Animal	4 m^2	5 m^2	6 m^2	7 m^2	7 m^2 >
C2: Social Relationship	Not group	Group	By gender	By age	Family heifer
C3: Barn Floor Type	Concrete	Plastic	Metal grid	25 cm straw	50 cm straw
C4: Ventilation	Very bad	Bad	Avarage	Good	Very good
C5: Lighting	Very bad	Bad	Avarage	Good	Very good
C6: Care	Very bad	Bad	Avarage	Good	Very good
C7: Nutrition	Very bad	Bad	Avarage	Good	Very good

Table 3. Weights of Criteria

Criteria	C1	C2	C3	C4	C5	C6	C7
Weight	0.15	0.14	0.14	0.14	0.14	0.14	0.15

Calculation of the decision matrix: In Table 5, there are 7 small-scale enterprises in the rows of the decision matrix in the TOPSIS method and the criteria applied in decision making (C1, C2, C3, C4, C5, C6, C7) in the columns.

Table 4. Decision Matrix in Small-Scale Cattle Farms by Animal Welfare

Businesses	Criteria						
	C1	C2	C3	C4	C5	C6	C7
B1	1	1	1	3	3	3	3
B2	1	1	1	3	3	4	3
B3	3	2	1	4	3	3	4
B4	1	1	1	2	3	3	3
B5	3	2	1	3	3	3	3
B6	3	1	1	2	3	4	3
B7	4	1	1	4	3	4	4

With 7 small scale enterprises and 7 evaluation criteria, the decision matrix is 7x7 and is formed as shown in equation 1. The decision matrix is created by taking the values in Table 4.

$$A = \begin{bmatrix} 1 & 1 & 1 & 3 & 3 & 3 & 3 \\ 1 & 1 & 1 & 3 & 3 & 4 & 3 \\ 3 & 2 & 1 & 4 & 3 & 3 & 4 \\ 1 & 1 & 1 & 2 & 3 & 3 & 3 \\ 3 & 2 & 1 & 3 & 3 & 3 & 3 \\ 3 & 1 & 1 & 2 & 3 & 4 & 3 \\ 4 & 1 & 1 & 4 & 3 & 4 & 4 \end{bmatrix}_{7x7} \quad (1)$$

Here, a_{ij} values in the $A = [a_{ij}]_{7x7}$ matrix show the values that the criteria take according to the enterprises. After the decision matrix is created, the Normalized decision matrix (R) is created by using the equation (1). The r_{11} value of the R matrix is obtained by dividing the a_{11} element of the A decision matrix by the square root of the sum of the squares of the first column elements of the A matrix and is calculated as an example in equation (11).

$$r_{11} = \frac{1}{\sqrt{1^2+1^2+3^2+1^2+3^2+3^2+3^2+4^2}} = 0.13 \quad (2)$$

R matrix is created by calculating all the criteria as in equation (2).
The deviation values of all the calculated alternatives are given in Table 5.

Table 5. Distance to Positive and Negative Ideal Solution Values

Alternatives	S^+_i	S^-_i
B1	0.104	0.024
B2	0.115	0.017
B3	0.047	0.084
B4	0.084	0.042
B5	0.028	0.092
B6	0.087	0.062
B7	0.038	0.092

The closeness values of all alternatives according to the ideal solution are shown in Table 6.

Table 6. Proximity Values According to the Ideal Solution

Alternatives	C^*_i
B2	0.128
B3	0.641
B4	0.333
B5	0.766
B6	0.446
B7	0.707

When the alternatives are ranked according to the closeness (C^*_i) value to the ideal solution, the ranking of the enterprises most suitable for animal welfare among small-scale enterprises is given in Table 7.

Table 7. Most Appropriate Small-Scale Cattle Farming Ranking

Rank	Business Name	Business Code	Result
1	Tözal	B5	0.766
2	Kayalı	B7	0.707
3	Kozluca	B3	0.641
4	Yağmurca	B6	0.446
5	Şekerköy	B4	0.333
6	Bereketli	B1	0.187
7	Bozburun	B2	0.128

When Table 7 is examined, it is concluded that the best Small-Scale Cattle holding is Tözal and the worst one is Bozburun.

DISCUSSION

The correct analysis and interpretation of data determines the future status of a business. Making the data applicable and analyzing and interpreting it with the most appropriate method provides an important competitive advantage to the business owners. For this purpose, MCDM method was applied to reveal the relationships between the data.
TOPSIS method, which is one of the MCDM methods, has been applied for the selection and ranking of Small-scale Cattle holdings suitable for Animal Welfare. As a result of all the calculations, it has been concluded that the TOPSIS method will be efficient and effective in solving the problem of evaluating animal welfare in cattle

enterprises. In addition, when studies on animal welfare were investigated, it was seen that the criteria evaluating animal welfare were not used in cattle enterprises and MCDM methods were not applied. Thus, it is thought that this study will be useful to researchers. In subsequent studies, it has been shown that MCDM methods can also be applied when evaluating animal enterprises according to animal welfare criteria. In addition, it is suggested that researchers can apply other MCDM methods besides TOPSIS method.

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Reproductive biology of whiting, *Merlangius merlangus* (Linnaeus 1758) in the Sea of Marmara

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Abstract

Merlangius merlangus is a key species economically and was the focus of this study. This species is found across various regions, including the northeastern Atlantic, southeastern Barents Sea, Iceland, Portugal, the Aegean Sea, the Adriatic Sea, and occasionally in the northwestern Mediterranean Sea. In Turkish waters, it inhabits the Black Sea, Marmara Sea, and Aegean Sea. Typically, M. merlangus prefers sandy and muddy substrates at depths between 10 and 200 meters. For this research, samples were collected using trawl nets from 34 stations in the Marmara Sea, spanning from March 2017 to December 2018. In total, 4978 M. merlangus specimens were examined. The study evaluated maturity stages, reproductive seasons, and the length at first maturity, finding that spawning predominantly occurs during the winter months based on GSI values and maturation stages. The length at first maturity was identified as 15.0 cm for females and 15.4 cm for males.

Key words: *Whiting, gonadosomatic index, maturity, first maturity length, Marmara Sea*

INTRODUCTION

Merlangius merlangus (Linnaeus, 1758), commonly known as whiting, is a commercially important species within the Gadidae family. In Türkiye, it is found in the Black Sea, the Marmara Sea, and the Aegean Sea (Mater et al., 2003). Its distribution extends to the northeast Atlantic, the southeastern Barents Sea, Iceland, Portugal, the Aegean Sea, the Adriatic Sea, and nearby waters, with occasional occurrences in the northwest Mediterranean Sea (Froese and Pauly, 2022). This species can grow up to 70 cm in length and has a lifespan of up to 20 years. Whiting typically inhabits sandy and muddy substrates and is found at depths ranging from 10 to 200 meters (Froese and Pauly, 2022).

Although *M. merlangus* is an economically important species, the amount of production in our country has been in decline from year to year. In the 2000s, the average production was 1000 tonnes. However, after 2010, it showed a rapid decline (1063 tonnes) and decreased to 110 tonnes in 2021 (TUİK 2023).

Despite its economic and ecological importance, there are only one study about reporductive time and fecundity of *M. Merlangus* in the Sea of Marmara (Göksungur 2004). The other studies about reproductive time and first reproductive length in the Black Sea (Bilgin et al., 2012; Sağlam and Sağlam, 2012; Mazlum and Bilgin, 2014; Mazlum and Bilgin, 2014; Yıldız et al., 2021).

The present study is to reveal and contribute the reproduction biology of the species distributed in the Marmara Sea. Therefore, our study is represents the first data about the first reproductive length of *M.merlangus* in the Sea of Marmara.

MATERIALS AND METHODS

Sampling was carried out by TAGEM (General Directorate of Agricultural Research and Policy of Turkey) under project number TAGEM/HAYSÜD/2014/05/01. Samples were collected between March 2017 and December 2018 at 34 stations of the trawl net in the Sea of Marmara. Sampling was conducted by bottom trawl according to the MEDITS (Mediterranean International Bottom Surveys) standards, with an average speed of 3 miles and a duration of 30 metres.

The total length (TL) of the specimens was measured to the nearest 0.1 cm and the total weight (W) was measured to 0.01 g. Gonad maturity stages were determined by Holden and Raitt (1974): immature, maturing, ripening, ripe, and spent.

The gonadosomatic index (GSI) was calculated using the formula developed by Gibson and Ezzi (1980):

GSI = (GW/W-GW)×100 (1)

where GW is the weight of the gonad (g).

Length at first maturity (L50) was estimated by fitting a logistic function using the Newton algorithm. This function is defined as follows;

P(1) = 1/1+e -(a+b1) (2)

where P(1) is the proportion of mature specimens at length 1, and a and b are the parameters of the logistic equation (Piñeiro and Saínza 2003).

RESULTS

A total of 4978 *M. merlangus* specimens were assessed and studied. Out of these, 1451 were identified as females, representing 29.1% of the sample, whereas 1488 were identified as males, which is 29.9%. The resulting sex ratio was 1:1.03, indicating a slight predominance of males.

GSI values ranged between 0.23 and 9.11. The highest GSI values for females were recorded in December 2018 and March 2017, while the lowest value was noted in June 2018 (Figure 1). Based on the GSI values and maturation stages (Figure 2) throughout the study period, spawning occurred throughout the year, with the most intense spawning observed during the winter months (see Figures 1 and 2). The length at first maturity was calculated to be 15.0 cm for females and 15.4 cm for males (Figure 3).

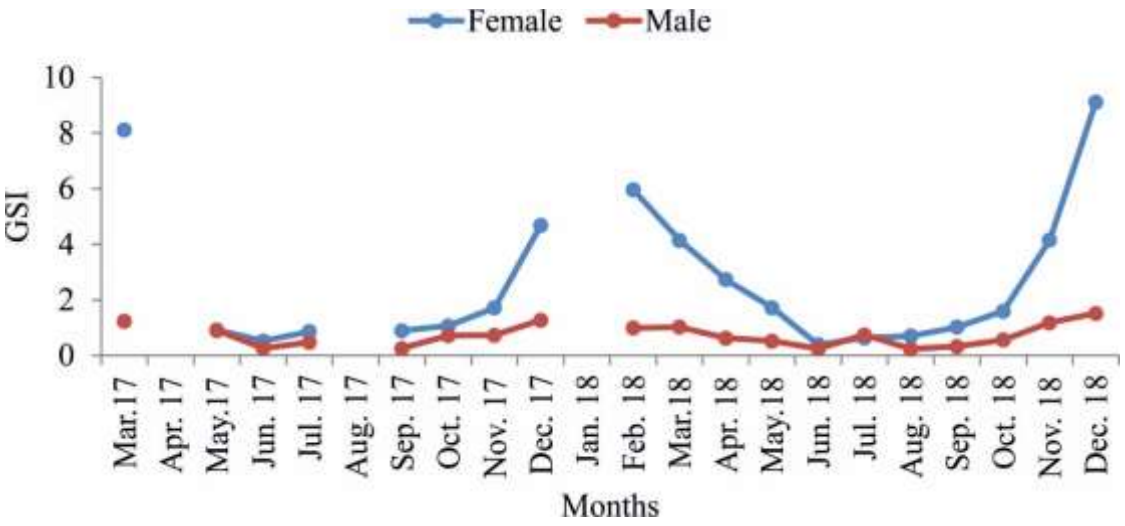

Figure 1. Monthly variation of gonadosomatic index in females and males of *M.merlangus.*

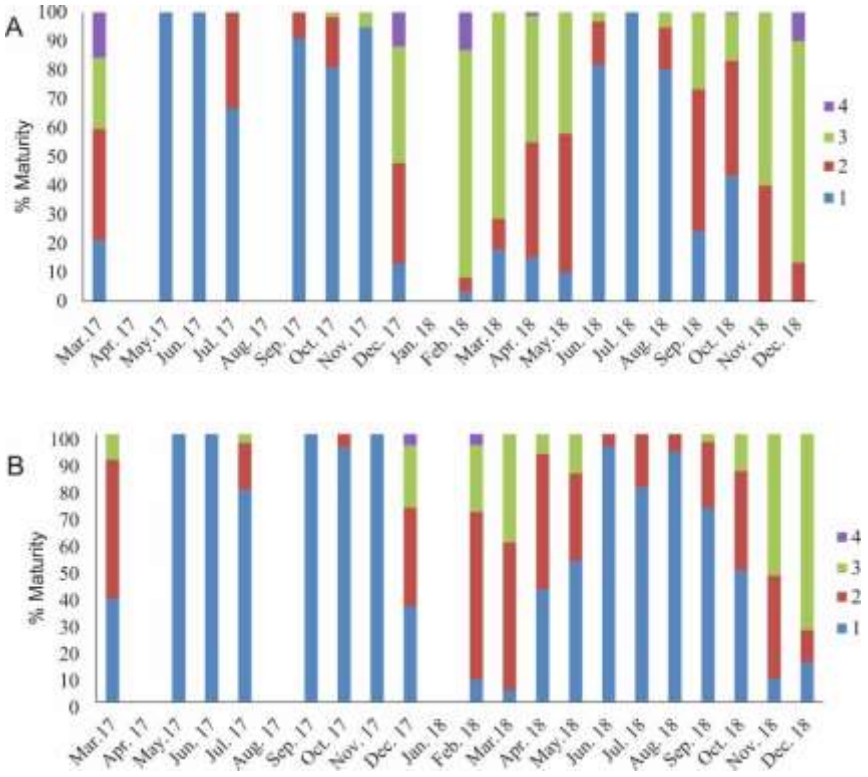

Figure 2. Montly maturity stages in females (A) and males (B) of *M.merlangus.*

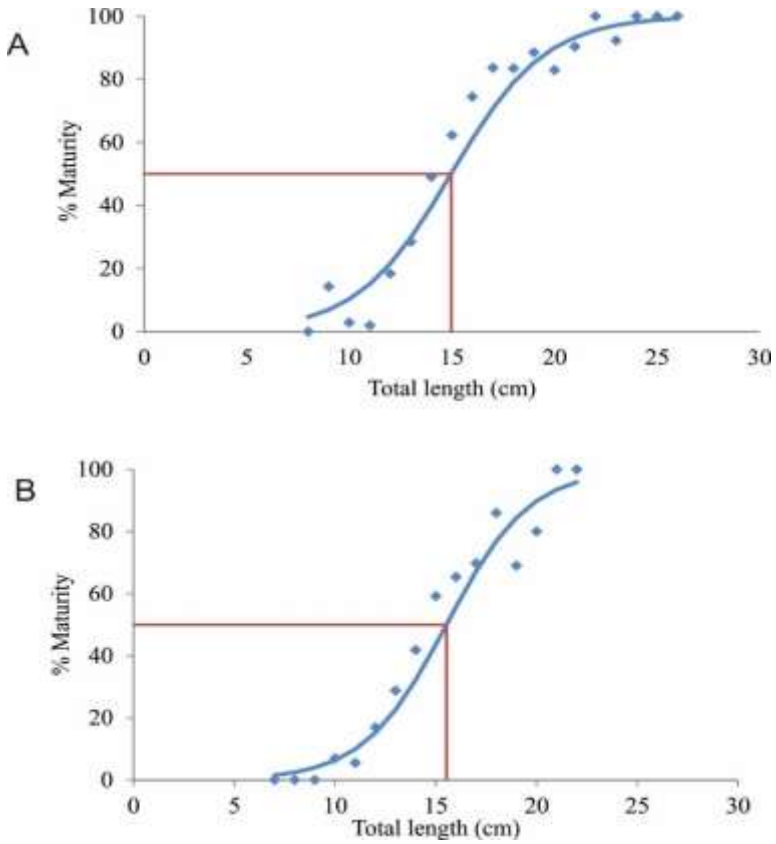

Figure 3. The first reproductive length (L50) of *M. merlangus* in female (A) and male (B) individuals.

DISCUSSION

The study sampled 4,978 whiting, with lengths ranging from 5.0 cm to 26.7 cm and weights from 2.03 g to 167.3 g. Females were more common than males. The highest gonadosomatic index (GSI) values were observed in December 2018 and March 2017, indicating peak spawning in winter. Whiting were found to be mature throughout the year, with the highest spawning activity in winter. The length at first sexual maturity was 15.0 cm for females and 15.4 cm for males. Cohen et al (1990) found that the spawning season for whiting in the Mediterranean was from January to May.

The first sexual maturity lengths for male and female were given as 20.2 cm TL and 28.4 cm in the North Sea (Jennings et al. 1998), 25.5 cm TL and 27.5 cm TL in the West Scheldt, Netherlands (Cattrijsse and Hampel 2000), 30.4 cm TL and 28.0 cm TL in the Celtic Sea, Ireland (Hehir, 2003). In the other studies in Turkish seas the first reproductive length were calculated as 14.6 for females and 13.9 for males (Bilgin et al., 2012), 13.9 for females and 12.5 for males (Yılddız et al., 2021) in the Black Sea.

Table 1 shows the reproductive periods and lengths in the different regions studied. It is assumed that regional and ecological differences account for the variations between the results of this study and those reported by other researchers. In the 5/1 communiqué for commercial fishery (2020/20) issued by the Ministry of Agriculture and Forestry, which regulates commercial fishing, the minimum landing size (MLS) of *M.merlangus* species is set at 13 cm TL (BSGM, 2020). In this study, the length at first sexual maturity was determined to be 15 cm TL for females and 15.4 cm TL for males. Stocks might be depleted by catching individuals that have not yet reached their first reproductive length. It seems that the MLS in the circulars should be increased in order to ensure the continuity of the stocks for *M. merlangus*.

Table 1. Reproductive parameters of whiting in different regions

Research	Area	Sex	Reprodcutive period	Lm (cm)
Cohen et al. 1990	Mediterranean Sea		January-May	
	Between the British Isles and the Bay of Biscay		January-September	
	Black Sea		Whole year	
	Northeast Atlantic		January-September	
Jennings et al. 1998	North Sea, Scotland	M		20.2 TL
		F		28.4 TL
Cattrijsse and Hampel 2000	West Scheldt, Netherlands	M		25.5 TL
		F		27.5 TL
Hehir 2003	Celtic Sea, Ireland	M	February-April	30.4 TL
		F	February-April	28.0 TL
Göksungur 2004	Marmara Sea		Whole year	
Bilgin et al. 2012	Black Sea	F	August, December and	14.6
		M	January	13.9
Sağlam and Sağlam 2012	Black Sea	C	March to July	
Mazlum and Bilgin 2014	Black Sea	C	February, April and November	-
Yıldız et al, 2021	Black Sea	F		13.9
		M		12.5
This study	Marmara Sea	F	Winter	15.0 TL
		M		15.4 TL

ACKNOWLEDGEMENTS

This study financially supported by the Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research TAGEM/HAYSÜD/2014/05/01.

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Software used for estimation of breeding values

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Abstract

A number of purpose specific software are available for estimation of breeding values where some of them have become obsolete due to innovation and upgradation of operating systems of computers. Nowadays ASReml is commonly employed in animal and plant breeding sciences for this purpose. While using ASReml we require data, pedigree and job control files in free format or txt format or in csv format usually formatted through Excel spreadsheet. The software ASReml is windows driven and can be operated on csv formatted files. The job file is directly written in its control panel where data are accessed directly. However, the software is simple to learn and run. After running job control file many files are generated along with solution file containing estimated breeding values which are further processed/edited and arranged in specific order in Excel to explain the results. This software also generate genetic parameters like heritability and correlations (genetic and phenotypic).

Key words: *EBVs, genetic evalaution, ASReml, genetic parameters*

Reproductive biology of European Hake, *Merluccius merluccius* (Linnaeus, 1758) in the Marmara Sea, Türkiye

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Abstract

Reproduction biology of marine teleost fish is a vital tool to determine spawning season, fecundity, first sexual maturity and related fisheries legislations. In this study, we aimed to estimate and present reproductive parameters of European Hake, distributed in the Marmara Sea. In this study, a total of 919 European Hake specimen were sampled with beam trawl scientific survey between March 2012 and July 2014 from the Marmara Sea. Individuals were ranged between 7.3 cm and 40.7 cm at total length (TL). Mean TL of males and females were detected as 19.3 cm TL and 22.5 cm TL, respectively. A 20 of 919 individual could not sexed due to damaged gonad structure. The sex ratio was estimated to be 1: 1.9, in favour of males. Gonadosomatic index (GSI) values were ranged between 0.90 (June) and 5.46 (November), with a mean of 3.16±0.40 for females, and distributed from 0.60 (April) to 1.21 (January), with a mean of 0.84±0.06, for males. For both sexes, the highest maturity stages of fish gonads were seen in December. When monthly variation of sexual maturity and GSI were revealed togather, it was detected that spawning occurred all year round, and peaked between November and January. The first sexual maturity lengths (L50) was estimated as 22.0 cm TL and 24.9 cm TL for males and females, respectively. The minimum landing size of European Hake was identied as a 20 cm fork length (FL) in the fisheries legislations. Due to fishing effort has been increased continuously, it can be thought that the minimum landing size should be increased and updated up to 22 cm FL or 25 cm TL.

Key words: *Gonadosomatic index, Sex ratio, First sexual maturity length, Spawning season*

INTRODUCTION

European Hake, *Merluccius merluccius* (Linnaeus, 1758) is a member of Merlucciidae family which has 2 genus (Merluccius and Lyconodes) and 17 species in the world. Whereas, *M.merluccius* is a single representative of the family Merluciidae in the Türkiye waters. It is distributed above equatorial line from Mauritania to Iceland and Norway, including Mediterranean and southern Black Sea coasts (Cohen et al., 1990). It is distribute at depths from 18 m to 1075 m, and mostly found between 70 and 400 m depths. It is known as one of the most economical demersal fish species in the Türkiye Seas. It mostly catch with demersal trawls, beam trawls and deep-water gillnets. It mostly catch with beam trawls in the Marmara Sea and with the demersal trawls in the Aegean Sea and Mediterranean coasts of Türkiye. According to Turkiye fisheries landing in 2023, *M.merluccius* caught 1107.7 tonnes. It is correspond to 0.4% of total fish landings and a 937.9 tonnes (85%) of total catch landed from the Aegean Sea and 123.4 tonnes (11.1%) was landed from the Marmara Sea (TUIK, 2023).

The previous studies reveal reproductive biology of the species was realized around Northeastern Atlantic (Murua and Motos, 2006), southern Mediterranean (Khoufi et al., 2014), Aegean Sea (Akalın, 2004; Soykan et al., 2015)I North Sea, Scotland and Iceland (Muss et al., 1999), Ireland (Svetovidov, 1986), Western Mediterranean (Pineiro and Sainza, 2003). Kahraman et al. (2017) and Yıldız et al. (2022) investigated reproduction biology of *M.merluccius* in the Marmara Sea.

The present study is to reveal and contribute the reproduction biology of the species distributed in the Marmara Sea.

MATERIALS AND METHODS

Sampling was carried out by TAGEM (General Directorate of Agricultural Research and Policy of Turkey) under project number TAGEM/HAYSÜD/xxxxxx. Samples were collected at 6 stations and 229 beam trawl tows between February 2012 and June 2014 in the Sea of Marmara.

The total length (TL) of the specimens was measured to the nearest 0.1 cm and the total weight (W) was measured to 0.01 g. Gonad maturity stages were determined by Holden and Raitt (1974): immature, maturing, ripening, ripe, and spent. The gonadosomatic index (GSI) was calculated using the formula developed by Gibson and Ezzi (1980):

GSI = (GW/W-GW)×100

where GW is the weight of the gonad (g) and W is the total weight.

Length at first maturity (L_{50}) was estimated by fitting a logistic function using the Newton algorithm. This function is defined as follows; P(1) = 1/1+e -(a+b1)

where P(1) is the proportion of mature specimens at length 1, and a and b are the parameters of the logistic equation (Piñeiro and Saínza, 2003).

RESULTS

A total of 917 *M. merluccius* specimens were assessed and studied. Between of them, 309 were identified as females, whereas 588 were identified as males. The sex ratio was found as a 1:1.9 in favour of males. Individuals were ranged between 7.3 cm and 40.7 cm at total length (TL). Mean TL of males and females were detected as 19.3 cm TL and 22.5 cm TL, respectively.

GSI values ranged between 0.90 (June) and 5.46 (November) in females and ranged from 0.60 (April) to 1.21 (January) in males.

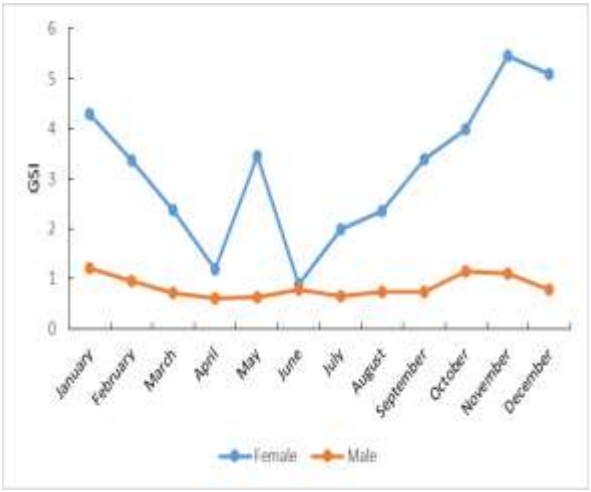

Figure 1. Monthly variation of gonadosomatic index in females and males of *M.merluccius*

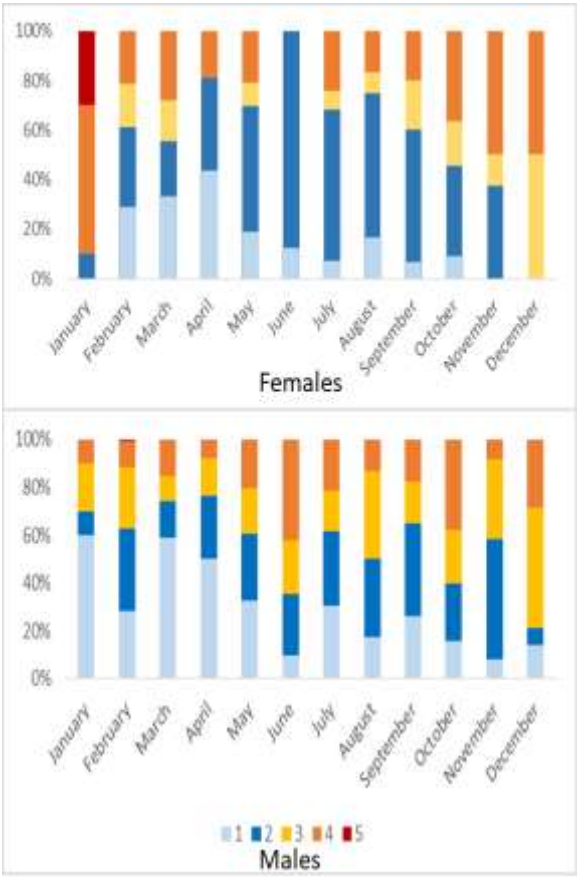

Figure 2. Montly Sexual maturity stages of *M.merluccius*

The highest GSI value for females was recorded in November while the lowest value was noted in June and for males the highest GSI value was detected in January,

and the lowest GSI value was in found in April (Figure 1).

Mean GSI values for females and males were found as 3.16±0.40 and 0.84±0.06, respectively .For both sexes, the highest maturity stages of fish gonads were seen in December. When monthly variation of sexual maturity and GSI were revealed togather, it was detected that spawning occurred throughout year and peaked between November and January (Figure 2).

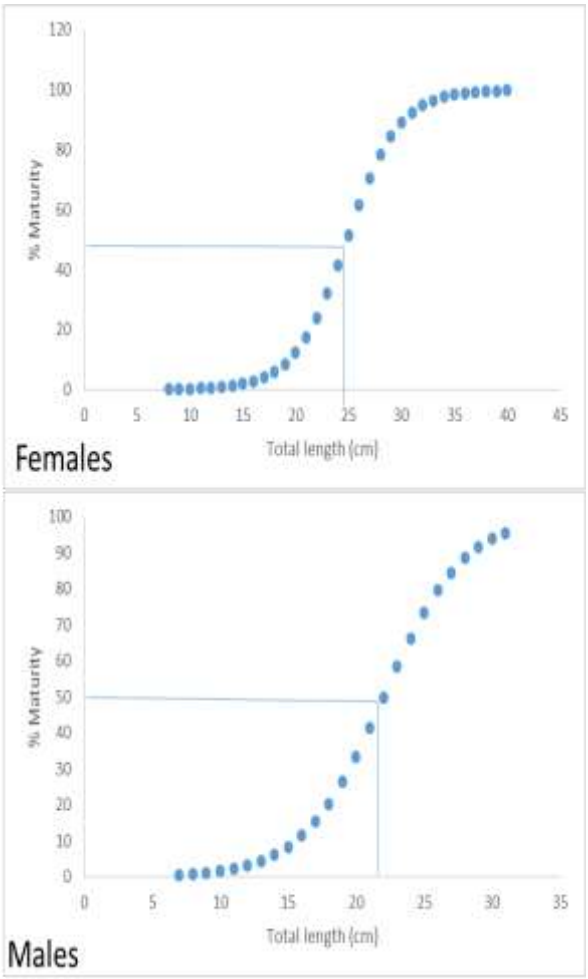

Figure 3. The first reproductive length (L50) of *M. merluccius*

The length at first sexual maturity was found to be 24.9 cm TL for females and 22 cm TL for males (Figure 3).

DISCUSSION

The protracted spawning period during whole year-round of the *M.merluccius* distributed in the Marmara Sea showed similarities with the previous studies. Independently of geographical location, mature gonads and high GSI revealed by nearly all previous studies (Murua and Motos, 2006; Khoufi et al., 2014; Akalın, 2004; Soykan et al., 2015; Muss et al., 1999; Svetovidov, 1986; Pineiro and Sainza, 2003; Yıldız et al., 2022. Although spawning activity ongoing all year-round, some clear spawning peaks were stated by previous studies. For example Khoufi et al. (2014) revaled that the spawning peaked in April in Tunisian coasts, peaked between January and March in Bay of Biscay (Murua and Motos, 2006), peaked from December to March in the Aegean Sea (Akalın, 2004). In the same study area, Kahraman et al. (2017) determined two major peak between December and January and one minor spawning peak in June. Similarly, highest spawning activity was detected between December and January by Yıldız et al. (2022) in the Marmara Sea. The given peak period of the spawning (from November to January) in the present study showed similarities with the previous studies both other areas and similar areas. The increase of Hake spawning activity between 10 °C and 12 °C was revealed by some authors due to physic-chemical requirement of winter-spawner fish species (Aldehit and Pincher, 1995). The mean sea water temperature values ranged 9 °C and 12 °C between December and April in the last 30 years in the Marmara Sea, whereas these temperature values only seen in February and March in 2022 and 2023. Thus, the increase of the mean sea water temperatures pose a significant threat for spawning of *M.merluccius*, especially in the Mediterranean.

The first sexual maturity lengths for male and female were given as 45.4 cm TL and 32.8 cm TL (Pineiro and Sainza, 2003) in the Iberia, 38 cm TL and 28.8 cm TL (Recasens et al., 1998) in the Gulf of Lions, 33.6 cm TL and) 27.8 cm TL (Akalın, 2004) in the Aegean Sea and 29.9 cm TL and 22.5 cm TL (Kahraman et al., 2017) in the Marmara Sea. The lower growth in the warmer areas may be a result of larger L_{50} values. The L50 results of the present study most close with the findings of Kahraman et al. (2017), and it can be stated that the geographical location can be the most important determinant for the L_{50} detection.

CONCLUSIONS

The results of global warming threat lots of areas such as Marmara Sea, which is defined as semi-enclosed basin and under the negative effect of industrial pollution. Beside, Marmara Sea has under the effect of high fishing effort stemmed from beam trawls especially in the winter months when the spawning of the *M.merluccius* peaked. Thus, in the near future, *M.merluccius* may abandon of the Marmara Sea and have to migrate colder areas for spawning. The minimum landing size of European Hake was identied as a 20 cm fork length (FL) in the Türkiye fisheries legislations. Due to fishing effort has been increased continuously, it can be thought that the minimum landing size should be increased and updated urgently up to 22 cm FL or 25 cm TL, at least.

ACKNOWLEDGEMENTS

This study financially supported by the Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research TAGEM/HAYSÜD/2011/09/02/04.

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Prediction of body weight from linear body measurement traits of nguni goats under four Agro-ecological zones of Limpopo Province using MARS and Cart

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Abstract

The study aims to determine the relationship between body weight and some linear body measurements traits, and to establish a model to predict body weight from linear body measurements traits using MARS and CART. A total of 426 Nguni goats aged between one to five years were used for body weight (BW) and some linear body measurements such as heart girth (HG), body length (BL), and withers height (WH). Correlation matrix was used to assess the relationship between BW and some linear body measurements traits. While MARS and CART were used to establish model to predict BW from some morphometric traits. Correlation results indicated that in female goats, BW had a significantly positive correlation (P<0.01) with HG (r = 0.84). While in male goats BW had a highly significant positive correlation (P<0.01) with BL (r = 0.79). CART model indicated that HG was the best predictor variable for BW, while MARS found BL > 77 cm as the best predictor of BW in the study. CART was the best model for prediction of BW with high R2 = 0.76, AdjR2 = 0.76 and r = 0.87. The study concludes that HG can be used as a predictor of BW.

Key words: *Nguni goats, MARS, CART, Linear body measurements, Goodness of fit*

Use of Whiteside Test Results in Determination of Bovine Bucket Milk Quality: A Case of the Black Sea Region of Türkiye

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Abstract

The objective of the present study was to determine the efficiency of Whiteside Test (WST) results to decide bovine milk quality. Total of thirty samples were collected from Amasya (n=15) and Samsun (n=15) provinces of Türkiye within February 2024. California Mastitis Test (CMT) was used to be reference method for comparison. To obtain WST data, 3 drops of NaOH solution were added to 5 ml milk for each sample and coagulation degrees after the reaction were recorded using 1 to 4 scale (1= no clot or gel formation; 2= weak gel formation; 3= moderate gel formation and 4= strong gel formation). According to locations, significant differences (P<0.05) were found for both methods. While strong (r=0.815) correlation was estimated between WST and CMT data, the linear regression model was calculated to be Y=0.477+0.869X. In conclusion, combining WST and CMT data is found as highly beneficial to achieve more accurate decision in determination of quality degree of bovine milk samples.

Key words: *Bucket milk, Dairy, Milk analysis, Milk quality, Raw milk*

INTRODUCTION

Bovine milk quality is one of the major items in dairy operations. Therefore, in addition to obtain high amount of milk, ensuring the high level by quality is regarded by dairy owners. Abnormality in raw milk generally shows its condition via abnormality in taste, vision or noise. However, dairy personal can only detect the abnormality by laboratory analyses during the inframammary infection that refers to mastitis case. In subclinical form, mastitis cases are 3–40 times more common than clinical form and cause the important yield losses by cow or herd bases (Bachaya et al., 2011). Pathogenic microorganisms including Staphylococcus aureus, *Streptococcus agalactiae*, *Klebsiella spp, Escherichia coli*, and *Enterobacter* spp are the common causative factors for mastitis. The bacteria attacks to the mammary glands during the unhygienic conditions on the barn or equipment (Ayuti et al., 2022).

Despite determination of bacteriological culture of milk samples is the gold technique for detecting mastitis or abnormality of milk, alternative methods are widely practiced due to their quick and easy using facilities (Sharma et al., 2010). Of these, CMT has been assumed to be a reliable, highly sensitive, cheap rapid screening test in field conditions even by less trained dairy staff (Senthilkumar et al., 2020). It has been informed by that the sensitivity of the CMT was 86.1 while specificity was 59.7% with percentage accuracy of 75.5% (Mpatswenumugabo et al., 2017). However, other alternative detection methods have still been investigating.

In spite of Whiteside Test (WST) has been declared to be one of the detection method since the old years (Whiteside, 1939; Astermark et al., 1969), the effectiveness is not clear and it is not widely used in the field in recent years. Revealing the reliability degree of WST will present clear information to dairy owners and researchers.

The aim of the present study was to determine the efficiency of WST results to decide bovine milk quality.

MATERIALS AND METHODS

Thirty bucket milk samples were collected from Amasya (n=15) and Samsun (n=15) provinces of Türkiye within February 2024. Approximately 50 ml raw milk sample was collected from the buckets and transferred

to the laboratory of Animal Science Department of Ondokuz Mayis University within the same days.

To compare, California Mastitis Test (CMT) was used to be reference method. (Qolbaini et al., 2014). In application, 2 ml milk from each sample was taken into a test cup and the equal amount CMT reagent was transferred on the same cup. After mixing, the coagulation was recorded with 1-4 scores (*1= negative; 2= trace, 3= moderate and 4=strong positive*).

In WST analysis (Yadav et al., 2000), 2 drops of NaOH solution was added to 5 ml milk for each sample and coagulation degrees after the reaction were noted with a 1 to 4 scale (Table 1) after 20 seconds.

The scores were recorded as follows: *1= no clot or gel formation; 2= weak gel formation; 3= moderate gel formation and 4= strong gel formation.*

Figure 1. Evaluation of milk samples by WST

To evaluate CMT or WST data by locations, t-test was applied.

Regression analysis was maintained after estimating Pearson correlation coefficient of two traits.

All statistical work was performed by SPSS 17.0 windows packet program.

RESULTS

The descriptive values of the elevated parameters are given in Table. 1.

Table 1. Descriptives of two parameters

Parameter	n	Min	Max	Mean±SE
CMT	15	1	4	1.86±0.164
WST	15	1	4	2.10±0.175

As seen, the highest and the lowest values for both variable were 1 and 4, respectively.

In the present study, both variables had significant (P<0.05) differences according to locations (Table 2).

According to obtained results, milk samples collected from Amasya province had lower mean when compared to milk samples of Samsun.

Table 2. Means (±SE) of the parameters by locations

Parameters	n	Mean±SE
CMT		
Amasya	15	1.53±0.191[a]
Samsun	15	2.20±0.243[b]
WST		
Amasya	15	1.73±0.228[a]
Samsun	15	2.46±0.236[b]

[a,b]: indicate statistical significance (P<0.05)

The association of both variable is presented in Figure 1.

In the figure, a distinct trend of WST by CMT is shown as a curve.

To calculate the amount and the way of the correlation of WST with CMT, a regression analysis was performed.

The linear regression analysis results are given in Table 3 and 4.

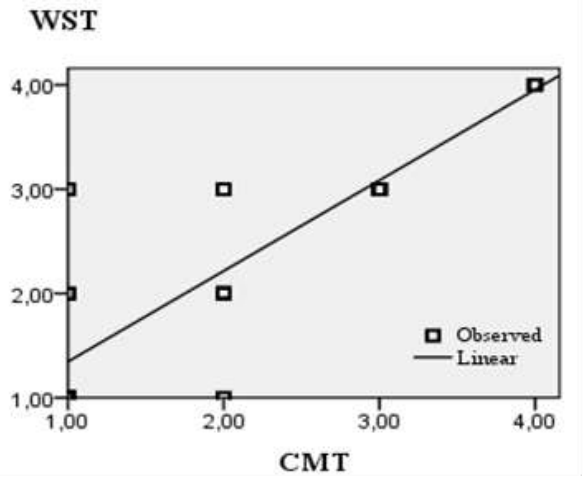

Figure 2. Associations of the evaluated parameters

Table 3. Model summary of the regression analysis

Model	R	R^2	Adjusted R^2	SE
1	0.815[a]	0.664	0.652	0.56587

a. Predictors: (Constant), CMT

Table 4. Coefficients of the variables

Unstandardized Coefficients		Standardized Coefficients		
B	SE	Beta	t	*P*
0.477	0.241		1.978	0.058
0.869	0.117	0.815	7.442	0.000

a. Dependent variable: WST

DISCUSSION

According to Table 1, the mean of the CMT values were calculated to be close to trace class. Similarly, WST mean pointed out to weak gel formation. The finding obtained here was found as harmonic for reflecting the class of each parameter. In a study conducted by Koçyiğit et al. (2016) in Bolu city of Türkiye, CMT positive percentage of dairy cows was 51.28% and 63% of these cows showed positive microbiological growth. Bakr et al. (2019), who studied subclinical mastitis cases in dairy cows, reported that CMT revealed 53% positive, and the WST revealed 49% positive with various degrees.

At this context, milk analysis results by chosen subclinical mastitis parameters could be noted within the wide range by different studies. Özenç (2019) emphasized that age of the cows and herd size were the main causative factors on subclinical mastitis incidence. Umar (2023) reported the effect of location on CMT positive cases in dairy cows. In a general statement, in addition to these factors, management, season or multi factors might be effective the different mastitis ratios in the field studies.

Especially, higher WST values of the milk samples of Samsun were found as remarkable (Table 2). In spite of similar region may be mentioned for both location, different herd size, farm staff and husbandry practices might be seen the major factors on this case. Senthilkumar et al. (2020) pointed out that environmental factors can be directly associated with the subclinical mastitis. At this point, sharing more time and focusing on the management may be given to be useful steps to decrease new mastitis occurrences and to boost milk quality level in herds of Samsun, whose milk samples were examined in this study.

According to Figure 1, a linear trend of WST by CMT, and a strong correlation between two parameters is attractive (r=0.815).

When the results of Table 3 and 4 were applied, the linear regression model was obtained to be Y= 0.477+0.869x. In addition, R^2 of the model was estimated to be 0.664. In other words, it can be assumed that changes in WST is linked to CMT data by 66.4%. This finding may be regarded as highly impressive case by the linkage of WST values with CMT data.

CONCLUSIONS

In this study, the effectiveness of WST test results in cow milk samples was investigated via comparison with CMT data. According to two different locations (Amasya and Samsun), both WST and CMT results were differed. The strong correlation that calculated two test results clearly revealed the reliability of WST data on detecting quality degree of bovine milk.

However, combining WST data with CMT results may be suggested to dairy farm directors to achieve more accurate decision for this process.

ACKNOWLEDGEMENTS

The authors thank to staff of the Cattle Breeders Association of Amasya and Samsun provinces for ensuring the milk samples.

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Usability of hemp seed meal in poultry

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Abstract

The nutritional value of hemp seed meal is partially similar to rapeseed meal, and it matures 28-35 days after hatching. It was concluded that the 30% additive rate did not have any negative effect on production or taste of the feed when fed on days (Kalmendal, 2008). Another study reported that inclusion of hemp seed meal in the diets of fast-growing organic broilers (10% at 10-28 days, 20% at 28-70 days) did not affect production performance or mortality. Several trials have been conducted to assess the beneficial effects of hemp seed meal on the fatty acid composition of egg yolk in laying hens. In Pakistan, the addition of 25% hemp seeds to layer feeds has been reported to significantly increase total and individual polyunsaturated fatty acids as well as omega-3 and omega-6 fatty acids, while reducing egg yolk total cholesterol and the content of monounsaturated fatty acids. In Canada, feeding laying hens up to 20% cold-pressed hemp seed meal had no effect on egg production, feed intake, feed efficiency, body weight change, or egg quality. Increased dietary inclusion of hemp seed meal resulted in the production of eggs containing lower concentrations of palmitic acid and higher concentrations of linoleic and alpha-linolenic acid. A 14% hemp seed ration was given to yearlings for 166 days, and as a result, there was no negative effect on live weight gain, feed conversion ratio, and carcass characteristics. However, increased conjugated linoleic acid and n-3 fatty acids were observed in the tissues. In calves and cattle, hemp seed meal (1 to 1.4 kg/day) resulted in similar yield and improved rumen function compared to a mixture of soybean meal and barley as a protein feed.

Key words: *Hemp seed, seed meal, poultry, alternative feed source*

Myf5 and Myf6 gene expression profiles in some skeletal muscles of male kids of Saanen and Honamlı goat breeds

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Abstract

Myogenic factor 5 (Myf5) and 6 (Myf6) genes, which are members of the myogenic regulatory factors family, are among the genes that regulate the development and differentiation of muscle cells and the main regulators of skeletal muscle tissue formation. Determination of the expression levels of Myf5 and Myf6 genes in goats of Saanen breed reared in goat milk production in our country and Honamlı kids reared for combined (meat-milk) production may help to reveal the fattening potential of both breeds at molecular level. Therefore, the aim of this study was to determine the expression level of Myf5 and Myf6 genes in Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles of 3-month weaning year-old Saanen (n=5) and Honamlı (n=5) kids. Total RNA in muscle samples was isolated using a commercial RNA extraction kit as recommended by the manufacturer. Isolated RNA was converted to cDNA using a commercial cDNA kit in a Thermal Cycler device. The Myf5 and Myf6 gene expression level in LD and ST skeletal muscles was determined by real-time quantitative polymerase chain reaction. The birth and 3-month weaning weight and daily body weight gain of the Honamlı male kids were higher than the Saanen kids (P < 0.05). In the study, although Saanen kids had higher (P < 0.05) RNA content in LD and ST muscles than Honamlı kids, Honamlı kids had higher Myf5 and Myf6 gene expression levels than Saanen kids (P < 0.05) in both muscles. The results of this study show that Honamli kids may have higher muscle growth than dairy Saanen kids due to higher Myf5 and Myf6 gene expression levels. As a result, it was concluded that Myf5 and Myf6 genes are expressed in Saanen and Honamlı kids and that animals suitable for fattening can be selected according to the expression differences of these genes.

Key words: *Saanen, Honamlı, Muscle development, Myf5, Myf6, Growth*

INTRODUCTION

The most important problem of the breeders dealing with goat breeding for milk and combined production in Türkiye is that they are suspicious of how much profit male kids born from female individuals will make under fattening, so dairy farms are very meticulously approaching the issue of fattening male kids. The biggest reason for this is that the fattening performance potential of male offspring of dairy breeds is not known enough and the studies on this subject are superficial or insufficient. If this problem is solved with studies that will enable to reveal the fattening potential of male offspring obtained from dairy breeds, our chances of finding a sustainable way for the future in meat production from dairy breeds will increase.

The Myf5 gene is a member of the myogenic regulatory factors (MRF) family, which plays an important role in the formation of muscle fibers and transcription of muscle-specific genes, and it has been reported to affect the level of meat fat (Fujisawa-Sehara et al., 1990; te Pas et al., 2007). ; Verner et al., 2007; Wang et al., 2017). The Myf5 gene is recognized as a core transcription factor involved in muscle development during embryonic myogenesis. Myoblasts proliferate during Myf5 regulation (Park et al., 2015). Tatusova and Madden (1999) revealed in their study that Myf5 is involved in the myogenic process, especially in the muscle differentiation stage (Wang et al., 2017). The Myf5 gene has been associated with meat yield and has been reported to have significant effects on lean meat content, fillet weight and muscle fat levels in animals (Te Pas et al., 2004; Verner et al., 2007). It has been reported that Myf5 gene polymorphism is associated with growth in

sheep (Nattrass et al., 2006). According to recent studies, SNPs in the exon region of Myf5 have proven to have significant associations with carcass and meat quality traits in animals (Hedayat-Evrigh et al., 2016). In recent years, many candidate genes have been identified that have an impact on many important economic yields, especially meat yield, milk yield and reproductive efficiency in cattle, sheep, goats, chickens and pigs. The most important of these are the genes belonging to the MyoD family. The myf5 and myf6 genes belonging to this family are the genes we targeted in the study.

As a result, it has been determined that Myf5 and Myf6 genes belonging to the MyoD gene family have very important effects on skeletal muscle development in the fetal and post-fetal period, as well as on the growth and development of Myf5 and MYF-6 genes belonging to the MyoD gene family in farm animals. It is thought to have an effect on muscle development and growth, which is associated with meat production. Based on the above-mentioned issues, the main aim of this study is to determine the expression levels of Myf5 and Myf6 genes, since studies on the performance of Saanen and Honamlı goats, which are being reared in Turkiye, are limited, will make a very strong contribution in this direction.

MATERIALS AND METHODS

In the study, Saanen and Honamli kids reaching the weaning age of 90 days were slaughtered according to standard slaughtering procedures in a commercial slaughterhouse. Longissimus-dorsi (LD) and Semitendinosus (ST) muscles on the right side of the carcass were isolated immediately after slaughter, and the muscle masses were divided into 2×5×2 cm samples and frozen in liquid nitrogen. Frozen samples were stored at -80 °C until the day of analysis. A commercial RNA kit (NucleoSpin® RNA kit) was used for RNA isolation in muscle samples and the process was carried out as recommended by the manufacturer of the commercial kit. After genomic DNA was eliminated by digestion with DNase I (Thermo Scientific, Waltham, USA), the RNA quality and quantity were determined using NanoDrop 2000 (Thermo Scientific, Waltham, USA), all RNA samples showed A260/A280 values within the range of 2.01 to 2.08 and A260/ A230 values above 2. Commercial cDNA kit (BIORAD iScript cDNA, 1708890) and Thermal Cycler (BIORAD) device were used for cDNA synthesis and the analysis was done as recommended by the manufacturer of the commercial kit. Primer and reference gene base sequences in the 5 ‘and 3’ directions used in Real-Time PCR are shown in Table 1.

Table 1. Primer and reference gene base sequences in the 5 ‘and 3’ directions used in Real-Time PCR

Genes r	Primer sequences	Product size
Myf5-F	5’ CACGACCAACCCTAACCAGAG 3’	101 bp
Myf5-R	5’ TCTCCACCTGTTCCCTTAGCA 3’	101 bp
Myf6-F	5’ CGGAGCGCCATTAACTACAT 3’	101 bp
Myf6-R	5’ AAATCCGCACCCTCAAGATT 3’	101 bp
GADPH-F	5’ GCA AGT TCC ACG GCA CAG 3’	118 bp
GADPH-R	5’ TCA GCA CCA GCA TCA CCC 3’	118 bp

In detail, the PCR was carried out in a reaction system of the total volume of 50 µL containing 25 µL premix TaqTM, 17.5 µL 0.1% 114 DEPC water, 2.5 µL forward primers (10 µmol/L), 2.5 µL reverse primers (10 µmol/L) and 2.5 µL cDNA template. PCR procedure was carried out as follows: 98 °C for 4 min, followed by 32 cycles of 98 °C for 40 s, 60 °C for 40 s, 65°C for 30 s, and then 90 °C extension for 10 min, finally 4 °C to terminate the reaction. Relative quantification of all transcripts was performed by qRT-PCR using the real-time PCR system. Realtime quantitative PCRs were run with SYBR Premix Ex TaqTM II. The reaction system was in a total volume of 10 µL containing 5 µL 2 × SYBR Premix Ex Taq II, 0.4 µL forward primer (10 µmol/L), 0.4 µL reverse primer (10 µmol/L), 0.2 µL 50 × ROX Reference Dye, 3 µL 0.1% DEPC water and 1

µL template cDNA. PCR amplification was carried out as follows: a denaturation of 98 °C for 30 s, followed by 40 cycles of 98 °C for 5 s, specific annealing temperature 60°C for 30 s. The 2–ΔΔCt method was used to analyze the mRNA expression levels. The data we obtained during the study were analyzed using SPSS 20.0 package license package programs. According to the results of Shapiro-Wilk test, it was determined that the distribution normality of the obtained data was suitable for normal distribution (P>0.05) and that the data were suitable for analysis of variance, and the variances were homogeneous (P>0.05) as a result of the Levene homogeneity of variance test. One-way analysis of variance was used to compare breeds.

RESULTS

Total RNA amounts in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı and Saanen male kids are presented in Table 2. In the study, the Saanen breed male kids had a higher (P < 0.05) RNA amount than the Honamlı male kids.

The expression levels of myogenic factor 5 (Myf5) and myogenic factor 6 (Myf6) gene in Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles of Saanen and Honamlı kids are presented in Table 2. In the study, there was a very significant difference between the two breeds in terms of the expression level of the Myf5 and Myf6 genes in LD and ST muscles. Honamlı kids had higher the expression level of the Myf5 gene than those of Saanen (P<0.05) in LD and ST muscles. Similarly the expression level of the Myf6 gene was higher in LD and ST muscles of Honamlı kids than Saanen kids (P<0.05).

Table 2. Total RNA amounts in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı and Saanen male kid

	Honamlı	Saanen
LD	86,98 ± 2,61[b]	130,92 ± 7,82[a]
ST	87,57 ± 9,58[b]	135,93 ± 17,10[a]

[a,b] Means in lines with different letters are significantly different at P<0.05.

Table 3. The expression levels of myogenic factor 5 (Myf5) and myogenic factor 6 (Myf6) gene in Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles of Saanen and Honamlı kids

Traits	Breeds	
	Honamlı	Saanen
Myf5	28.39±3,54[a]	0.59±0,11[b]
Myf6	16.35±2,47[a]	3.53±0,94[b]

[a,b] Means in lines with different letters are significantly different at P<0.05.

DISCUSSION

Growth, which is related to carcass weight, which is one of the important indicators of meat yield, is classified in two categories as prenatal and postnatal growth. The attachment of the embryo to the uterus during muscle development, growth, maturation and development of functions is a multidimensional chain of events involving cellular increase and specialization (Ujan et al. 2011a). This chain of events is primarily controlled by the myogenic determination MyoD gene family. It is known that MRFs regulate myogenesis from stages in the formation, development and proliferation of muscle fibers to postnatal muscle maturation, differentiation and function (Zhong et al., 2013; Patel et al., 2014; Siqin et al., 2017). The growth and development of muscle cells in farm animals is regulated by the MyoD gene family, MyoG, Myf5 and Myf6. Evolutionary analyzes of the amino acid sequences of this transcriptional activator family have reported that the vertebrate genes MyoD1, Myf5, Myog (myogenin), and Myf6 are derived by gene copies from a single ancestral gene (Haghes and Schiaffino, 1999). These genes are

shaped in the formation of muscle cells in the embryonic period and control the maturation and functions of muscle fibers after birth (Haghes and Schiaffino, 1999).

Comprehensive studies are needed to more precisely determine the relationships between meat production and meat quality parameters of the MRF gene family. Muscle expression of certain genes, such as myogenic transcription factors, can significantly affect the meat content and meat quality of carcasses. Therefore, transcription analysis of genes related to muscle development will provide valuable genetic information for meat production.

In this study, it was determined that there were differences between breed in terms of expression levels of Myf5 and Myf6 genes in LD and ST muscle. Because of this result, the comparison of the expression level of Myf5 and Myf6 genes can show that each Honamli breed has high fattening potential and can also reveal the possibility of using them as molecular markers in breeding studies on meat yield traits. The genetic information from this research can be used to develop ways to accelerate genetic improvement in breeding and possible future research directions. In other words, determining the expression levels of the MRF gene family, especially Myf5 and Myf6, can provide important information to more descriptively and clearly define the meat production of our domestic goat breeds and to increase meat production. However, by comparing with dairy breeds, it will be an important tool in determining the breeds that are thought to have poor meat yield. In addition, the fact that the genes responsible for muscle fiber development of Honamlı kids and Saanen kids will be compared for the first time, and this will pave the way for the elimination of a deficiency in this area. Moreover, it will contribute especially to breeding and crossbreeding studies in terms of determining the meat production potential of goat breeds other than meat productive breeds in Turkiye.

CONCLUSIONS

Significantly different degrees of expression of Myf5 and Myf6 in goat skeletal muscle were presented in this study. As a result of this study, it is thought that Honamlı kids may have higher muscle growth due to their higher Myf5 and Myf6 gene expression levels compared to Saanen kids, and positive results will be obtained as a result of correct feeding programs to be applied to male kids of this breed. Finally, the determination of the expression level of Myf5 and Myf6 genes is not only limited to the breeds with high meat yield, but the determination of the meat yield potential of the breeds bred for different purposes has been proven with the study that will make great contributions to the sustainability of meat production with the increasing population of today.

ACKNOWLEDGEMENTS

This study was supported within the scope of TUBITAK 2209/A University Students Research Projects Support Program.

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The effect of medicinal plants on the health of broiler chickens: current research and advances

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Abstract

The utilization of medicinal plants in poultry farming has garnered increasing attention due to their potential health benefits for broiler chickens. Current research in this field highlights the significant impact of various plant extracts on the overall health, immunity, and performance of broiler chickens. Studies have demonstrated that medicinal plants such as turmeric, ginger, and thyme possess antioxidant properties and can promote digestive health and boost the immune system of broiler chickens. These effects are attributed to the bioactive compounds present in medicinal plants, including phenolic compounds, flavonoids, and essential oils, which exhibit antimicrobial and anti-inflammatory activities. The supplementation of medicinal plants in broiler diets has been shown to improve growth performance parameters such as body weight gain, feed conversion ratio, and carcass characteristics. Moreover, medicinal plants have been found to mitigate the negative effects of stressors such as heat stress and disease challenges in broiler chickens. The immunomodulatory effects of medicinal plants contribute to enhanced resistance against various pathogens, thereby reducing the reliance on antibiotics in poultry production. Furthermore, the inclusion of medicinal plants in broiler diets can improve meat quality by reducing lipid oxidation and enhancing flavor attributes. However, the efficacy of medicinal plants in broiler production is influenced by factors such as dosage, duration of supplementation, and the specific plant species used. Standardization of herbal preparations and optimization of dosage regimens are essential for ensuring consistent results across different studies. Additionally, further research is needed to elucidate the mechanisms of action of medicinal plants and their interactions with the gut microbiota of broiler chickens. In conclusion, current research and advances in the use of medicinal plants underscore their potential as natural alternatives to conventional growth promoters and antimicrobials in broiler production. However, more comprehensive studies are warranted to validate their efficacy, safety, and practical application in commercial poultry farming systems. Overall, the integration of medicinal plants into broiler diets has the potential to enhance animal health, welfare, and sustainability while reducing the environmental impact of poultry production.

Key words: *Broilers, medicinal plants, health effects*

Determining the relationship between the expression levels of MyoD and MyoG Myomarker genes and the fattening performance in Saanen goat kids

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Abstract

The aim of this study is to determine the expression level of MyoD and MyoG genes in Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles of Saanen kids at different slaughter weights. In the study, 10 male Saanen kids were fed for 60 days (up to 5 months of age) after 3 months of weaning. From the end of fattening, the kids were divided into two groups as low and high at 1 standard deviation according to their live weights. Total RNA in post-slaughter muscle samples was isolated using a commercial RNA extraction kit as recommended by the manufacturer. Isolated RNA was converted to cDNA using a commercial cDNA kit in a Thermal Cycler device. MyoD and MyoG gene expression level in LD and ST skeletal muscles was determined by real-time quantitative polymerase chain reaction. In the study, no difference was detected between the two groups in terms of total RNA amount of LD and ST muscles. There was no difference between the two groups in terms of the expression level of the MyoD gene in LD and ST muscles. However, expression level of MyoG gene in both muscles was higher in high body weight kids than low body weight kids (P<0.05). The results of the study showed that the difference in body weight at the end of fattening in Saanen kids might be due to the difference in the expression level of the MyoG gene and there might be a positive correlation between the fattening performance and the MyoG gene.

Key words*: Myomarker, MyoD, MyoG, Skeletal muscle, Goat kids*

INTRODUCTION

It is possible to use all available resources effectively and efficiently to increase the standard of living of human beings. While doing this, protecting the existing ecosystem, sustainable economic development and sustainable environmental management are a must. One of the species in the ecosystem that needs to be protected is undoubtedly the goat. In addition, goat breeding is a livestock activity that is suitable for sustainable agricultural practices.

Goat is of great importance to Türkiye. Türkiye is an important country in the world in terms of goat breeding and goat breeding constitutes an important part of the Turkish agricultural sector.

Goat meat, contrary to popular belief, is a healthy type of meat. Since its fat and cholesterol levels are lower than other types of meat, it eliminates the factors that pave the way for cardiovascular diseases.

By performing gene expression analyses on farm animals, the skeletal muscle development profiles (muscle fiber type, diameter, intramuscular fat ratio) of the animals to be fattened can be changed, thus affecting the feed utilization rate for higher amounts of meat production or higher amounts of meat production can be achieved with lower amounts of feed from the animals. In addition, determining the cellular or molecular characteristics of the skeletal muscle tissue of the animal species and breeds to be used in red meat production is of great importance in terms of predetermining the meat production potential of the animals to be fattened, increasing meat production, reducing production costs and reducing meat prices.

In recent years, many candidate genes that have an effect on many important economic yields, especially meat yield, milk yield and fertility, have been identified in cattle, sheep, goats, chickens and pigs. The most important of these are the MyoD and MyoG

myomarker genes, which are members of the Myogenic Regulatory Factors (MDF) gene family. MyoD and MyoG, together with other MDF gene family members, act as the main regulators of skeletal muscle myogenesis, regulating the development of skeletal muscle precursor cells and subsequent muscle fiber differentiation and development through the expression of their encoded genes. Gene expression analyses to be performed on farm animals can be used to change the skeletal muscle development profiles (muscle fiber type, diameter, intramuscular fat ratio) of the animals to be fattened, and the feed utilization rate can be affected for higher meat production, or higher meat production can be achieved with lower amounts of feed from the animals.

In this study, we focused on determining the expression levels of the myomarker genes MyoD and MyoG in relation to fattening performance in Saanen goat kids. MyoD and MyoG genes play important roles in the differentiation of myoblasts, which are critical for the development of muscle tissue and muscle fiber formation. Evaluating whether the expression levels of these genes are related to fattening performance may provide valuable information for livestock breeders to design a more efficient and effective fattening program.

As a result, studies suggest that MyoD and MyoG myomarker genes, which are members of the MDF gene family, have significant effects on skeletal muscle development in the fetal and postnatal period and even on the development and growth process in farm animals, and may have an effect on meat yield. Studies on the determination of growth, meat yield and fattening performance of the Saanen goat breed are based on racial characteristics or environmental factors. For this reason, determining the expression levels of MyoD and MyoG myomarker genes, which are effective on skeletal muscle development in goat breed, which is an important source of red meat in our country, may provide important information on the meat production potential of the Saanen goat breed and on the clear determination of increased meat yield.

MATERIALS AND METHODS

In the study, 10 male goats born from Turkish Saanen goats were used as animal material. After birth, all goats were kept in the farm with their mothers for 15 days so that they could receive sufficient colostrum. After this period, the goats were treated with protective sprays against internal and external parasites and standard health protection practices were applied. In order to ensure adequate rumen development of the goats, they were first offered quality dry grass (dry alfalfa hay) in the pen for the second week after birth until the age of 90 days weaning, and then they were allowed to graze in the pasture with their mothers. Until the age of weaning, the goats were allowed to suckle their mothers freely and during this period, they were offered kid growth feed (at least 90% dry matter, 16% crude protein and 2500 kcal/kg metabolic energy) and quality dry alfalfa hay adlibutum.

In the study, birth and 90-day weaning weights of the male kids included in the experiment were determined. After weaning age (90th day), all kids were fattened for 60 days (up to 150 days of age). All kids in the experiment were given commercial kid fattening feed (at least 90% dry matter, 12% crude protein and 2700 kcal/kg metabolic energy) and quality dry alfalfa hay in increasing amounts 4–5 days before fattening to get used to the feeds before being fed. All kids included in the experiment were fasted for 1 day before fattening and weighed to determine their live weights before fattening. During the fattening period, commercial kid fattening feed and roughage were mixed and offered to the kids as ad libitum. The live weights of the kids included in the fattening will be determined at 10-day intervals throughout the fattening period. At the end of the 60-day fattening, the kids were starved for 1 day and their live weights were determined, and the kids were divided into two groups as low and high according to their live weights at the end of the fattening. The groups were formed according to 1 standard deviation of the average slaughter weight of all kids.

At the end of the fattening, the kids taken for testing were slaughtered in accordance

with the regulations and in a commercial slaughterhouse authorized by the Ministry of Agriculture and Forestry. Immediately after slaughter, approximately 50 g samples were taken from the Longissimus dorsi (LD) and Semitendinosus (ST) muscles on the left side of the carcass. The fat and connective tissues in the muscle samples were cleaned and 2×5×2 cm pieces were taken, frozen in liquid nitrogen and stored at -80 ºC until analysis.

Total RNA of LD and ST muscle samples were isolated by a commercial RNA (PureLink ™, RNA Mini Kit, Invitrogen™, 12183018A) purification kit using the TRIzol Reagent (Thermo Fisher Scientific, US) as suggested by the manufacturer. Genomic DNA was eliminated by digestion with DNase I (Thermo Fisher Scientific Inc., Waltham, MA, USA). The purity and concentration of isolated RNA were evaluated by the A260/A280 ratio using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and all RNA samples showed A260/A280 values within the range of 2.01 to 2.08 and A260/ A230 values above 2. The integrity of collected RNA was checked with 1 % w/v agarose gel electrophoresis. Total RNA was converted to cDNA using a commercial cDNA kit (BIORAD iScript cDNA, 1708890) following the manufacturer's instructions in the Thermal Cycler (BIORAD) device. For GADPH the prepared cDNA samples were further purified, quantified, diluted to the same initial concentration, and stored at −20 °C until subsequent quantitative real-time PCR analysis.

GADPH was selected as housekeeping gene to normalize the expression of target genes. All primers were synthesized by Sentebiolab (Ankara, Türkiye). The specificity of each of the designed primers was checked via online Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and melt curve analysis was carried out during qRT-PCR. Relative quantification of all transcripts was performed by qRT-PCR using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Real-time quantitative PCR were run with EvaGreen mastermix (5× HOT FIREPol EvaGreen qPCR Mix Plus, Solis BioDyne, Tartu, Estonya). The reaction mix was in a total volume of 10 µL comprising 5 µL of 5X HOT FIREPol mix, 0.5 µL of forward primer (10 µmol/L), 0.5 µL of reverse primer (10 µmol/L), Dye, 2 µL of DEPC treated water, and 2 µL of template cDNA. PCR amplification was carried out as follows: denaturation of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, specific annealing temperature of 60 °C for 30 s. The relative mRNA expression levels of the genes were calculated by the $2-\Delta\Delta Ct$ method.

qRT-PCR Analyses

Primers used for the amplification of genes were designed using online tools (https://www.ncbi.nlm.nih.gov/tools/primerblast/) based on the related gene sequences of caprine (Table 1).

Table 1. Primer Sequences for the mRNA expression analysis of genes

Genes	Primer sequence (5'-3')		PS (bp)
	Forward	Reverse	
MyoD	GCCTGAGCAAAGTCAACGAG	GAGTCGCCGCTGTAGTGTTC	229
MyoG	GCAGCGCCATCCAGTACATAG	GAAGGCCGCAGTGACATCC	284
GAPDH	GCAAGTTCCACGGCACAG	TCAGCACCAGCATCACCC	118

PS= product size

The data obtained during the study were analyzed using SPSS 20.0 package license package programs. The distribution normality of the obtained data was determined according to the Shapiro-Wilk test results as the data were suitable for normal distribution ($P>0.05$) and the suitability of the data for variance analysis was determined according to the Levene variance homogeneity test as the variances were homogeneous ($P>0.05$). Pearson correlation coefficient was used to determine the relationships between variables. One-way variance analysis was used to compare the groups.

RESULTS

The mean threshold cycle (CT) values obtained as a result of simultaneous RT-PCR of MyoD, MyoG and GAPDH (reference gene) genes in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of low and high weight Saanen kids are presented in Table 2. No statistically significant difference was detected between the two groups in terms of threshold cycle (CT) values obtained as a result of simultaneous RT-PCR of MyoD, MyoG and GAPDH (reference gene) genes in LD and ST muscles.

Table 2. Cycle Threshold (CT) mean values obtained as a result of simultaneous RT-PCR of MyoD, MyoG and GAPDH (reference gene) genes in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of low and high weight Saanen kids

	Low	High
GAPDH - LD	19,90 ± 0,20	19,28 ± 0,17
GAPDH - ST	18,75 ± 0,41	19,33 ± 0,29
MyoD - LD	28,75 ± 0,19	29,01 ± 0,23
MyoD - ST	28,82 ± 0,40	29,12 ± 0,08
MyoG - LD	35,67 ± 0,70	34,56 ± 0,83
MyoG - ST	31,61 ± 0,52	33,15 ± 0,47

The expression levels of MyoD gene in Longissimus-dorsi (LD) skeletal muscle of low and high weight Saanen kids are shown in Figure 1. In the study, no significant difference was detected between the two groups in terms of expression level of MyoD gene in LD muscle. As a result of quantitative real-time polymerase chain reaction (Real-Time qPCR), the expression levels of MyoD gene in LD muscle of low and high weight kids were calculated as 3.79 ± 0.36 and 2.02 ± 0.85 fold change, respectively.

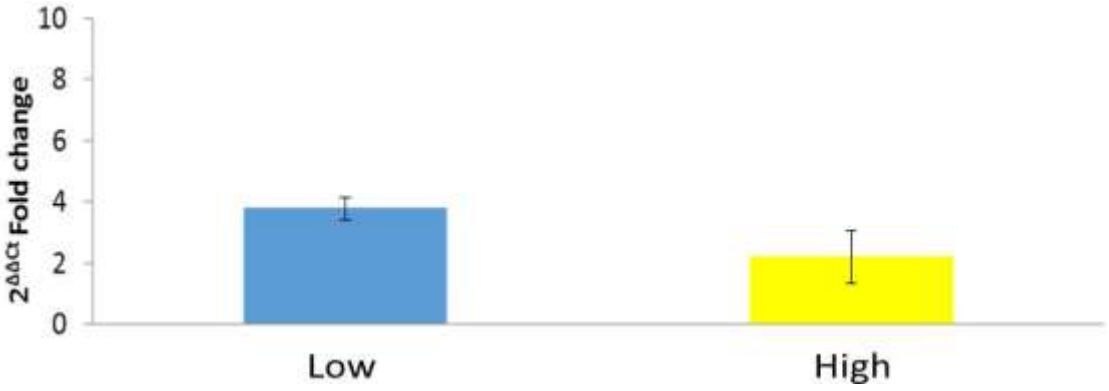

Figure 1. Expression levels of MyoD gene in Longissimus-dorsi (LD) skeletal muscle of low and high weight Saanen kids.

The expression levels of MyoD gene in the skeletal muscle of low and high weight Saanen kids are shown in Figure 2. In the study, no significant difference was found between the two groups in terms of the expression level of MyoD gene in the ST muscle. As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of MyoD gene in the ST muscle of low and high weight kids were calculated as 4.15 ± 0.41 and 3.62 ± 1.34 fold change, respectively.

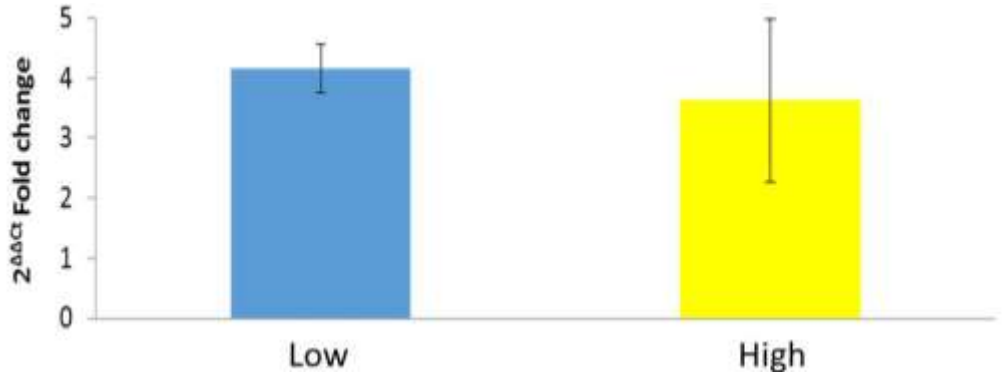

Figure 2. Expression levels of MyoD gene in Semitendinosus (ST) skeletal muscle of low and high weight Saanen kids.

The expression levels of MyoG gene in Longissimus-dorsi (LD) skeletal muscle of low and high body weight Saanen kids are shown in Figure 3. In the study, it was found that there was a very significant difference in the expression level of MyoG gene in LD muscle between the two groups ($P < 0.05$). As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of MyoG gene in LD muscle of low and high body weight kids were calculated as 4.68 ± 0.76 and 17.41 ± 5.32 fold change, respectively ($P < 0.05$). MyoG gene was expressed approximately 4-fold less in ST muscle of low body weight kids compared to the reference gene in high body weight kids ($P < 0.05$).

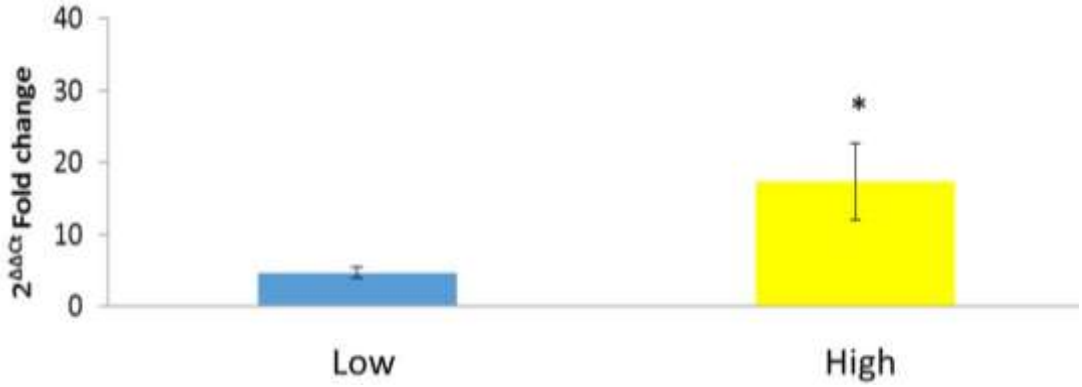

Figure 3. Expression levels of MyoG gene in Longissimus-dorsi (LD) skeletal muscle of low and high weight Saanen kids. *$P < 0.05$.

The expression levels of MyoG gene in Semitendinosus (ST) skeletal muscle of low and high weight Saanen kids are shown in Figure 4. In the study, it was determined that there was a significant difference in the expression level of MyoG gene in ST muscle between the two groups ($P < 0.05$). As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of MyoG gene in ST muscle of low and high weight kids were calculated as 2.96 ± 0.77 and 6.13 ± 4.57 fold change, respectively. MyoG gene was expressed approximately 2-fold less in ST muscle of low weight kids compared to the reference gene in high weight kids ($P < 0.05$).

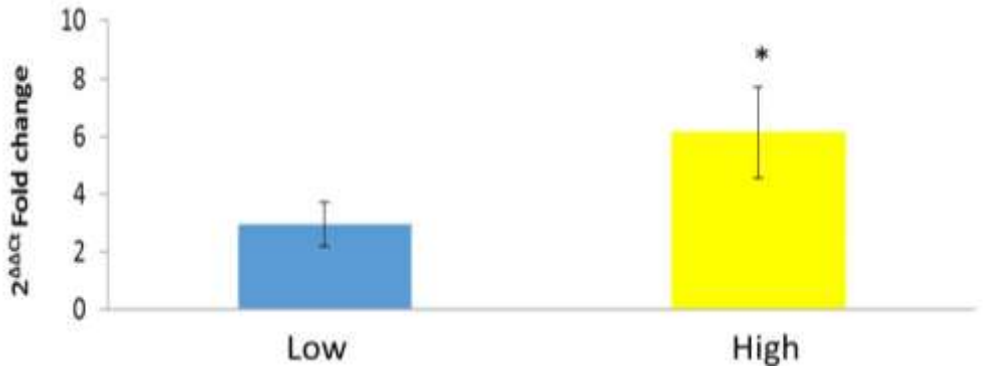

Figure 4. Expression levels of MyoG gene in Semitendinosus (ST) skeletal muscle of low and high weight Saanen kids ($P < 0.05$).

DISCUSSION

Many genetic and environmental factors affect meat quality and yield in farm animals. The development and growth of muscle fibers in living beings are regulated by four conserved basic helix-loop-helix (bHLH) transcription factors, Myf5, Myf6, MyoD and MyoG, of the MDF gene family (Zhong et al., 2013).

MyoD is a transcription factor and is primarily involved in the development and differentiation of muscle cells. The MyoD gene triggers the differentiation of muscle cell precursors called myoblasts, which leads to the formation of muscle fibers. MyoD is activated by cellular signals that regulate gene expression. When activated, it initiates the expression of other genes, allowing myoblasts to transform into muscle cells. It also stops the proliferation of myoblasts and directs them to differentiate into muscle fibers. During this process, MyoD activates myogenic (muscle-forming) factors and promotes the synthesis of muscle-specific proteins. MyoG is also a transcription factor and has similar functions to MyoD. The MyoG gene enables myoblasts to differentiate into muscle fibers at more advanced stages. MyoG steps in to maintain and complete the myoblast differentiation initiated by MyoD. MyoG changes the gene expression in the nuclei of myoblasts, allowing them to acquire the typical characteristics of muscle cells. It also helps myoblasts come together to form muscle fibers called myotubules. MyoG also plays an important role in regulating the size and contractility of muscle fibers.

MyoD and MyoG genes play key roles in the development and differentiation of muscle cells. MyoD triggers the differentiation of muscle cell precursors, while MyoG supports the further differentiation of myoblasts and the formation of muscle fibers. The interaction of these two genes determines the structural and functional properties of muscles by ensuring the proper development of muscle cells. In farm animals, live weight gain and carcass weight are associated with growth characteristics, and these characteristics are closely related to the increase in the number or diameter of muscle fibers of the animal and the development of muscle mass. Growth, which is associated with carcass weight, which is the greatest indicator of meat yield, is divided into two parts: prenatal and postnatal growth. Muscle development is a multidimensional chain of events that includes cellular increase and specialization during growth, maturation and development of functions, and the attachment of the embryo to the uterus (Ujan et al., 2011). This event is mainly controlled by the MDF gene family. It is known that MDFs regulate myogenesis from the stages of muscle fiber formation, development and proliferation to postnatal muscle maturation, differentiation and functions (Patel et al., 2014; Siqin et al., 2017; Zhong et al., 2013). The growth and development of cells that make up the muscle mass in farm animals are regulated by the MDF gene family members MyoG, MyoD, Myf5 and Myf6. These genes control the formation of muscle cells in the embryonic period and the maturation and functions of muscle fibers after birth

(Hughes and Schiaffino, 1999). Extensive studies are needed to determine the relationships between MyoD and MyoG genes and meat yield and meat quality parameters more precisely. Muscle expression of certain genes, such as myogenic transcription factors, can significantly affect meat quality as well as meat content in the carcass.

In the current study, although there was a big difference between the groups in terms of the expression levels of both genes MyoG and MyoD in LD and ST muscle, they presented very close values. This study will shed light on the definitive solution of the question marks that will arise regarding live weight in the breeding studies to be carried out in the future. I think that using the genetic information obtained in this study, genetic progress paths in fattening can be accelerated and future research can be developed. In other words, since the Saanen goat is a breed widely used in terms of milk yield in our country, such studies will also allow it to increase meat yield. In addition, this study conducted on the Saanen goat and the determination of the expression levels of the MyoG and MyoD genes that we focused on, which will be the first to be done, has a great potential to eliminate a major deficiency in this field. If progress is made in this direction, it can contribute to competition with highly developed countries in this regard. In addition, while the skeletal muscle fiber defined during prenatal development occurs in two separate stages, postnatal growth is limited to hypertrophic muscle fiber growth. Skeletal muscle fibers are the main cell type of meat mass obtained after slaughter. Therefore, differences in the activity of the MDF gene family may be very important for the amount of meat stored in these animals, which is of great economic importance. Therefore, MDF genes, especially MyoG and MyoD genes, can be considered as potential candidate genes to investigate the relationship between genomic variation and skeletal muscle mass and hence meat mass.

The smallest cellular unit that constitutes skeletal muscle mass is defined as muscle fiber, and the molecular processes that occur in muscle fibers reveal the growth and development tendency of the organism (Handel and Stickland, 1987). The number of muscle fibers, the type of muscle fibers that skeletal muscle tissue has, and even the diameter of muscle fibers can affect the cellular molecular process of this tissue or its DNA and RNA content and RNA/DNA ratios, thus affecting the transcriptional and translational capacity of muscle tissue (Sen et al., 2015). Differentiation in cellular properties such as DNA and RNA content of muscle fibers can directly affect the growth and development of the organism. Studies have shown that in tissues consisting of multinucleated cells such as skeletal muscle, the protein, DNA and RNA content of the cell and their ratios can be used as an index in determining cell size (Greenwood et al., 2006). In the current study, when the total genomic DNA amount among breeds was examined, it was found that the total genomic DNA amount in the LD and ST muscles of high-weight goats was higher than that of low-weight goats. In short, it was determined that high-weight goats in LD and ST muscle had 15% higher DNA amount than low-weight goats. Although skeletal muscle mass cells or muscle fibers have a multinucleated structure, the amount of DNA in muscle mass can be directly related to the number of muscle fibers. It may be an indication that high-weight goats have more skeletal muscle fibers than low-weight goats, and high-weight goats may indicate a high fattening potential. In the current study, when the total RNA amounts in LD and ST muscles were examined among breeds, it was found that the total RNA amount was higher in high-weight goats than in low-weight goats. High-weight goats in LD and ST muscles contained 17% more RNA than low-weight goats. It shows that high-weight goats are more metabolically active and have a greater capacity for growth in muscle fibers. All these results may indicate that high-weight goats may have a higher growth or fattening potential.

CONCLUSIONS

This study presented significantly different expression of MyoG and MyoD genes in goat skeletal muscles. This study showed that high live weight goats may have higher muscle growth due to higher MyoG and MyoD gene expression levels and this goat

breed can be recommended for more efficient fattening application. As a result of this study, it was predicted that the Saanen breed, which is reared as dairymen with the increasing world population, may contribute to increasing meat yield in line with this and similar studies.

ACKNOWLEDGEMENTS

This study was supported within the scope of TUBITAK 2209/A University Students Research Projects Support Program.

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Liposome-based transfection for CRISPR-Cas9 gene editing in livestock

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Abstract

With the increasing use of gene editing technologies on livestock, the CRISPR-Cas9 system has become a crucial tool for making targeted genetic modifications and understanding biological processes. Although CRISPR genome editing technologies have been applied in various species and different cell types, the effective delivery to specific cells remains a major challenge. In recent years, biomaterial-based functional vector systems have been particularly preferred for delivering CRISPR components to target cells. One of the most common biomaterial-based vector systems is liposome-based vectors. Liposomes offer significant advantages such as efficient encapsulation of genetic material, low cytotoxicity, and compatibility with various cell types. However, there are concerns regarding these vector systems, such as inadequate cellular uptake and low transcription efficiency. In this study, the advantages and disadvantages of liposome-based transfection in livestock genome editing studies are addressed, shedding light on future research directions.

Key words: *Livestock, CRISPR, Gene editing, Transfection, Liposome*

INTRODUCTION

One of the fundamental components of agriculture, farm animals play a pivotal role in the production process, fulfilling vital needs in meeting human nutritional requirements. This vital importance has increased interest in genetic editing technologies to understand the genetic structure of farm animals and make potential improvements for future agricultural productivity. Among these techniques, the CRISPR-Cas9 gene editing system has emerged as a powerful tool, offering the capability to precisely and targetedly edit the genome of farm animals.

The CRISPR-Cas9 system is an RNA-guided DNA endonuclease system consisting of the Cas9 nuclease and a customizable single guide RNA (sgRNA). The sgRNA can be programmed to contain 18-20 nucleotides of sequence complementary to a target sequence, immediately preceding a protospacer adjacent motif (PAM) (Tian et al., 2017). The Cas-sgRNA complex guided by sgRNA searches along the genome for its target and creates a blunt-ended double-strand break approximately 3 base pairs upstream of the PAM region (Jinek et al., 2012). The created DNA break stimulates the DNA repair pathway. The ensuing DNA repair results in either non-homologous end joining or homologous recombination-based repair, leading to the knockout of the target gene or the addition of the desired mutation (Symington and Gautier, 2011).

The delivery of CRISPR complex to target cells is a crucial stage in terms of the feasibility of this technology. However, the limited loading capacity and high immunogenicity of carriers can limit the implementation of gene editing strategies. Non-viral delivery systems, on the other hand, may offer greater advantages for in vivo application due to their higher biocompatibility and more controllable preparation compared to viral vector systems (Miller et al., 2017; Yin et al., 2020).

In recent years, the use of liposomal reagents as non-viral delivery systems has become increasingly widespread (Zuris et al., 2015; Wang et al., 2018). Liposomes are lipid-based vesicles characterized by effectively encapsulating genetic material and low cytotoxicity. These characteristics suggest that liposomes could be an effective tool for delivering gene editing material to animal cells. However, there are also concerns such as insufficient cellular uptake

and low transcription efficiency associated with liposomal transfections (Saffari et al., 2016; Yin et al., 2020). This review extensively addresses the advantages and challenges of liposome-based transfection, particularly in CRISPR-Cas9 gene editing applications in livestock.

LIPOSOME-BASED TRANFECTION

Lipofection, also known as liposome-based transfection, is a technique that delivers genetic material into cells through liposomes, which can readily fuse with the cell membrane due to their structure consisting of a phospholipid bilayer (Figure 1). Depending on the type of liposome and the cell, liposomes can undergo endocytosis or directly fuse with the cell membrane to deliver genetic material into the cells (Carter and Shieh, 2015).

Liposomes have become one of the most studied and popular non-viral gene delivery methods due to their versatility, high biocompatibility, biodegradability, and ability to protect loads (Goodwin and Huang, 2014; Sioson et al., 2021). With commercial kits, transfection reactions can be completed within 30 minutes and gene expression can be tested within hours (Carter and Shieh, 2015). Since the development of the lipofection technique, it has been used in various cell types (Felgner et al., 1987), including primary endothelial cells (Young et al., 2002), secretory epithelial cells (Lu et al., 1989), endometrial stromal cells (Lascombe et al., 1996), primary astrocyte cultures (Yu et al., 1991; Bochelen et al., 1992), oligodendrocytes (Guo et al., 1996), spermatogonial stem cells (Nakami et al., 2022), and sperm cells (Harel-Markowitz et al., 2009; Arias et al., 2017; Konoval et al., 2019). Successful examples of liposomes include Lipofectamine, Lipofectin, and Lipofectace (Saffari et al., 2016).

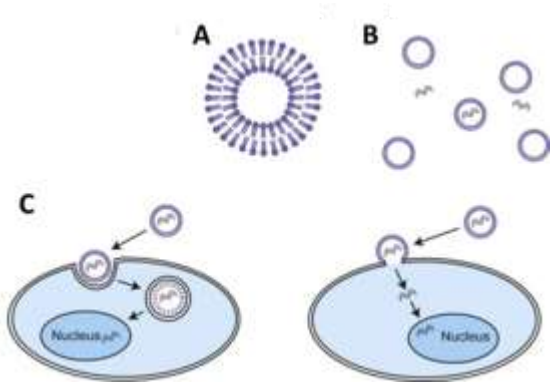

Figure 1. The delivery of genetic material into cells through liposomes. a) Double-layered lipid vesicle of liposomes; b) The encapsulation of genetic material by liposomes, fusion with the cell membrane, and direct release of DNA into cells; c) Endocytosis of genetic material encapsulated by liposomes into cells (Carter and Shieh, 2015).

In recent years, cationic lipids have also been widely used in immunotherapy and vaccine research. An example: new mRNA vaccine technologies developed against COVID-19 also utilize lipid nanoparticles to deliver mRNA into cells (Verbeke et al., 2021).

LIPOSOMAL TRANSFECTION FOR CRISPR-CAS9 IN LIVESTOCK

Lipofection emerges as one of the most attractive methods in various livestock species, ranging from cattle to honeybees. Szillat et al. (2020), successfully established a stable Madin-Darby Bovine Kidney (MDBK) CD46-knockout cell line using the CRISPR/Cas9 RNP approach to investigate the evasion ability of different pestivirus species A and B (BVDV-1 and -2) from CD46-dependent cell entry. The MDBK cells were transfected according to the manufacturer's instructions using Lipofectamine CRISPRMAX transfection reagents. In their findings, they reported a clear decrease in the infection rate after inoculation of four different BVDV-1/2 isolates into the knockout cells. Wang et al. (2015), aimed to demonstrate the feasibility of the CRISPR-Cas9 system by targeting the MSTN and FGF5 genes in goats. In the study, they injected single-cell stage embryos with Cas9 mRNA along with sgRNAs targeting two functional genes

(MSTN and FGF5). The transfection procedure was carried out using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer's instructions. In primary fibroblasts, the targeting efficiency of MSTN and FGF5 was reported to be as high as 60%, while the disruption efficiency of MSTN and FGF5 in 98 tested animals was reported to be 15% and 21%, respectively, and 10% for dual gene modifications. In their study, Abu-Bonsrah et al. (2016), performed editing on genes (CDKN1B, DGCR8, DICER, DROSHA, EZH2, HIRA, KIAA1279, KIF1BP, MBD3, RET, STMN2, TYRP1) involved in embryonic development and the pathogenesis of embryonic diseases in the chicken DF-1 cell line using the CRISPR/Cas9 system with Lipofectamine 3000. Additionally, the study demonstrated that targeted modifications can be achieved through homology-directed repair (HDR) by generating MEN2A and MEN2B mutations in the RET gene. Konoval et al. (2019), aimed to achieve HDR-directed integration of the EGFP gene into the duck genome via SMGT (Sperm Mediated Gene Transfer) using CRISPR-Cas9. For the experiment, 24 ducks of Shaoxing breed (13 males and 11 females) were used. The CRISPR complex was transfected into sperm cells using Lipofectamine 2000. As a result, 31 ducks carrying the EGFP gene were obtained, out of which 19 were confirmed to carry the EGFP gene. In F2 analyses, it was found that 16 (F1) ducks were able to transmit the transgenic DNA to their offspring. As a result, it was reported that 27.6% (56/203) of individuals from the F2 offspring contained exogenous DNA structures. Thus, the researchers demonstrated that exogenous DNA can be successfully transferred into the duck genome using CRISPR-Cas9. Challagulla et al. (2023), achieved germ line targeting of the endogenous IFNAR1 (Interferon Alpha and Beta Receptor Subunit 1) gene in chickens through in vivo transgenic expression of high-quality Cas9 and guide gRNAs. First, they developed a Tol2 transposon vector carrying Cas9, gRNA (IF-gRNAs), and green fluorescent protein (GFP) transgenes (pTgRCG), which was validated in chicken fibroblast DF1 cells. The transfection of fibroblast cells was carried out using Lipofectamine 2000 Reagent (Invitrogen). They subsequently injected the pTgRCG plasmid directly into the dorsal aortas of embryonic day (ED) 2.5 chicken embryos by targeting circulating primordial germ cells (PGCs). The resulting chimeric roosters produced completely transgenic generation 1 (G1) chickens with structural expression of Cas9 and IF-gRNA (G1_Tol2-Cas9/IF-gRNA). An indel spectrum was identified at gRNA-targeted loci in G1_Tol2-Cas9/IF-gRNA chickens, and these indels were stably inherited by the G2 generation. Piñeiro-Silva et al. (2023), analyzed the production of genetically edited porcine embryos using zona pellucida-intact oocytes through lipofection with Lipofectamine CRISPRMAX Cas9, comparing it with the electroporation method. In this context, two factors were evaluated: (i) an increase in the concentration of lipofectamine-ribonucleoprotein complexes (LRNPC) (5% versus 10%), and (ii) the concentration of ribonucleoprotein within the complexes (1xRNP versus 2xRNP). It was found that an increase in LRNPC concentration had a detrimental effect on embryo development and subsequently influenced the number of mutant embryos. The 5% group had a similar mutant blastocyst rate to the electroporation method (5.52% and 6.38%, respectively, $p > 0.05$). It was reported that an increase in ribonucleoprotein concentration within the complexes had no effect on blastocyst rate and mutation rate; the mutant blastocyst rate was similar in both 1xRNP and 2xRNP lipofection groups as well as the electroporation group (1.75%, 3.60%, and 3.57%, respectively, $p > 0.05$). Yıldız (2023), aiming to develop a sustainable approach to Varroa control, successfully knocked out the JHAMT gene in honey bee workers by transfecting honey bee sperm cells with the CAS9 and sgRNA complex using Lipofectamine CRISPRMAX transfection reagents.

CONCLUSIONS

In summary, lipofection emerges as a promising method for CRISPR/Cas9-mediated gene editing in various animal species and cell types due to its ease of use and cost-effectiveness. While studies demonstrate its comparable efficiency to other non-viral transfection methods, further

optimization of transfection protocols is warranted for maximizing its potential. Future research endeavors should focus on exploring the application of lipofection in diverse biological contexts and refining the techniques to enhance its efficiency and versatility in genetic editing.

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The Discriminant analysis of some egg parameters from different chicken genotypes

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Abstract

Although there are intensively selected lines in terms of production characteristics in the chickens, there are limited information on the comparison of their egg-related characteristics. This study aimed to compare by discriminant analysis using the parameters of egg weight, egg width, egg length, and egg shape index in some Meat-type (Anadolu-T, Ross 308, Dam Line and Sire Line), Egg-type (Atak-S, Lohmann Brown and Lohmann Selected Leghorn), and Standard breeds (Sultan and Ameraucana). While meat and egg-type breeds had similar management practices under commercial conditions, similar practices had been applied to standard breeds in controlled poultry houses. The content of laying feed given to all breeds was 14-15% crude protein, 3.2-3.5% calcium and 2750-2850 kcal/kg metabolic energy. In this context, the data of 2476 eggs obtained from 50-55 week old chickens belonging to 9 genotypes under classified 3 main types were analyzed. According to the results, egg weights of Sire Line, Ross 308, Anadolu-T, Lohmann Brown, Atak-S, Dam Line, Lohmann Selected Leghorn, Sultan and Ameraucana were 69.89a, 69.10a, 62.84b, 59.59c, 59.58c, 59.51c, 56.81d, 45.87e and 43.03f g, respectively ($p<0.01$). In the same order, the egg width was found to be 44.71b, 45.61a, 43.41bc, 43.32bc, 43.62c, 43.17d, 42.46e, 39.89f and 39.17g mm ($p<0.01$). The egg length was found to be 61.97a, 58.79c, 59.72b, 56.12ef, 57.58d, 56.59e, 55.99f, 51.30g and 51.47g mm ($p<0.01$). The egg shape index was determined to be 72.27c, 77.68a, 72.82c, 77.32a, 75.75b, 76.38b, 75.85b, 77.84a and 76.11b % ($p<0.01$). The egg weights of Meat-type, Egg-type and Standard breeds were 64.61a, 58.36b, and 45.42c g, respectively ($p<0.01$). The egg width was found to be 44.06a, 42.97b and 39.78c mm ($p<0.01$). The egg length was found to be 59.05a, 56.20b and 51.35c mm ($p<0.01$). The egg shape index was calculated to be 74.78c, 76.50b and 77.55a % ($p<0.01$).We also detected significant positive correlations ($p<0.01$) between the egg weight and the egg width (0.88), and the egg length (0.83). In the discriminant analyses, the success of assigning eggs to their groups was relatively low (52.4%) in terms of genotypes, but high (78.5%) in the type groups (Meat-type, Egg-type, and Standard breeds).

Key words: *Egg weight, Discriminant Analysis, Egg Shape, Genotype*

Investigation of cattle animal species and milk production by discriminant analysis

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Abstract

In this study, it was aimed to determine which groups were different from each other in terms of categorical variable groups milked in Türkiye and the independent variables, the number of milked cattle and milk yield, within the specified years. For this purpose, data were obtained from the types, number and milk yield of cattle milked in the statistical tables between the years 2000-2018 published by the Turkish Statistical Institute (TUIK). In the research, the data set was analyzed with the help of the Discriminant method using Mahalanobis distance for multiple normality assumption, Boxs'M test for covariance matrix equality, VIF test for multicollinearity.
According to the estimation results, in the data set obtained from the milked bovine species, number and milk yield, in the classification made, the first function distinguishes cattle crossbred and buffalo, while the second function distinguishes cattle culture and cattle native from each other. In addition, it was determined by the discriminant analysis that the classification accuracy rate was 90%.

Key words: *Cattle, milked cattle, discriminant analysis, milk yield.*

INTRODUCTION

Animal husbandry has been the most important source of livelihood and economic activity of humanity since the time it landed on the earth. Animal husbandry is an extremely important factor in terms of adequate, healthy, and balanced nutrition of the population, which tends to increase continuously. In addition, the livestock sector has an important place in terms of raw material supplies in livestock related industries. Livestock is a sector that benefits the country's economy, creates the highest added value for unit investment and provides employment opportunities at the lowest cost (Demir, 2012).

In our age, animal husbandry has an important place both in economy and industry all over the world, especially in developed societies, while the demand for the food needs of the world population, which tends to increase, is increasing day by day. It has important social and economic functions such as increasing national income and employment in terms of balanced and adequate nutrition of people, supplying raw materials to milk, meat, leather, cosmetics, textile and pharmaceutical industries, contributing to development and increasing foreign currency inflow through exports (Anonymous, 2020).

Livestock farming is divided into two groups as cattle and sheep farming. In addition to being the most prominent source of livelihood for people living in rural areas, cattle breeding is also known as the economic source of especially developing societies (Güven et al., 2017).

Almost the bovine stock in Türkiye consists of cattle.

High milk yield of the cattle, providing milk production throughout the year the long lactation period, high ability to convert roughage and concentrate into meat and milk, the possibility of being raised in different climatic zones of our country, and it’s very good adaptation to the practices for genetic improvement and control of reproduction. advantages make cattle breeding important in cattle breeding. Cattle

breeding in Türkiye was mainly carried out as a family business until the 1980s, and cattle breeding enterprises with an economic size have been established since 1980. As a result of the increase in state support in recent years, the number of large-capacity modern cattle farms has increased rapidly (Anonymous, 2020).

While the number of cattle milked in Türkiye was 5349171 heads in 2000, this number increased by approximately 20% in 2018 6413789 heads. While the milk yield of the cattle milked in 2000 was 8799371 tons, this number increased by 128% in 2018 20112619 tons (TUIK, 2021).

In this study, it was aimed to determine which groups were different from each other in terms of categorical variable groups milked in Türkiye and the independent variables, the number of milked cattle and milk yield, within the specified years.

MATERIALS AND METHODS

The data set in this study was obtained from the milked bovine species, number and milk yield in the statistical tables between the years 2000-2018 published by the Turkish Statistical Institute (TUIK). The number of cattle milked by year is given in Table 1. and the milk yield of cattle milked by is given in Table 2.

Table 1. Number of cattle milked by years

Years	Cattle Culture (head)	Cattle Crossbreed (head)	Domestic Cattle (head)	Buffalo (head)
2000	904849	2335119	2039601	69602
2001	912411	2248877	1924526	65356
2002	850725	1971740	1570103	51626
2003	1034817	2236680	1768865	57378
2004	832711	1699804	1343206	39362
2005	925618	1717309	1355170	38205
2006	1106679	1799409	1281843	36553
2007	1299750	1698801	1230889	30460
2008	1385730	1665189	1029324	31440
2009	1470886	1686064	976198	32361
2010	1626412	1787012	948417	35362
2011	1868274	1962713	930155	40218
2012	2211242	2263400	956758	46959
2013	2314278	2395897	897097	51940
2014	2427909	2428708	752623	54891
2015	2500880	2314061	720833	62999
2016	2542163	2235501	654051	63329
2017	2940907	2426764	601377	69497
2018	3185959	2554947	597001	75882

In this study, Mahalanobis distance analysis was used for the multiple normality assumption in the first stage. Mahalanobis distance; It is the more general version of the Euclidean distance at the moment when the relationship between the values is ignored. It is one of the most common statistical techniques used to determine the central parameter criterion of multivariate vectors and the distances of the variance-covariance matrix (Aggarwal, 2013). X is the data matrix consisting of p-dimensional values, $\bar{x}$ the mean vector calculated from the obtained data and the sample variance-covariance matrix calculated from the data that are identical in S.

Mahalanobis distance i. for observation

$$\sqrt{D_i} = (x_i - \bar{x})^* S^{-1} (x_i - \bar{x}) \qquad (1)$$

form (Penny, 1996).

Box's M test was used for the assumption of equality of covariance matrices. Box's M test; $H_0 : \sum_1 = \sum_2 = \cdots \sum_k$ H_1 hypothesis will have at least one variance-covariance matrix different from the others. For this purpose, first the common S matrix is obtained.

Table 2. Milk yield of cattle milked by years

Years	Cattle Culture Milk Yield (ton)	Cattle Hybrid Milk Yield (ton)	Cattle Native Milk Yield (ton)	Buffalo Milk Yield (ton)
2000	2639113	4591861	1501067	67330
2001	2660282	4410758	1418042	63327
2002	2467889	3867656	1155088	50925
2003	3215859	4568252	1730027	48778
2004	3231461	4608293	1769571	39279
2005	3596017	4646857	1783328	38058
2006	4295367	4884590	1687345	36358
2007	5050533	4608728	1620079	30375
2008	5380715	4520465	1353996	31422
2009	5713004	4585859	1284450	32443
2010	6309065	4861835	1247644	35487
2011	7239644	5341224	1221560	40372
2012	8554402	6166762	1256673	46989
2013	8946131	6531573	1177305	51947
2014	9383812	6628337	986701	54803
2015	9672573	6315366	945581	62761
2016	9825300	6101826	859137	63085
2017	11355933	6620540	785846	69401
2018	12301080	6957715	778082	75742

$$S=\frac{\sum_{i=1}^{k}(n_i-1)S_i}{\sum_{i=1}^{k}(n_i-1)} \quad (2)$$

k: Number of groups
p: Number of variables in groups
Si: Covariance matrices
ni: Number of observations
is in the form.

With the help of S, Box's M test is performed:

$$M=\sum_{i=1}^{k} n_i - 1 \ln|S| - \sum_{i=1}^{k}(n_i - 1)\ln|S_i| \quad (3)$$

(Alpar, 2013).

Increase factors (VIF) test was used for the assumption of multicollinearity. Variance increase factors (VIF) test; whether the high correlation caused by the connections between the values is a problem in terms of analysis. It is known that the multicollinearity problem is not mentioned when the variance increase factors (VIF) values are less than 10 (Hair et al., 2006).

Afterwards, discriminant analysis was used to determine which groups were different from each other in terms of the categorical variable groups milked bovine species and independent variables, the number of milked cattle and milk yield. Discriminant analysis: It is used to determine the discriminant variables that have the greatest effect on the discrimination between groups and to determine which group will join the data that cannot be determined from which group. Its general task is separation, and it is one of the multivariate statistical methods used to determine the groups they belong to by making use of the p features of the data or to determine the most significant function that can separate the existing groups from each other (Çamdeviren, 2000). It is the process of revealing the differences between two or more groups with the help of discriminant variables (Klecka, 2000).

The assumptions used in discriminant analysis for the probability of correct grouping are:

1) The data have multiple normal distributions,
2) The covariance matrices of all groups are equal, and
3) There should be no multicollinearity problem between independent variables (Alpar, 2013).

Discriminant analysis also enables to determine in which variables the difference is concentrated compared to other variables in separation and therefore to determine the factors that affect the differentiation between groups. Comparing the cluster classes obtained the analysis with the base classes allows to analyze whether the existing component is sufficient (Erçetin, 1993).

RESULTS

In this study, it was aimed to determine which groups were different from each other in terms of categorical variable groups milked in Türkiye and the independent variables, the number of milked cattle and milk yield, within the specified years. For this purpose, data were obtained from the types, number and milk yield of cattle milked in the statistical tables between the years 2000-2018 published by the Turkish Statistical Institute (TUIK). In the research, the data set was analyzed with the help of the Discriminant method using Mahalanobis distance for multiple normality assumption, Boxs'M test for covariance matrix equality, VIF test for multicollinearity.

According to the results of the research, a correlation of 0.758 was found between the Mahalanobis distances and the inverse cumulative chi-square value. This showed that the data conformed to the multiple normality assumption. Box's M test was used in the test of equality of covariance matrices. As a result of the analysis, the significance value was found to be 0.021. Since this is greater than 0.01, it was determined that the covariance matrices were equal. After the analysis of multiple normality and equality of covariance matrices, it was determined that there was no multicollinearity in the analysis of multicollinearity, since the VIF value was 4.5351 less than 10.

In the data set obtained from the milked bovine species, number and milk yield in the statistical tables between 2000-2018 published by the Turkish Statistical Institute (TUIK), in the classification made, the 1st function distinguishes cattle crossbred and buffalo, while the 2nd function distinguishes cattle from culture and cattle. distinguishes the native from each other. In addition, it was determined that 15 out of 19 of the milked animal species in the 1st group, all 19 milked animal species in the 2nd group, 17 out of 19 of the milked animal species in the 3rd group and all 19 milked animal species in the 4th group were correctly classified. The correct classification percentage was found to be 90% in total, and it was determined by the analysis that 65% and above is a good rate since it is sufficient for discriminant analysis in the literature. Obtain better results in the data set obtained from the bovine species, number, and milk yield in the statistical tables between the years 2000-2018 published by the Turkish Statistical Institute (TUIK), the number of observations in the data set should be larger, the group in the selected categorical variables and in each group. Increasing the data, increasing the independent variables in the model, and natural events and global warming that have occurred in cattle breeding in Türkiye in recent years should be consider. Avoid these and similar problems in future articles or thesis research, the deficiencies mentioned should not be ignored.

DISCUSSION

The data set in this study was obtained from the milked bovine species, number and milk yield in the statistical tables between the years 2000-2018 published by the Turkish Statistical Institute (TUIK). In the data set obtained, Mahalanobis distances were found for the multiple normality assumption in the first step, and a correlation of 0.758 was found between the reverse cumulative chi-square value.

Box's M test was used in the assumption of equality of covariance matrices. As a result of the analysis, the significance value was found to be 0.021.

After the analysis of multiple normality and equality of covariance matrices, the VIF value was found to be 4.5351 in the analysis of multicollinearity

Categorical variable groups according to years, cattle species milked and independent variables, number of cows milked and milk yield, were tested with the help of Discriminant analysis.

As a result of the analysis:

Table 3. Chi-square and wilks' lambda values of separation functions.

Function Test	Wilks' Lambda	Chi-Square	df	Sig.
1 through 2	.074	187.798	6	.000
2	.281	91.494	2	.000

Since the significance values in Table 3 were less than 0.05, it was determined that both function groups were significantly separated from each other.

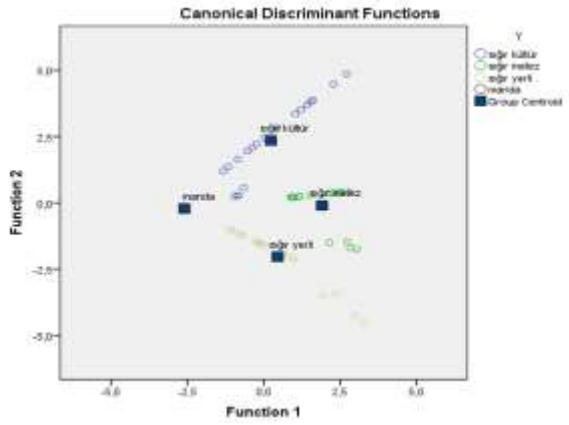

Figure 1. Group values allocated by each function

In Figure 1, the 1st function distinguishes cattle crossbred and buffalo, while in the 2nd function, cattle culture and cattle native distinguish each other.

Table 4. Eigenvalue, variance, cumulative variance and canonical correlation values of the discrimination functions.

Fuction	Eigenvalue	Percentage Of Variance	Cumulative %	Canonical Correlation
1	2.810	52.3	52.3	0.859
2	2.564	47.7	100.0	0.848

When the Canonical correlation value is squared in Table 4, the explained variance value is obtained, which is 0.7378 for the first function. For the second function, it is 0.7191. These values explain the dependent variable at the rate of 73.78% and 71.91%, respectively.
In addition, the 1st function in eigenvalues has more power to explain the variances in all variables than the 2nd function. In percentages of variance, the first function explains 52.3% of the total variance, while the second function explains 47.7%.

Table 5. Variable coefficients of separation functions.

	Functions	
	1	2
Number of Milked Bovines (head)	1.542	-1.431
Milked Bovine Milk Yield (ton)	-0.676	1.992

In Table 5, separate loads of each independent variable in the 2 separation functions are given. In the absolute value, the number of milked cattle has the highest load in the 1st function, while the milk yield of the milked cattle in the 2nd function.

Table 6. Structure matrix of each variable of the separation functions.

	Functions	
	1	2
Number of Milked Bovines (head)	.947*	.322
Milked Bovine Milk Yield (ton)	.680	.733*

In Table 6, while the variable with the highest correlation in the 1st function was the number of milked cattle, it was also the milk yield of the milked cattle in the 2nd function.
Table 7; 78.9% of the milked animal species in the 1st group are correct 21.1% are incorrect, the milked animal species in the 2nd group are correct, 89.5% of the milked animal species in the 3rd group are correct, 10.5% are incorrect, and all of the milked animal species in the 4th group are correct correctly grouped.

Table 7. Grouping results in analysis.

	Estimated Group Membership				
Groups	**Cattle Kulture**	**Cattle Crossbreed**	**Domestic Cattle**	**Buffalo**	**Total**
Cattle Kulture	15	0	0	4	19
CattleCrossbreed	0	19	0	0	19
Domestic Cattle	0	0	17	2	19
Buffalo	0	0	0	19	19
Cattle Kulture %	78.9	.0	.0	21.1	100.0
CattleCrossbree%	.0	100.0	.0	.0	100.0
Domestic Cattle%	.0	.0	89.5	10.5	100.0
Buffalo%	.0	.0	.0	100.0	100.0

In a study, the canonical separation function of Karayaka and Bafra (Sakız x Karayaka G1) sheep was determined by using body weight and body measurements. As a result of the research, it has been concluded that the canonical separation function of sheep

breeds has a 100% accuracy rate (Kılıç et al., 2013). In a study, it was investigated that discriminant analysis may be suitable for predicting feather color in Japanese quails. As a result of the research, it was concluded that the feather color of Japanese quails has an accuracy rate of 72.20% (Çelik et al., 2015). In a study, the comparison of the progress of the cattle breeding sector in Turkey in 2000 and later Europe and the world was investigated. As a result of the research, it was concluded that the cattle sector in Türkiye is not yet at a competitive level (Güven, 2018). In a study, the evaluation of the scientific studies on the structural and mechanization characteristics of dairy cattle enterprises in the last forty years in Türkiye was investigated in terms of their practical requirements.

More conscious evaluation of the methods and inputs/outputs that are missing in practice in dairy cattle farms has been examined. It has been concluded that in the context of environment and energy use in terms of livestock, information that can be used as a data set for subject workers, policy makers and decision makers will be compiled (Bilge and Aybek, 2018). In study, the comparison of livestock enterprises in terms of forage cultivation and pasture use habits in a selected district in Tekirdağ and Kırklareli provinces was investigated. As a result of the research, it was concluded that 75.80% of all enterprises were classified correctly (Öztürk et al., 2019). In a study, it was aimed to determine the morphological characteristics of some genotypes in the Eastern Black Sea, Central Black Sea and Western Black Sea Regions. As a result of the research, 3 genotypes formed 3 separate clusters that did not overlap, Korgan genotype; It was concluded that the Camili genotype was closer to the Yığılca genotype (Gençer and Günbey, 2020). In a study, it was aimed to determine the current situation in terms of breeding practices in dairy cattle enterprises. As a result of the research, it has been determined that although the fertility and reproduction practices are well implemented in the mentioned enterprises, calf care and selection practices continue at an insufficient and traditional level. In addition, it has been concluded that it is important for agricultural engineer employed in private or public institutions to inform breeders and provide consultancy services on animal breeding and breeding (Kıyıcı and Çınar, 2020).

In study, it was aimed to determine the structural features of dairy cattle farms that are members of Balikesir Province Gonen Dairy Producers Union, to identify the problems they encounter and to propose solutions for these problems. As a result of the research, feed costs constitute 56.4% of dairy cattle costs in enterprises. 82.3% of the enterprises benefit from fodder crops and 85.5% from other livestock supports. It was determined that 37.9% of the enterprises used loans for livestock. The enterprises face the most problems in the supply of roughage. It was concluded that dairy cattle enterprises should benefit from more and support should be increased (Özdemir, 2021).

The difference this research from the studies in the literature is to determine which groups are different from each other in terms of the categorical variable groups milked in Türkiye and the independent variables, the number of milked cattle and milk yield, within the determined years.

AUTHOR CONTRIBUTIONS

The authors declare that they have contributed equally to the article.

CONFLICT OF INTEREST

The authors of the article declare that there is no conflict of interest between them.

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The Effect of growing in different rearing systems on egg production, egg quality traits and hatching results in japanese quails

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Abstract

This study was carried out to determine the effect of growing breeding quails in different housing systems on egg production-related characteristics, egg quality-related characteristics and incubation-related characteristics. At the age of 3 weeks, a total of 360 quails were distributed equally to 3 different housing systems (cage, enriched cage and floor) in a male:female mixed. When the quails reached 6 weeks of age, 30 females were taken from each system (90 females in total) and placed in individual cages. Again, 10 males were taken from each system. Then placed in such a way that each male was given to 3 females (changing the pen every 2 days and 1 female from each system). The study continued for 3 months and the eggs laid were individually weighed and recorded every day (approximately 5500 eggs were obtained). A total of 744 eggs were broken to determine egg quality characteristics. A total of 1252 eggs were incubated. The average quail weight at 3 weeks of age was 48.6 g and there was no difference between systems ($p>0.05$). At the age of six weeks, average weights were weighed in Cage, Floor and Enriched systems, 193.9, 177.2 and 198.5 g, respectively ($p<0.01$). Age of first egg according to these groups were 52.8, 65.4 and 57.3 days ($p<0.01$). Body weight at first egg was 270.1, 273.9 and 276.8 g ($p>0.05$). Weight of first egg was 9.7, 10.6 and 9.8 g ($p=0.08$). Total egg yield was 71.5, 61.1 and 69.9% ($p<0.05$). Broken-cracked egg yield was 2.8, 2.0 and 2.2% ($p>0.05$). Hatching egg yield was 69.0, 59.2 and 67.7% ($p<0.05$). Egg weights were 9.24, 9.07 and 9.34 g ($p<0.05$). Egg shape index was 79.09, 78.96 and 80.14% ($p<0.01$). Yolk height was 11.14, 11.01 and 11.24 mm ($p<0.01$). Yolk diameter was 23.09, 22.29 and 22.93 mm ($p<0.01$). Yolk index was 48.43, 49.58 and 49.16 % ($p<0.01$). Albumen length was 72.4, 67.4 and 69.2 mm ($p<0.01$). Albumen index was 7.74, 8.38 and 8.15 ($p<0.01$). No difference was detected in some egg quality characteristics such as haugh unit, egg shell thickness, albumen height and albumen width ($p>0.05$). In the study, where the general average of hatchability was 73.3%, no difference was detected between fertility rate, hatchability of fertile egg and embryo mortality rates ($p>0.05$). As a result, it was determined that quails raised on the Floor were weaker and started laying eggs later, and as a result, their egg productivity remained at a very low level compared to other groups. The group with the highest body weight development and egg weight of females was Enriched. The differences in egg quality characteristics vary according to groups.

Key words: *Quail, rearing system, egg yield, egg quality, enriched cage.*

Behavioral resistance against *Varroa destructor* in honey bee colonies

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Abstract

Honey bees, known as eusocial organisms, have developed behavioral strategies for various purposes in the course of evolution. These strategies have been shaped through adaptation processes to fulfill important functions such as maintaining colony life, protecting their offspring, finding resources, and coping with various environmental challenges. Bee behaviors, particularly, encompass specific behavioral resistance mechanisms against diseases and ectoparasites. Behavioral resistance is generally examined under two main categories: hygienic and grooming behaviors. Hygienic behavior involves the detection of diseased or parasitized larvae and pupae, the removal of infected/infested brood, thus reducing the spread of infection/infestation. Grooming behavior is classified into two categories based on the performer: auto-grooming and allo-grooming. Auto-grooming refers to self-grooming behavior, while allo-grooming describes mutual grooming between two bees or the grooming of one bee by several bees acting socially together. This review emphasizes the potential of honey bee populations in struggling the honey bee ectoparasite Varroa destructor by examining how hygienic and grooming behaviors are influenced by genetic and environmental factors. Additionally, it can serve as an important resource to guide future research and to better understand the effects of Varroa destructor challenges on the sustainability of bee colony populations.

Key words: *Honey bee, Honey bee health, Behavioral resistance, Grooming behavior, Hygienic behavior*

INTRODUCTION

Honey bee (*Apis mellifera*) is the most useful insect species, both with its role in the healthy and balanced functioning of the ecosystem as a pollinator and with its production of valuable bee products such as honey, beeswax, pollen, royal jelly and propolis (Yıldız, 2023). However, honeybee losses have been a significant issue since the inception of modern beekeeping. Various factors have been identified as causes for this, including climate change, habitat loss, diseases, ecto- and endoparasites, and pesticides. (Brown et al., 2016; Potts et al., 2016; Pătruică et al., 2021). Parasites and pathogens are fundamental factors underlying honeybee losses that threaten both the beekeeping industry and agriculture as a whole. To date, Varroa destructor has been identified as the primary culprit responsible for the majority of losses experienced (Eliash, 2017).

V. destructor is an obligate ectoparasite that feeds on the fat body of both larvae and adult honeybees (previously thought to feed on hemolymph) (Anderson and Trueman, 2000; Ramsey et al., 2019). Varroa mites invade honeybee larvae before the cells are sealed and feed on developing larvae, thereby multiplying within the brood cells. Varroa mites invade honeybee larvae before the cells are sealed and reproduce by feeding on developing bee larvae. Female mites that attach to emerging bees also accompany them out of the cells, transferring to another bee or another bee larva (Shimanuki et al., 1994; Rosenkranz et al., 2010). If appropriate control methods are not employed, a colony infected with Varroa will collapse within 2-3 years (Boecking and Genersch, 2008; Rosenkranz et al., 2010). Beekeepers use chemicals such as organophosphate coumaphos, tau-fluvalinate, and formamidine amitraz to control Varroa mites. However, due to beekeepers not using chemicals at the correct time and in a synchronized manner, as well as the development of resistance to

chemicals by Varroa (Pettis 2004; Maggi et al. 2010), the desired results are not achieved. Additionally, residues of chemicals in bee products pose a risk to human health.
Honey bee colonies have natural resistance mechanisms against various diseases and parasites. Some colonies may exhibit higher levels of resistance or susceptibility to certain diseases or pests compared to others. This variability arises from differences in the composition of genetic diversity within colonies. The raw natural structures comprising the colony shape the disease and parasite resistance genotypes of honeybees through natural selection. Resistance mechanisms operate through physical, behavioral, and immune system pathways. It is known that these mechanisms are genetically based. The most important defense mechanisms against diseases and parasites in honeybees are hygienic and grooming behaviors.

BEHAVIORAL RESISTANCE MECHANISMS IN HONEYBEES

Hygienic Behavior

The most well-known behavioral resistance mechanism in honeybees is hygienic behavior. This behavior was first described by Park (1937). It has been demonstrated that hygienic behavior is an effective behavioral mechanism against many diseases and Varroa mites (Laidlaw and Page, 1997).
Hygienic behavior is defined as worker bees detecting and removing dead or infected brood from the colony (Rothenbuhler, 1964a, b). This behavior is an inheritable trait controlled by multiple genetic loci (Jones and Rothenbuhler, 1964; Rothenbuhler, 1964a, b; Momot and Rothenbuhler, 1971; Wilson-Rich et al., 2009). Researchers have reported that honeybees bred for hygienic behavior have positive effects on Varroa infestation, and breeding efforts in this direction are rapidly increasing (Büchler vd., 2010; Rinderer vd., 2010; Sumpter ve Martin, 2004). In honeybee colonies exhibiting hygienic behavior, bees infested with Varroa are removed by uncapping cells (Varroa Sensitive Hygiene - VSH), thereby limiting the Varroa infestation rate and reducing the reproductive success of the mites (Harbo and Harris, 2009). It has been reported that this behavior does not have a negative impact on colony performance and even increases honey production (Spivak and Reuter, 1998).
It is reported that a relatively small percentage of colonies (≤10%) exhibit hygienic behavior in nature, and there is limited data on hygienic behavior in naturally infested hives (Spivak and Gilliam, 1998). In Mexico, Africanized honeybees (*A. mellifera scutellata*) remove naturally infested brood at a rate of 32%, while under the same conditions, European honeybees (*A. mellifera)* remove them at a rate of 8% (Vandame et al., 1996). In Tunisia, *A. mellifera intermissa* bees remove naturally infested brood at a rate of 15.5%, while *A. mellifera carnica* colonies remove them at a rate of 16.6% (Boecking and Drescher, 1992). Additionally, it has been reported that 20% of Australian bees exhibit hygienic behavior (Oxley et al., 2008). Due to the insufficient data obtained through natural means, researchers have conducted various experiments in recent years to unravel the principles of tolerance to Varroa. These studies have mainly been conducted in hives where disease or parasite-infested or killed broods are artificially introduced. In a study conducted in 76 colonies that were not subjected to any selection for hygienic behavior, the average rate of removal of experimentally infested brood with one live mite per cell was found to be 23.5±18.2. Only 9.2% of these colonies removed more than 50% of infested brood (Boecking and Drescher, 1998).
Various studies aimed at understanding the molecular mechanisms underlying hygienic behavior allow us to gain a deeper understanding of the defense capabilities of honey bees. Mondet et al. (2015), investigated the mechanisms of Varroa Sensitive Hygiene (VSH) behavior by comparing the antennal transcriptomes of bees exhibiting and not exhibiting VSH behavior. Their findings suggest that antennae may play a key role in the execution of VSH behavior. Hygienic bees, specific odorants better detection, can be predicted from transcriptional patterns and antennal motor activity. These results shed light on the importance of the peripheral nervous system, providing a new perspective

on understanding VSH behavior and the evolution of collective defense. Tsuruda et al. (2012), conducted a study to map quantitative trait loci (QTL) in order to identify genes influencing VSH. They identified a major QTL on chromosome 9 (LOD score = 3.21) and a significant QTL on chromosome 1 (LOD = 1.95). The confidence interval of the QTL on chromosome 9 encompasses the "no receptor potential A" gene and a dopamine receptor. The "no receptor potential A" gene is involved in vision and olfaction in Drosophila, while dopamine signaling has been shown to be necessary for the inhibitory olfactory learning required to identify mites in brood cells in honey bees. Sepehri et al. (2023), conducted SNP analysis on the candidate gene NorpA2, associated with vision and olfaction, in Varroa-sensitive (SUS) and resistant (RES) colonies. Interestingly, the results revealed three specific differences between the SUS and RES groups in the nucleotide sequence of the promoter region: SNP (C/T) at position 308, and SNP/deletion at positions 504 and 563. Teixeira et al. (2021), conducted RNA sequencing on the brains of worker honeybees observed exhibiting hygienic behavior to determine expression changes associated with hygienic behavior in African-derived Apis mellifera. Additionally, they used the transcriptome data to investigate single nucleotide polymorphism (SNP) variation in genes previously associated with traits in other A. mellifera populations. The analysis revealed 49 differentially expressed genes (DEGs), most of which were upregulated during hygienic behavior. Apidaecin, a DEG, also exhibited SNP variation among samples, emerging as a promising candidate gene for both expression-based and heritable variation in hygienic behavior. Additionally, they reported 27 additional SNPs in the coding regions of candidate genes previously implicated in hygienic behavior, including Abscam, cac, Syn1, Pka-C1, and Obp4. Oxley et al. (2010), identified the Hyg1 locus on chromosome 2 associated with hygienic behavior. This locus includes genes associated with behavior, odor, neuron development and function, as well as receptor and transcriptional activity within a 95% confidence interval. Harpur et al. (2019), conducted whole-genome sequencing of male bees obtained from two bee colonies selected for hygienic behavior and one unselected bee colony, identifying 73 candidate genes. On chromosome 6, the abscam gene, which plays a significant role in axonal guidance, particularly in olfactory neurons, is noteworthy, along with genes such as goosecoid (Hoxgene) and tropomyosin-2-like genes essential for nervous system development. Additionally, attention is drawn to the orthologue of the Drosophila dyschronic gene (GB45054), which is involved in biological processes like the sensory perception of sounds and light stimuli on chromosome 11, as well as the insulin-like receptor (GB53353) functioning in protein phosphorylation on chromosome 9 and in the transmembrane receptor protein tyrosine kinase signaling pathway.

Grooming Behavior

Grooming behavior is typically a straightforward process, involving the cleaning and incapacitation of mites found on the bodies of adult bees (Pritchard, 2016). Another behavioral resistance mechanism in honey bees, grooming behavior, is a common strategy observed among vertebrates and arthropods to rid themselves of ectoparasites. This behavior has evolved to protect both individual and colony health (De Figueiró Santos et al., 2016). Grooming behavior is classified into two different forms: self-grooming and allo-grooming. Self-grooming is the behavior of cleaning oneself through the movement of mouthparts or pro-/mesothoracic legs. Allo-grooming, on the other hand, occurs when one or more bees groom each other either individually or socially. In many studies, grooming behavior has been shown to be closely associated with a decrease in mite levels. Andino and Hunt (2011), reported a correlation between the rate of mites cleaned by bees in laboratory grooming assays and the proportion of damaged Varroa on sticky boards placed under hives. In their findings, they demonstrated that colonies exhibiting high grooming behavior had a high proportion of incapacitated mites on the sticky boards placed under the hives. Also, in colonies exhibiting higher levels of grooming behavior, mites are incapacitated

by being bitten (Arechavaleta-Velasco and Guzmán-Novoa, 2001).

Grooming behavior in honeybees holds critical importance for both bee health and colony organization, with various studies revealing how this behavior varies across different bee breeds and conditions. Rosenkranz et al. (1997), recorded an average mite damage rate of 45% in Italian (*A. mellifera ligustica*) and Carniolan (*A. mellifera carnica*) bees, whereas African honeybees (*A. mellifera scutellata*) exhibited a 38.5% mite damage rate in their grooming behavior study. Aumeier (2001), reported that 66% of artificially infested Carniolan bees (Apis mellifera carnica) responded to the presence of mites on their bodies within the first 30 seconds. Bak and Wilde (2015), reported that 86% of artificially infested Caucasian bees (Apis mellifera caucasica) exhibited grooming behavior to remove mites. van Alphen and Fernhout (2020), reported an average mite damage of 5.75% in the Italian race (*A. mellifera ligustica*). Yıldız and Karabağ (2022), reported that in their individual grooming behavior study conducted on different races under in vitro conditions, the Italian honeybee (*A. mellifera ligustica*) exhibited the highest grooming behavior, with 65% of the tested individuals showing grooming behavior.

Molecular research sheds light on genetic and neurobiological mechanisms, aiding in our deeper understanding of the origins and workings of this complex behavior. Various studies have aimed to unravel the molecular mechanisms of grooming behavior by investigating various candidates, including Neurexin-1 (AmNrx1), Atlastin, Ataxin-3, poly U binding factor kd 68, Vitellogenin, autophagy-linked FYVE protein, blue cheese (BlCh), and genes encoding immunity-related hymenoptaecin, which are believed to have potential neurodevelopmental and behavioral implications associated with grooming behavior in honey bees (Yıldız and Karabağ, 2022). In general, the expression levels of genes have been investigated in bees treated with Varroa mites. As a result of such studies, it has been revealed that many of the genes differently expressed between tolerant and susceptible bees are involved in the development of the nervous system. Arechavaleta-Velasco et al. (2012), identified a region on chromosome 5 associated with honeybee grooming behavior using a QTL mapping approach. This region contained 27 genes, including potential neurodevelopmental and behavioral candidates such as neurexin-1, ataxin-3, and atlastin. Hamiduzzaman et al. (2017), invastegated the relationships between grooming behavior and the expressions of genes related to immunity, nervous system, and detoxification. In bees exhibiting intense grooming behavior, significant upregulation of Neurexin-1 expression, which plays a key role in synaptic information transmission and maintenance (Craig and Kang, 2007; Dean and Dresbach, 2006), has been identified. As a result, Neurexin-1 has been reported as a potential biomarker for behavioral traits in bees. Morfin et al. (2020) reported that in selectively bred Indiana mite-biting honeybee colonies, the rate of mite damage inflicted by mite-biting bees, the severity of injury, and the colony overwintering rate were higher compared to the control group. Additionally, the expression of the Neurexin-1 gene, associated with grooming behavior, was found to be significantly higher in Indiana mite-biting bees. Although the molecular mechanism of grooming behavior remains incompletely understood, it is evident that grooming behavior is negatively correlated with the number of mites in colonies.

CONCLUSIONS

This review article emphasizes the importance of behavioral resistance observed in honeybee colonies against the harmful effects of Varroa. Natural behaviors of bees such as hygienic behavior and grooming behavior play a significant role in controlling Varroa populations. Hygienic behavior reduces the reproductive success of Varroa by enabling bees to identify and clean infected larvae. Additionally, through grooming behavior, bees can physically remove the Varroa parasite, thereby reducing its detrimental impact within the colony. These natural behaviors reduce dependence on chemical treatments while enhancing the health and resilience of colonies. In this context, focusing on biological and behavioral factors in combating Varroa destructor is important

for bee health and colony productivity. Molecular research plays a critical role in uncovering the mechanisms underlying behavioral resistance. Research findings suggest that genes involved in the nervous system play a crucial role in determining both hygienic and grooming behaviors. Future research focusing on neural genes could be key to better understanding and strengthening these natural resistance mechanisms, paving the way for developing a sustainable Varroa control strategy in the beekeeping industry.

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Prevalence of multi-drug resistant (MDR) Escherichia Coli and Klebsiella Pneumoniae isolated from pets in Eastern Algeria

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Abstract

The rampant use of antibiotics by healthcare providers has led to a significant animal and public health issue: antibiotic resistance. This problem is escalating as various bacterial groups in particular Escherichia coli and Klebsiella pneumoniae which exhibit heightened resistance levels by expressing genes that confer multi drug resistance (MDR) profile, posing a serious challenge to treatment in companion animals. In the present study, 409 pets were studied (209 cats and 200 dogs) from various east regions of Algeria. Strains were isolated on Hektoen enteric agar supplemented with cefotaxime (1ug/ml) and identified using the API 20E system. The phenotypic resistance was evaluated using the Kirby-Bauer disc diffusion method against 16 antibiotics and the results were interpreted according to the EUCAST guidelines. Forty-five E. coli (35 from cats10 from dogs) and 6 k. pneumoniae (5 from cats and 1 from dog) were isolated, with a prevalence of 11% and 1.46% respectively. All the strains were producers of extended spectrum beta lactamases and all of them presented the multidrug resistance profile. The highest levels of resistance were observed towards cefazoline and cefotaxime (100%) in E.coli and K. pneumoniae in both dogs and cats and 5 strains (2 E.coli and 3 K. pneumoniae) presented an intermediate resistance against Imipenem. Meanwhile low resistance for aminoglycosides, all the strains were susceptible to gentamicin except one K. pneumoniae isolated from a dog. These results suggest that the presence multidrug resistant bacterial strains in companion animals poses a significant risk to their owners with a potential transmission of resistance genes and therapeutic failure.

Key words: *Escherichia coli, Klebsiella Pneumoniae , ESBL, multidrug resistance.*

Street animals rehabilitation center procedures, Kahramanmaras example

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Abstract

In this study, it was aimed to evaluate the diseases frequently seen in these animals with the numerical data of stray or stray dogs brought to the Stray Animals Rehabilitation Center of Kahramanmaras Metropolitan Municipality between 2021-2022. Male and female dogs from Andırın, Afşin, Çağlayancerit, Dulkadiroğlu, Elbistan, Göksun, Nurhak, Onikisubat, Pazarcık and Türkoğlu districts of Kahramanmaras province in 2021 and 2022 were evaluated. In 2021, a total of 2065 dogs (1071 females and 994 males) came to the street animal rehabilitation center. In 2022; Dogs of 3052 different breeds, including 1551 female and 1501 male dogs, were received. The most common cases encountered in dogs brought to stray animals rehabilitation center in 2021 were 0.68% tumoral formations, 5.4% traumatic disorders, 3.29% skin diseases, 1.3% nervous tissue diseases, 1.11% eye diseases, 6.5% ear diseases, 1.3% amputations, 2.17% formal disorders, 16% general inflammatory cases, 3.05% foot diseases and 0.93% prolapse vagina. In 2022, 0.52% tumoral formations, 5.70% traumatic disorders, 3.74% skin diseases, 2.09% nervous tissue diseases, 1.21% eye diseases, 1.44% ear diseases, 1.99% amputations, 2.98% shape disorders, 20.8% general inflammatory cases, 3.11 foot diseases and 1.09% prolapse vagina were the most common cases.
In addition, in order to control the reproduction of the dogs that came to the rehabilitation center in 2021, 48% of the male dogs were castrated and 55% of the female dogs were ovariohysterectomy. In 2022, 55% of the incoming male dogs were castrated and 44% of the female dogs were ovariohysterectomy.

Key words: *Stray animals, stray and stray dogs, illness, rehabilitation*

INTRODUCTION

Streets are used not only for transportation purposes, but also as living spaces by some living things, such as animals and plants.This situation brings along a phenomenon that reflects the identity of the city as the streets are interaction spaces.However, with modernization, streets can offer an alienated impression to their users due to socio-cultural changes.

In addition, city streets can create an unsafe and undefined atmosphere for users (Tiftik et all., 2015). Vehicles parked on the sidewalks, congested traffic and the presence of luxury and expensive cars on rough roads appear as a facet of the country's unplanned urbanization reflected in the street image.In this context, streets have meanings beyond just being a means of transportation. The habitat of both animals and plants is important as a place of interaction and a reflection of the identity of the city. Factors such as modernization and irregular urbanization can affect the unique character and use of streets. Stray animals are a phenomenon that creates serious problems both among municipalities and the public today. Many individuals, especially animal lovers, believe that this world not only for humans, but also for other living things to have a right to exist.

According to this perspective, animals have the right to life just like humans. However, another part of the society thinks that stray animals, i.e. stray cats and dogs, should live in more secluded and secluded areas, such as forests, rather than between neighborhoods. Veterinarian Dodurka

defined the concept of "stray animal" in his study carried out in 2009 as follows: He defined stray animals as stray animals that live on the streets and/or were born on the street, or were removed from the home by their previous owners. In the same context, stray animals trying to survive in rural areas are also included in this group. Both groups can be classified as "free range animals" (Kandır, 2014). These creatures, who continue their natural lives on the streets, fall into a needy situation apart from their natural lives with the changes in the streets. Cultural and spatial changes occurring rapidly in urban areas indicate that the definitions and values of streets are rapidly transforming. This process of change points out that the concept of the street, defined in the past, has become uncertain today, and this situation both negatively affects the life of the city residents and complicates the living conditions for the creatures living on the street.

These rapidly developing changes negatively affect the positive interaction between street people and street animals. The street should be considered as a space that covers not only the means of transportation, but also the relations it contains.

We can say that the street is a place where contact, communication, interaction and mutual relations meet. If we need to sort the users of the street from a social perspective, people, who are the creators of the physical environment that creates the streets, can be listed as both humans and street animals and plants that are among the social groups that interact with each other.

Within the framework of these rapidly developing situations, there has been a serious increase in the number of stray animals. With this increase, it becomes difficult to feed and care for stray animals. In order to prevent the number of these friends from getting out of control, the importance of sterilization and vaccination is once again revealed.

In this context, Kahramanmaras Municipality Temporary Animal Nursing Home operates as a facility established by the Veterinary Affairs Directorate on the World Animal Protection Day on October 4, 2012, based on the Animal Protection Law No. 5199 and the relevant implementation regulation. The main purpose of this center is to function as a center for the rehabilitation of stray animals. In this context, in order to protect the health of stray stray animals and to ensure reproductive controls, it performs the following duties in accordance with the current legal regulations: Collecting stray animals

- Sterilization process
-Vaccination against zoonotic diseases (Rabies)
-Using marking methods,
-Adoption or leaving them back to their environment,
-Registering the owned animals, keeping the animals under surveillance by cooperating with the Public Units when necessary, and following them throughout the surveillance process.

The aim of this study was to evaluate the most common cases of stray animals wandering in Kahramanmaras province between the years 2021-2022.

MATERIALS AND METHODS

The research material consisted of 5617 dogs brought to Kahramanmaras Metropolitan Municipality Stray Animals Rehabilitation Center between 2021-2022. Dogs coming to the rehabilitation center are grouped as male and female. However, in our study, evaluations were made without considering the age of the dogs. The rehabilitation center has 2 operating rooms, 1 x-ray room, 1 examination room, laboratory and necessary administrative buildings. In addition, this center provides services with 4 Veterinarians, 4 Veterinary Health Technicians and 8 animal care personnel, as well as 8 emergency response personnel.

RESULTS and DISCUSSION

The numbers of male and female dogs coming from 10 districts to Kahramanmaras Metropolitan Municipality Stray Animals Rehabilitation Center in 2021-2022 are given in Table 1.

When Table 1 is examined, it is seen that there is a difference between the number of animals brought to the Kahramanmaras Metropolitan Municipality Street animals rehabilitation center in 2021 and the number of animals brought in 2022.

When the officials working in the stray animal rehabilitation center were interviewed, they reported that this difference was due to the fact that there were no active dog collection teams in 10 of the 11 districts of Kahramanmaras in 2021, which are listed in Table 1, and that there were active collection teams in 2022.

Table1. Number of dogs brought to Kahramanmaras Metropolitan Municipality Stray Animals Rehabilitation Center between 2021-2022

DISTRICT	YEARS			
	2021		2022	
	FAMELE	MALE	FAMELE	MALE
Andırın	0		71	64
Afşin	62	61	145	143
Çağlayancerit	34	31	117	143
Dulkdairoğlu	318	297	381	359
Elbistan	154	144	273	235
Göksun	106	108	61	63
Nurhak	0		12	8
Onikişubat	309	241	362	317
Pazarcık	115	144	197	220
Türkoğlu	73	68	92	89
Total	1171	1094	1711	1641
	2265		3352	

The total number of dogs brought to the Stray Animals Rehabilitation Center of Kahramanmaras Metropolitan Municipality between the years 2021-2022 was 5617, approximately 2.46 times the number reported by Oğrak and Alici (2022) for the Sivas Municipality Shelter.

In the study, the diseases frequently encountered in 5617 dogs brought to the Stray Animals Rehabilitation Center between 2021-2022 are presented in a table 2.

When Table 2 is examined, it is seen that traumatic disorders come first among the diseases that are frequently encountered between the years 2021-2022. Post-traumatic behavioral disorder has become a concept that we hear frequently, especially after the earthquakes we have experienced recently. However, this concept was thought to be unique to humans, whereas the reality was not like that at all.

Many animals, especially dogs, are very emotional creatures. They may experience psychological problems associated with people, such as trauma and depression. Post-traumatic mood disorders are very common, especially in dogs.

Table 2. Diseases frequently encountered in dogs brought to Kahramanmaras Metropolitan Municipality Street Animal Rehabilitation Center between 2021-2022.

DISEASES	DOG	
	2021	2022
Tumoral Formations	(%0.61) 14	(%0.47) 16
Traumatic Disorders	(%5) 113	(%5,2) 174
Skin Diseases	(%3) 68	(%3,4) 114
Nervous Tissue Diseases	(%1,2) 27	(%1,9) 64
Eye Diseases	(%1) 23	(%1,1) 37
Ear Diseases	(%1,5) 136	(%1.3) 44
Sterilization	1094	1641
Ovariohysterectomy	1171	1711
Amputations	(%1.2) 27	(%1.8) 61
Shape Disorders	(%2) 45	(% 2.7) 91
General İnflammatory Cases	(%15) 340	(%19) 637
Eye Diseases	(%1.12) 26	(%1.7) 57
Ear Diseases	(%0,95) 22	(%0,78) 26
Foot Diseases	(%2,75) 63	(%2,83) 95
Prolapse Vagina	(%0.44) 10	(%0.51) 17

These traumatic disorders were grouped by Parlak et al., (2015) as traffic accidents, fights of animals, injuries with fire and sharp objects, falls, freezing, burning, all kinds of poisoning, and falling from high places. It is seen that the number of sterilizations in male dogs between 2021-2022 is 2735.

It is seen that the number of sterilizations applied in male dogs is higher than that reported by Oğrak and Ali (2022) for Sivas Municipality Shelter. Ovariohysterectomy operation applied in female animals is the process of removing the uterus and ovaries from the body by ligating and cutting in

accordance with the technique. This operation is an irreversible surgical intervention in the female dog. It is seen that the number of ovariohysterectomy operations is 2882 in total in the years 2021-2022.

It was found to be considerably higher than the number of Ovariohysterectomies reported by Oğrak and Ali (2022) for the Sivas shelter. It is thought that the number of veterinarians and veterinary health technicians is among the reasons for the high rate of sterilization and ovariohysterectomy procedures in male and female animals.

It is also thought to be due to the speed of manual dexterity of veterinarians. When Table 2 is examined, it is seen that the least encountered disease in Stray animals rehabilitation center in 2021 is prolapse vagina. However, when Table 2 is examined, it is seen that the least encountered case in 2022 is tumoral formations.

Vaginal prolapse is described in the literature as a phenomenon in which edematous vaginal tissue tends to the uterine lumen and often to the lips of the vulva (Özenç et al., 2016). When Table 2 is examined, it is seen that there is an increase in the number of animals brought to the Stray Animal Rehabilitation Center in 2022 compared to 2021. When the veterinarians in Kahramanmaras Metropolitan Municipality Stray Animal Rehabilitation Center were asked about the reasons for the increase in these numbers; They reported that the main reasons for the increase were the desire of dogs to come to the city center to feed themselves, as people could not leave their homes during the COVID-19 pandemic and people could not go to rural areas.

CONCLUSIONS

The data of this study show that; It is seen that the number of dogs brought to Kahramanmaras Metropolitan Municipality Stray Animals Rehabilitation Center is increasing every year. In this situation, the sterilization and treatment of stray animals coming from all districts of Kahramanmaras cannot be undertaken by the Metropolitan Municipality alone and the district municipalities should take part as a common stakeholder in these processes. In addition, in order to prevent the increase in these numbers, it is necessary to control the reproduction by neutering more male dogs and Ovariohysterectomy in female animals. With neutering processes, we can both take reproduction under control and protect stray animals against rabies, which has a very important place in the world.

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Morphometric trait-based body weight of south african bapedi breeding ewes

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Abstract

Morphometric traits help rural farmers who lack weighing equipment to anticipate the body weights of their animals for a variety of reasons, including feeding, medication, and breeding purposes. Regression analysis is often used in animal research to describe quantitative relationships between a response variable and one or more explanatory variables such as live body weight and morphometric traits. The study aimed to develop a model that can be employed to predict the live body weight from morphometric traits of breeding Bapedi sheep ewes using a regression method. The study was conducted in Limpopo Province of South Africa (AREC 46/2023: PG). A total of 102 Bapedi ewes were used as experimental animals to measure body weight (BW), body length (BL), heart girth (HG), sternum height (SH), head length (HL), withers height (WH) and rump height (RH). Pearson correlation and stepwise regression were used to analyze the data. Correlation findings displayed that BW had a highly positive remarkable association (P < 0.01) with HG (0.87) followed by WH (0.65). Regression results revealed that the model including HG, WH, RH, SH, and HL, was the best-fitted regression model (R2 = 0.79, RMSE = 3.72, AIC = 274.49, BIC = 287.66) for estimation of live body weight in Bapedi ewes. The study concludes that the improvement of HG, WH, RH, SH, and HL might enhance the body weight of the Bapedi sheep and HG can be used as the predictor of live body weight. This study´s outcomes may assist farmers in the selection of breeding stock and precision in day-to-day activities such as feeding, marketing, and medications.

Key words: *Best-Fitted Model, Correlation, Goodness of Fit, Heart Girth, Regression*

Effectiveness of enrichment the NAE broilers diets with yeast probiotics

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Abstract

NAE stands for "No Antibiotics Ever" and refers to a type of poultry diet and management practice in which chickens are raised without the use of any antibiotics from hatch to harvest. This practice is part of a broader trend in the poultry industry to meet consumer demand for antibiotic-free meat and to address concerns about antibiotic resistance. Instead of antibiotics, NAE programs rely on enhanced biosecurity measures, vaccination programs, and the use of probiotics, prebiotics, and other natural health-promoting additives to maintain bird health.
The study of the effectiveness and safety of the use of probiotic yeast strains was conducted on 5 broiler chickens. Blood, tissue, and droppings samples were taken from poultry for the analysis of biochemical, immunological, and productive indicators

Key words: *Probiotic, Yeast, Broiler, Biochemical parameters, Immune response,*

INTRODUCTION

The "No Antibiotics Ever" (NAE) initiative in poultry feed is a crucial issue today due to the increased consumer demand for antibiotic-free meat and the growing concern over antibiotic resistance. Antibiotic resistance, fueled by the overuse of antibiotics in animal agriculture, poses significant public health risks as it can lead to the development of resistant bacterial strains that affect humans (Van Boeckel et al., 2017). The NAE program aims to eliminate the use of antibiotics at any stage of poultry production, necessitating alternative methods to ensure bird health and growth.

Probiotics, including yeast probiotics such as *Saccharomyces cerevisiae*, have emerged as viable replacements for antibiotics. These probiotics enhance gut health, boost the immune system, and inhibit pathogenic bacteria, thereby maintaining bird health and performance without the need for antibiotics (Gaggìa et al., 2010; Allen et al., 2013). The implementation of yeast probiotics in poultry diets aligns with consumer expectations and helps mitigate the risks associated with antibiotic use in agriculture.

Yeast probiotics, particularly those from the *Saccharomyces* genus, offer several advantages over bacterial probiotics in animal nutrition. Yeast probiotics are more robust and can survive the harsh conditions of the gastrointestinal tract better than many bacterial probiotics, ensuring they reach the intestines alive where they exert their beneficial effects (Klemenčič et al., 2012). Additionally, yeast probiotics such as *Saccharomyces cerevisiae* have shown superior abilities in enhancing gut health and boosting immune responses due to their complex cell wall structure, which includes β-glucans, mannan-oligosaccharides, and other bioactive components that promote the growth of beneficial bacteria and inhibit pathogens (Chee et al., 2010).

Recent research has identified several new strains within the Saccharomyces genus, such as *Kluyveromyces marxianus,* that hold significant potential as probiotics. These strains exhibit strong probiotic properties, including the ability to survive gastrointestinal conditions, adhere to intestinal epithelial cells, and modulate the host's immune response (Fonseca et al., 2008). *Kluyveromyces* strains have shown

promising results in improving gut health and enhancing nutrient absorption due to their unique metabolic activities and bioactive compounds. Studies have demonstrated that these yeast strains can inhibit pathogenic bacteria and promote the growth of beneficial microbiota, thereby contributing to a healthier gut environment (Loureiro & Malfeito-Ferreira, 2003). The utilization of these new strains in animal nutrition could lead to more effective and sustainable probiotic formulations, reducing the reliance on antibiotics and improving overall animal health and performance.

MATERIALS AND METHODS

Animal Experimental Design.

A total of 14-day old Ross 308 chicks were divided (by similar weight) into 5 replicates (experimental units) of 15 birds each and assigned to one of the 5 experimental diets. Animals were raised for 49 days (d) according to the Experimental design and probiotic usage recommendation.

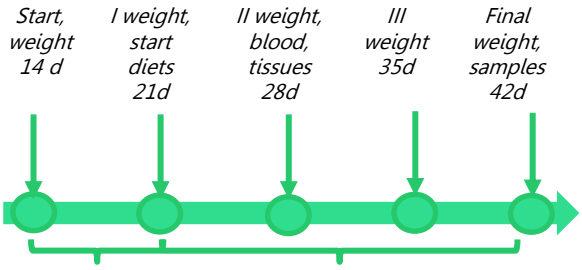
The experiment was carried out under the supervision of certified veterinarians. Chickens were vaccinated before dividing into groups at hatchery according standarts, then placed in floor pens with wood shavings as litter, supplemental heat in the first period, plastic waterer, and feed ad libitum according to the experimental groups.

Animals were fed the starter formula from d1 to d14 and grower from d21 to d28 and finisher formula from d28 to d49. The dietary treatments after the beginning of the experimental period reported in Table 1, as well as rations formulations.

Table 1. Groups differentiation by diets.

Groups	ALL DIETS WITHOUT ANY ANTIBIOTICS OR OTHER GROWTH PROMOTERS	
	14- 28 days old	28 - 49 days old
Control	Basic balanced diet– Grower (GBd)	Basic balanced diet – Finisher (FBd)
Experimental 1,2,3,4	Basic balanced diet - Grower (GBd) + 0,1% of probiotic	Basic balanced diet - Finisher (FBd) + 0,1% of probiotic

The health and vitality status of the subjects were evaluated twice daily and at the end of the trial, a post-mortem exam was performed on all animals. On days 15, 21, 28 and 40, all the birds were weighed, and average daily gain (ADG) was calculated. On days 28 and 49, five broilers from each replicate were sacrificed and samples of blood and tissues were collected for further analyses.



Experimental 1	*S. Cerevisiae - EnzActive*
Experimental 2	*Kl. marxianus - KM S1*
Experimental 3	*Kl. marxianus - KM S2*
Experimental 4	*Commercial Kl. marxianus - KM CS*

Figure 1. Trials scheme.

All procedures on animals were carried out in compliance with European Union regulations (EU Council. Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purposes, 1986. EU Commission).

Yeast strains origin

Yeasts *Saccharomyces cerevisiae* was grown at the facilities of Enzym Group, on a molasses medium in accordance with the developed technology regulations to produce this strain. were grown at the facilities of Enzym Group, on a molasses medium in accordance with the developed technological regulations for the production of this strain. After receiving the yeast cream, it was dried by special technology to obtain a final product capsulated in the outlier of inactive yeast cells. As a result, we received product EnzActive – live *Saccharomyces cerevisiae* with a yeast cell count ≥ $1.5*10^{10}$CFU/g.

Yeasts *Kluyveromyces marxianus* (KM S1 and KM S1), had been isolated, selected,

identified and grown in R&D center of Enzym Company. *Kluyveromyces marxianus* (B0399) is a commercial strain from the yeast market, were used to compare with Enzym's strains.

Blood sampling.

At days 14 and 21 of trials blood samples were collected in to tubes with EDTA anticoagulant tubes. Portions of each blood sample were immediately used for determining biochemical and immunological parameters. The remaining was centrifuged, and their plasma was collected and stored at −80°C for further enzymatic and chemical analyses.

Biochemical Parameters.

Malondialdehyde (MDA): The blood was centrifuged at 1,500 × g for 5 min; plasma was collected in labeled tubes and stored at −80°C until analysis. After thawing, 500 µL of plasma was placed in
a labeled glass tube and mixed with the reagents of a commercial kit for the measurement of thiobarbituric acid reactive substances (TBARS). Each tube was covered with a glass marble and
incubated at 95°C for 45 min. The tubes were removed from incubation and allowed to cool in an ice bath for 10 min. Once cooled, the tubes were centrifuged at 3000 × g for 10 min and the supernatant carefully removed from the tubes for analysis. The absorbance of the supernatants was measured at 532 nm using a UV/VIS spectrophotometer (Gildford Instrument Laboratories, Inc., Oberlin, OH) and the results were compared against a standard curve made with 100, 50, 25, 12.5, and 0 nmol/mL of malondialdehyde dimethyl acetyl.

Lipid hydroperoxides (LHP): Determination of the content of lipid hydroperoxides in biological material is achieved by precipitation of proteins with a solution of trichloroacetic acid and extraction of lipids with ethanol followed by the interaction of the studied extracts with ammonium thiocyanate (Vlizlo, V. V., 2012). Tissue collection and preparation of samples for extraction are carried out at t<4 °C. 0.2 ml of blood plasma, which contains 0.5 mg/ml of sodium oxalate in a buffer solution of pH 7.4, placed in a centrifuge tube with well-fixed cork, add 2.8 ml of ethanol and 0.05 ml of 50% THOK solution. The test tube is closed and shake for 5–6 min. A protein precipitate is formed isolated by centrifugation for 10 min at 3000 rpm. The obtained supernatant, which is an ethanol extract of lipids, is used as an object for determination of lipid hydroperoxides and total lipids, which contain polyunsaturated fatty acids.

Enzymes analysis.

Superoxiddismutase spectrophotometric assays based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals - measures the decrease in absorbance at 560 nm, reflecting SOD activity (Beauchamp and Fridovich, 1971).

Transferases: serum was separated by centrifugation at 6000 rpm for 1.30 minutes and serum analysis was carried out immediately. The serum Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) were measured using commercial kits (AST (AS101) and ALT (AL100) - Randox, United Kingdom) and a spectrophotometer. AST and ALT were measured at 546 nm while ALP measured at 405 nm.

In vitro trials

To study the potential probiotic properties of yeast strains, testing was conducted to assess:

- sensitivity to acidity,
- digestive tract conditions, and high temperatures,
- ability to grow on specific media and accumulate biomass and protein.

Simulation of the Gastrointestinal Tract

Methods and Materials

Composition of the stomach medium in flask №1: 0.2 g – Hydrochloric acid (HCl); 0.32 g – Pepsin from porcine gastric mucosa; 90 ml – Distilled water. The final pH ~2.5

Composition of the intestine medium in flask №2: 0.5 g – Bile salts; 0.1 g – Pancreatin (for biochemistry, powder); 100 ml – Distilled water. The final pH of the medium ~6.5

Preparation of OGYE nutrient medium: Dissolve 18.5 g Oxytetracycline - glucose yeast extract agar, brand OXOID (CM0545) in 500 ml distilled water and bring to a boil for complete dissolution. Sterilize in an autoclave at 121°C for 15 minutes. Cool to 50°C and under sterile conditions, add the content of 1 vial of oxytetracycline

supplement (SR0073A) or alternatively, 1 vial of chloramphenicol supplement (SR0078E). (Adjust pH if necessary). Mix thoroughly and pour into sterile Petri dishes.

Dried yeast at a quantity of 10 g was added to flask #1, simulating stomach conditions, at a ratio of 1:9. The flask was placed in an orbital shaker-incubator at 37°C and 110 revolutions per minute for two hours. Every 30 minutes, 1 ml of suspension was taken from the flask, serial dilutions were made, and plated on selective OGYE nutrient medium. After two hours and the final sampling point, the contents of flask #1 were transferred to flask #2, simulating small intestine conditions, and incubated for an additional four hours with sampling points taken every hour. Serial dilutions were made from all sampled points and plated on selective OGYE nutrient medium.

Determination of heat resistance.

This method is used to determine the CFU of yeast under the influence of temperature in both dry and dissolved forms.

Composition and Preparation of Nutrient Media: Peptone-Salt Solution (PSS) Composition: sodium chloride – 8.5 g, peptone – 1.0 g, distilled water – 1000 ml

Preparation method: Dissolve 8.5 g of sodium chloride and 1.0 g of peptone in 1 dm3 of distilled water with slow heating. Filter the obtained solution through filter paper if necessary, cool it down, adjust the pH to 7.0±0.1, dispense into containers, and sterilize at (121±1)°C for 30 minutes.

Yeast and Mold Agar Composition: Bacteriological agar – 15 g, yeast extract - 3 g, maltose extract – 3 g, enzymatic peptone - 5 g, glucose – 10 g, distilled water – up to 1000 ml

Preparation method: Add 36 g of the mixture to 1 dm3 of distilled water, stir, boil for 1 minute until the agar is completely melted, sterilize in an autoclave at (121±1)°C for 20 minutes. After cooling the medium to (45-50)°C, pour into sterile Petri dishes at 13-15 ml each.

Conducting the Determination. Prepare three samples of 10 g each. One sample is dissolved in 90 ml of PSS, while one remains in dry form. The flasks with dissolved yeast and dry samples are placed in a thermostat at 80°C for 20 minutes. The control sample is dissolved but not placed in the thermostat. After cooling, prepare appropriate tenfold dilutions (10-1 to 10-7). Inoculate 0.1 ml of the test sample from the corresponding dilution onto a Petri dish with nutrient medium. Spread the sample over the agar surface using a Drigalski spatula. Incubate at 25°C under anaerobic conditions for 48-72 hours.

Results Evaluation. Th dishes with 30-300 colonies and count the yeast colonies were selected, and results were converted to CFU per gram (average number of colonies × Dilution × 10 (conversion per ml) 114 × 107 × 10 = 1.14 × 1010 CFU/g of yeast sample).

Study of poultry feces

At the final stage of the research, fecal samples were collected to analyze the content of short chain fatty acids (SCFA).

Sample Collection: Fresh fecal samples from broilers at the same time each day to ensure consistency were collected.

Immediately stored samples in airtight containers to prevent contamination and volatilization of SCFAs.

Sample Preparation: We weighed a specific amount of the fecal sample (e.g., 1 g). Then added an appropriate volume of distilled water or buffer (e.g., 10 mL). and homogenized the mixture using a homogenizer. Centrifuged the homogenate at a high speed (e.g., 10,000 rpm) for a set period (e.g., 10 minutes) to separate the solid and liquid phases. After that we collected the supernatant for SCFA analysis.

SCFA Analysis

Gas Chromatography (GC): The samples were prepared by filtering the supernatant through a 0.45 µm filter to remove any remaining particulates. Derivatize the SCFAs if required by the GC method being used.

A measured volume of the sample (e.g., 1 µL) were injected into the GC system equipped with a flame ionization detector (FID) or mass spectrometry (MS) detector. We used a suitable column for SCFA separation, such as a capillary column with a polyethylene glycol (PEG) stationary phase. We identified and quantified SCFAs by comparing retention times and peak areas with those of known standards.

Data Analysis: The concentration of each SCFA in the samples was calculated by using the calibration curve obtained from standards. The results were expressed t as

µmol/g of feces or any other suitable unit. (Zhao et al., 2019; Wang et al., 2020).

Statistical analysis.

All data were analyzed by t-Test, using analysis when the overall P value of the experiment was below the value of significance ($P < 0.05$), t test was applied in order to assess the significance of results of single pairs of data.

RESULTS

In Vitro Trials

Growth abilities. The graph presents the growth results of three different yeast strains (*Kluyveromyces marxianus* KM S2, commercial *Kluyveromyces marxianus*, and the commercial probiotic strain of Saccharomyces cerevisiae produced by Enzym Group - EnzActive) cultivated in a Solaris M30 laboratory fermenter. Two key parameters were evaluated: the amount of active dry biomass (ADB) in g/L and the yield in percentage (Figure 2).

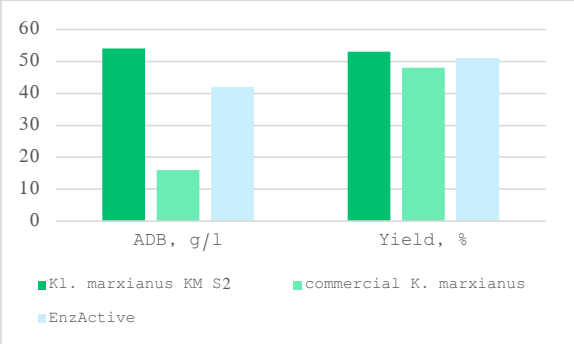


Figure 2. Growth of probiotic strains in R&D.

The *K. marxianus* KM S2 strain exhibited the highest productivity, generating approximately 50 g/L of active dry biomass. This indicates its high capability for growth and biomass production under the conditions provided by the Solaris M30 fermenter. Additionally, this strain had the highest yield, around 53%. The high yield signifies efficient substrate conversion into biomass, making KM S2 a promising candidate for further research and potential commercial applications.

In contrast, the commercial *K. marxianus* strain demonstrated significantly lower productivity, producing about 10 g/L of active dry biomass. Its yield was also considerably lower, approximately 45%. This indicates its lower efficiency in converting substrate into biomass, rendering it less attractive for use under similar conditions.

The EnzActive strain showed intermediate results, producing approximately 30 g/L of active dry biomass. The yield for this strain was around 51%, slightly lower than KM S2 but significantly higher than the commercial strain. This suggests that while EnzActive may be beneficial under certain conditions, *K. marxianus* KM S2 remains the most efficient option for achieving maximum productivity.

In conclusion, the cultivation of these strains in the Solaris M30 laboratory fermenter revealed that *K. marxianus* KM S2 is the most effective among the strains studied. Its high productivity and efficiency in laboratory conditions highlight its potential as a probiotic for further research and commercial use.

Thermostability

The *K. marxianus* KM S2 strain demonstrated the highest activity in dry yeast (4.25×10^{10} cfu/g) and the highest level of thermostability (3.1×10^{10} cfu/g). This strain showed a remarkable ability to withstand high temperatures without significant reduction in the number of viable cells, making it a promising candidate for probiotic applications and industrial use (Table 2).

Table 2. Thermostability of K. marxianus KM S2 compared to other strains

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Strains	Activity of dry yeast, cfu/g	Thermostability (85°C/20min), cfu/g
EnzActive (*S. cerevisiae*)	$2,7*10^{10}$	$1,3*10^{10}$
Kl. marxianus KM S1	$1,4*10^{10}$	$3*10^{5}$
Kl. marxianus KM S2	$4,25*10^{10}$	$3,1*10^{10}$
Commercial strain *K. marxianus* grown in R&D	$1,2*10^{9}$	$3,4*10^{6}$

EnzActive (*S. cerevisiae*) also exhibited high activity (2.7×10^{10} cfu/g) and thermostability (1.3×10^{10} cfu/g), confirming its suitability for high-temperature conditions, although it is surpassed by K. marxianus KM S2 in these parameters.

The *K. marxianus* KM S1 strain showed high activity (1.4×10^{10} cfu/g) but significantly

lower thermostability (3×10^5 cfu/g), limiting its applicability in high-temperature environments.

The commercial K. marxianus strain exhibited the lowest activity (1.2×10^9 cfu/g) and thermostability (3.4×10^6 cfu/g), indicating its lower efficiency compared to the other strains under conditions requiring high productivity and heat resistance.

Simulation of Gastrointestinal Tract Conditions

The strain *K. marxianus* KMS2 demonstrates significant positive characteristics under conditions simulating passage through the gastrointestinal tract. From the start of the experiment to the end of the six-hour period, KMS2 maintains stable viability, with several key aspects highlighted:

Resistance to Stomach Conditions

Stomach conditions are characterized by low pH, an aggressive environment for many microorganisms. KMS2 shows the ability to retain a substantial portion of its viability during the first two hours in the acidic stomach environment. The initial count is 1.4×10^{10} cfu/g, and even after two hours of exposure to the acidic medium, KMS2 retains 5.7×10^9 cfu/g. This indicates a high level of resistance, which is a crucial quality for probiotic strains, as the ability to survive stomach conditions is critical for reaching the intestines.

Viability in the Intestines

After passing through the stomach, KMS2 exhibits stable viability in the less acidic environment of the intestines. The counts decrease only slightly, from 5.7×10^9 cfu/g at the two-hour mark to 3.0×10^9 cfu/g after six hours. This ability to maintain viability in the intestines is a vital aspect, as it is in the intestines that probiotics exert their beneficial effects, including enhancing the microbiota and strengthening the immune system.

Overall, the strain *K. marxianus* KMS2 demonstrates significant positive qualities, making it a promising candidate for use as a probiotic, particularly given its ability to maintain viability under the challenging conditions of the gastrointestinal tract (Table 3) .

Table 3. *In vitro* testing the viability of probiotic strains under the conditions of the gastrointestinal tract

Time Points	Strains			GIT sections
	EnzActive	KMS1	KMS2	
Start	2,05*10^10	4.25*10^10	1,4*10^10	
30 min	2,0*10^10	5,5*10^9	5,8*10^9	Stomach
1 h	1,9*10^10	5,3*10^9	5,8*10^9	
1 h 30 min	1,8*10^10	5,0*10^9	5,75*10^9	
2 h	1,7*10^10	5,,0*10^9	5,7*10^9	
3 h	1,6*10^10	4,5*10^9	4,5*10^9	Intestine
4 h	1,55*10^10	4,0*10^9	4,5*10^9	
5 h	1,45*10^10	3,4*10^9	4*10^9	
6 h	1,4*10^10	3*10^9	3*10^9	

Overall Conclusion to the *in vitro* trials.

Based on the presented data, it can be concluded that the *K. marxianus* KM S2 strain possesses the best characteristics for use as a probiotic. Its high productivity, dry yeast activity, and excellent thermostability combined with good survival in the simulated gastrointestinal tract make it a promising candidate for further research and potential commercial application. These properties ensure its ability to effectively colonize the gut and maintain viability under conditions typical for the gastrointestinal tract and during the processing and storage of probiotic products.

In Vivo **Trials**

Studying ALT and AST activity in broilers' blood when feeding them probiotics is crucial for evaluating liver health and overall

metabolic status. ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) are enzymes that indicate liver function, and their activity levels can reveal potential liver damage or stress. In our studies, ALT activity on the 14th day of the experiment was within the normal range. However, on the 28th day, the ALT activity in the control group and the group fed a commercial probiotic from the market exceeded the upper allowable limit. In contrast, in broilers that received experimental probiotics in their diet, including the product EnzActive, the ALT level remained within the normal range (Figure 4).

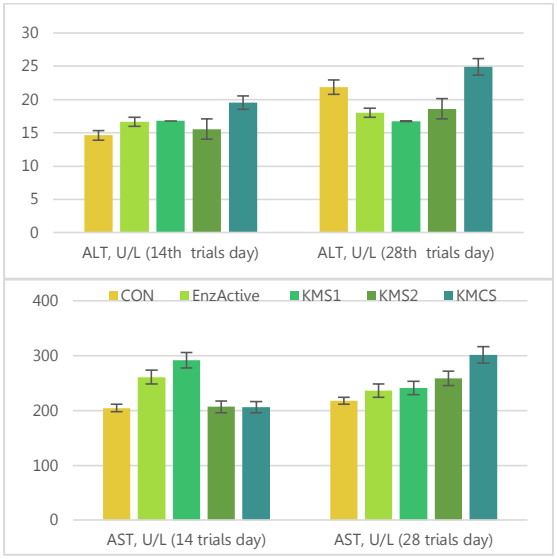

Figure 4. The activity of transferase enzymes in the serum of broilers during the experiment

As for AST activity, on the 28th day of the experiment, the enzyme activity in the blood of the birds fed EnzActive and KM S1 normalized. The activity slightly intensified in the blood of the broilers that received KM S2 probiotic, but the changes remained within physiological norms. But the last group showed excessive enzyme activity growth, which may indicate metabolic disturbances.

Probiotics are known to influence gut health and immune response, which can, in turn, impact liver function. Monitoring these enzymes helps in understanding the safety and efficacy of probiotics in poultry diets, ensuring they promote overall health without causing adverse effects. Research has shown that probiotics can modulate enzyme activity, highlighting their role in maintaining liver health in broilers (Swiatkiewicz et al., 2014, Mountzouris et al., 2010).

It is crucial to investigate changes in oxidative stress and antioxidant defense in the blood of broilers when studying the addition of probiotic yeast to their diets. Oxidative changes, provoked by the influence of various stress factors (including thermal), can cause significant cellular damage, leading to impaired growth, health issues, and reduced overall performance in broilers. Probiotic yeast has been shown to enhance antioxidant defense mechanisms, helping to neutralize harmful free radicals and protect cellular integrity. By understanding the impact of probiotic yeast on these parameters, we can optimize broiler diets to improve health outcomes, enhance growth performance, and ensure the sustainable and efficient production of poultry. The oxidative status can be assessed based on primary oxidation [i.e., through the measurement of lipid hydroperoxide (LHP) value] or secondary oxidation [i.e., through the measurement of malondialdehyde (MDA). Our research showed that by the end of the study, the level of lipid peroxidation products in the blood of broilers in the experimental groups was significantly lower than in the control group (Figure 5).

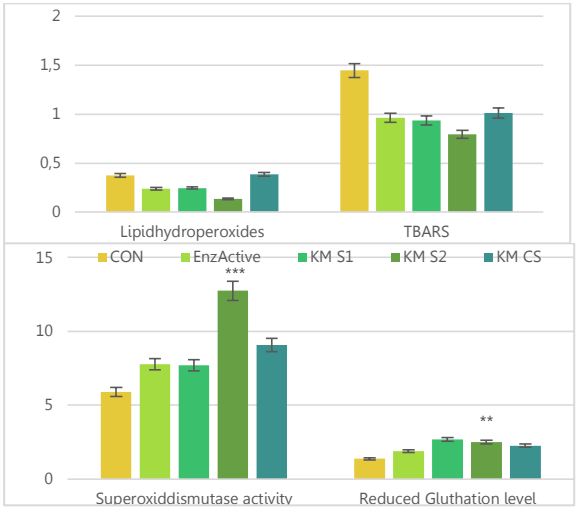

Figure 5. The level of lipid peroxidation products, the activity of superoxide dismutase, and the content of reduced glutathione in the blood of broilers
* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$ compared to the CON group

This effect was especially pronounced with the addition of the probiotic KM SC2 to the diet, which was our target strain in this research. Consequently, the activity of superoxide dismutase (SOD) was higher in the blood of broilers with probiotics in their diet, as was the content of reduced glutathione compared to the control. The highest SOD activity was recorded in the group that consumed the target probiotic in their diet.

Short-chain fatty acids (SCFAs) in the feces of broilers are critical parameters in evaluating the efficacy of probiotics in poultry trials. SCFAs, such as acetate, propionate, and butyrate, are key metabolic products of gut microbial fermentation and serve as indicators of a healthy and balanced gut microbiome. The presence and concentration of SCFAs reflect the probiotic's ability to enhance beneficial microbial activity, improve nutrient absorption, and support gut health. Higher SCFA levels often correlate with better gut integrity, reduced pathogen load, and overall improved growth performance in broilers. Thus, measuring SCFAs in feces provides a valuable metric for assessing the functional impact of probiotics on poultry health and productivity (Hassan et al., 2020).

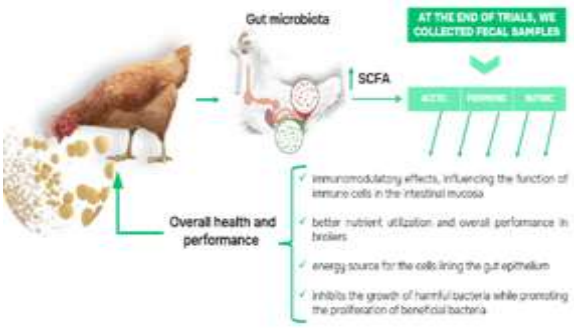

Figure 6. SCFA evaluation

A statistically significant increase in acetate and propionate levels was found in all experimental groups (Figure 7).

Generally, indicates several positive effects on gut health and overall broiler performance. Enhanced fermentation activity: Probiotics, enhance the fermentation of dietary fibers and other substrates in the gut. This produces short-chain fatty acids (SCFAs), including propionic acid and acetic acid, as metabolic byproducts. Higher levels of SCFAs like propionic and acetic acid can lower the pH in the intestines, creating an environment that is not favorable for pathogenic bacteria and more favorable for beneficial microbes. This helps maintain a healthy balance of gut microbiota. SCFAs are absorbed by the intestinal cells and used as an energy source, which can enhance the health and function of the gut lining. A healthier gut lining improves the absorption of nutrients, leading to better feed efficiency and growth performance in broilers.

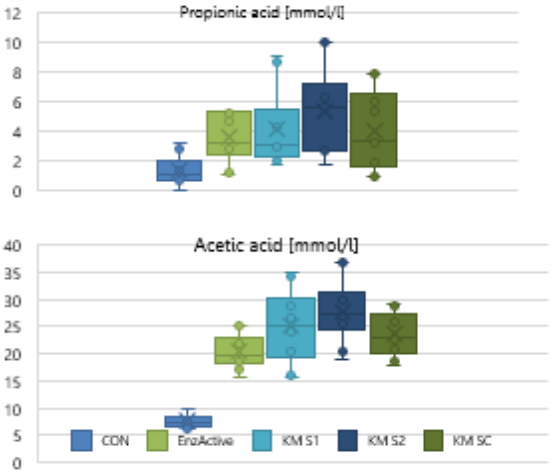

Figure 7. Propionic and Acetic acids content in the feces of broilers

A higher level of butyric and valerianic acids was detected in the feceses of birds fed the probiotic, especially in the group with *K.marxianus* isolated in R&D (Figure 7).

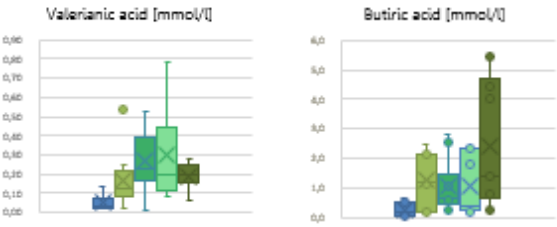

Figure 8. Butiric and Valerianic acids and total SCFA content in the feces of broilers

SCFAs have been shown to have immunomodulatory effects, enhancing the function of immune cells and promoting the production of antimicrobial peptides. This supports the overall immune system of broilers, making them more resistant to

infections and diseases. The presence of butyric and valeric acids lowers the gut pH, creating an environment that is less favorable for pathogenic bacteria. This helps maintain a healthy balance of gut microbiota, reducing the risk of infections. Butyric acid is an important energy source for the cells lining the gut, supporting their growth and function. This can lead to better nutrient absorption and overall improved growth performance in broilers. Enhanced Growth Performance: The combined effects of improved gut health, enhanced nutrient absorption, reduced inflammation, and better immune function contribute to overall better growth performance and feed efficiency in broilers.

In summary, the increase in SCFA levels when broilers are fed probiotics suggests that the probiotics are effectively enhancing gut fermentation, promoting a healthier gut environment, and contributing to better overall health and performance of the birds (Figure 8). The most interesting result for us showed *K.marxianus* S2 isolated in R&D.

DISCUSSION

In the context of using probiotic strains of the yeast *K. marxianus* in agricultural animals, it is important to highlight several key aspects that define their potential as probiotics. Research demonstrates that *K. marxianus* can significantly improve animal health and productivity by influencing gut microbiota, enhancing immune response, and combating pathogens.

For example, *K. marxianus* yeasts exhibit significant probiotic potential for improving the health and productivity of broilers. The addition of *K. marxianus* to the diet of chicks led to improvements in growth, nutrient digestion, and immune response modulation. Specifically, birds receiving a diet supplemented with *K. marxianus* showed increased body weight, improved feed conversion ratio, and strengthened immune system due to increased relative mass of immune organs and elevated activity of specific immunoglobulins (Lane & Morrissey, 2010). Recent studies have also shown that the addition of probiotic yeasts *K. marxianus* can significantly enhance the performance of broiler chicks. Specifically, one study revealed that including *K. marxianus* in the diet positively affected weight gain and improved feed conversion ratio in chicks. In this experiment, the use of different doses of *K. marxianus* (0.25-2.5 g/kg) contributed to increased average daily weight gain and reduced feed conversion ratio, indicating more efficient nutrient utilization (Bolla, et al., 2013).

Additional research has shown that the introduction of *K. marxianus* may also impact immune response improvement and intestinal tract structure. For instance, experiments demonstrated that the addition of *K. marxianus* leads to increased weight of immune organs such as the spleen and thymus, as well as improved intestinal villi structure, which may contribute to better nutrient absorption (Wang et al., 2018).

A study conducted in Korea showed that *K. marxianus* yeast, isolated from kefir, can survive in the gastrointestinal tract of animals better than other probiotic yeasts, such as *Saccharomyces boulardii* (Youn et al., 2022). The goal was to compare the survival and adaptation properties of different yeasts under the complex conditions of the gastrointestinal tract, including high acidity and the presence of bile acids. Among all the samples, *K. marxianus* yeasts demonstrated high cell surface hydrophobicity, allowing them to interact better with intestinal epithelial cells, which improves their adhesion and survival in the gastrointestinal tract. As in our studies, when comparing several strains, we established better viability and stability of *K. marxianus*, other authors also successfully prove this ability of the strains.

Probiotic strains were found to be effective in reducing liver injury and normalize the levels of AST and ALT activity (Hong et al., 2024).

There is enough evidence that broilers show inflammatory changes in the liver through their life. Some authors showed that the increased ALT and AST rate in the serum of the COBB 500 broiler could be the cause for general liver lesions. The increased level of AST might be lowered due to lessened growth rate caused by reduced food intake with increased temperature.

The increased AST/ALT ratio indicates that increased heat may cause chronic liver damage in fast growing broilers (*Dudley,*

R.F., 1982) and this might relate with the occurrence of sudden death syndrome (SDS) when compared with the enzyme levels of dead broilers from sudden death syndrome in other studies (Qujeq, & Aliakbarpour, 2005).

ALT enzyme is found in highest amount in liver and is used to identify acute liver failures (Orlewick, & Vovchuk, 2012) as the enzyme is released into the serum immediately after a hepatocellular damage.

The changes in the activity of both enzymes recorded in the blood of birds in the experimental groups that received probiotics produced by the Enzyme company are within physiological norms and do not indicate any protein metabolism disorders or liver and myocardium dysfunctions. In contrast, the enzyme response upon the addition of the commercial product obtained for comparative evaluation prompts consideration of the underlying cause of these changes, as the alterations in activity were significant.

The impact of yeast probiotics on ALT and AST activity in broilers is significant in assessing liver health and overall metabolic function. Studies have shown that incorporating yeast-based probiotics into broiler diets can positively influence liver enzyme levels, potentially reducing liver stress and enhancing metabolic efficiency. For instance, research by Zhang et al. (Zhang et al., 2020) demonstrated that broilers fed with yeast probiotics exhibited lower ALT and AST levels compared to control groups, indicating improved liver function and reduced tissue damage.

The results of our experiment prove that the addition of our target probiotics to the diet does not cause destructive or other pathological changes in the liver due to the normalization of the activity of transferases in the blood.

Oxidative metabolism is a normal process in all tissues. During the normal oxidative metabolic process, various reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced. During this normal metabolism, 1 to 2% of oxygen is converted to ROS. ROS are implicated in different disorders, including thermal injury, inflammation, sepsis, mutagenesis, carcinoma, autoimmune diseases and ischemia reperfusion injury. The role of ROS in the injury induced by ischemia reperfusion has been convincingly shown in different organs, including the brain, liver, skin, muscle, lung, intestine, kidneys and heart. However, under some circumstances, increased ROS/RNS production or decreased antioxidant defenses may lead to oxidative stress, in which case the generated reactive species can alter the properties of lipids, proteins and nucleic acids, leading to cellular dysfunction.

Probiotic yeasts in broiler diets can significantly influence oxidative stress parameters, including lipid peroxidation and superoxide dismutase (SOD) activity. By enhancing antioxidant defenses, probiotic yeasts help mitigate oxidative damage and support overall health. Research has shown that dietary supplementation with probiotic yeasts reduces lipid peroxidation products and increases SOD activity in broilers. This effect is attributed to the probiotic's ability to improve the antioxidant status of the birds, thus promoting better growth performance and health outcomes (Borges, J. T., & Borges, C. A., 2020; Gao et al., 2015). Aluwong, T with his team proved that administering yeast probiotic supplement increased body weight and enhanced serum antioxidant enzyme activities of broiler chickens. Antioxidant enzymes are most effective when acting synergistically with one another or with other components of the antioxidant barrier of the organism when their activity remains balanced. It has been shown that nutrition plays a vital role in maintaining the pro-oxidant-antioxidant balance (Cowey, 1986). In the present study, there was increased in both GSH content and SOD activity. The most importantly growth processes in early life are characterized with the generation of ROS through cellular division and apoptosis (Buetler, 2004). A similar study in turkey reported that mannanoligosaccharides a component of S. cerevisiae used as dietary additive stimulate the mechanisms of oxidative defense and improve the growth performance of the birds. Our research has corroborated numerous literature data on the positive effect of probiotic yeast on reducing the effects of oxidative stress in the poultry body. Moreover, the new strain *K.*

marxianus, isolated and grown in R&D caused a significant increase in antioxidant protection and a decrease in the accumulation of the final metabolites of peroxidation.

Short-chain fatty acids (SCFAs) in the feces of broilers are important indicators of gut health and microbial activity, particularly when yeast-based probiotics are added to their diet. The addition of yeast probiotics, such as Saccharomyces cerevisiae, has been shown to enhance the production of SCFAs, including acetate, propionate, and butyrate. These SCFAs play a crucial role in maintaining gut integrity, promoting beneficial microbial populations, and inhibiting the growth of pathogens. Increased SCFA production in the gut leads to improved nutrient absorption, better immune responses, and overall enhanced performance in broilers. Studies have consistently demonstrated that the inclusion of yeast probiotics in poultry diets results in higher SCFA concentrations in feces, reflecting a more efficient and healthier gut environment (Awad et al., 2009; Ding et al., 2019; Kim et al., 2020).

Our research demonstrates that the inclusion of probiotics in broiler diets, with the complete absence of antibiotics during the growing period, contributes to an increase in the levels of short-chain fatty acids in the feces. As mentioned above, SCFA play a crucial role in ensuring optimal digestion, immune response, pathogen protection, nutrient absorption, and high productivity levels.

CONCLUSIONS

Thus, the application of *K. marxianus* as a probiotic for broiler chicks presents a promising strategy for enhancing both productivity and health. These findings support the efficacy of *K. marxianus* integration into feeding programs, which could improve economic performance in broiler production and enhance disease resistance.

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Runs of Homozygosity Islands in Nili Ravi Buffalo

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Abstract

Inbreeding is a major challenge to enhancing the efficiency of breeding programs, especially where recording is difficult. Buffalo breeding in Pakistan is challenging and faces inbreeding depression effects, particularly on reproductive efficiency. In this study, we identified the distribution of runs of homozygosity (ROH) in 96 Nili Ravi buffaloes registered under the progeny testing program (PTP) in Punjab province. We identified putative genes and QTLs under selection. The mean length of ROH was 4.23 ± 1.88 MB. Signatures of selection were identified in six genomic regions located on chromosomes 1, 3, 6, 15, 16, and 18. A total of 201 genes and 180 QTLs were harbored on these chromosomes. Many genes are related to milk production (e.g., APRT and DGAT). Some genes are associated with reproduction and adaptability traits in buffalo. The proximity of genes linked with milk and reproductive traits suggests positive selection and might be included in buffalo breeding programs, especially under challenging climate change conditions.

Key words: *Runs of Homozygosity, Nili Ravi Buffalo, Inbreeding Depression, Climate Change Adaptation*

Effects of aqueous extracts of *Urtica Dioica L.* on bovine and ovine semen quality during cryopreservation

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Abstract

Aqueous plant extracts are recognized to enhance sperm function during cryopreservation. This study aimed to evaluate the effects of adding an aqueous extract of UrticaDioica L.(AED) to the preservation medium on the quality of refrigerated and frozen semen in bovine and ovine species. In ovine species, fresh semen from 15 adult rams of the Sarda breed was diluted in an egg yolk-based medium supplemented with 2% (G2) and 5% (G5) aqueous extract of nettle. Physiological parameters were evaluated using the CASA system, including motility, progressivity, and morphology, along with the hypo-osmotic swelling (HOS) test for membrane integrity. In bovine species, the effect of adding the same aqueous extracts was tested on the spermatozoa viability of Tarentaise bulls (n=3) after freezing-thawing, with membrane integrity assessed using the HOS test.
CASA system results for ovine frozen semen indicated that AED supplementation improved sperm motility, with values of 38.5±4.5 for G2 and 41.9±3.86 for G5, compared to 33±1.44 for the control. Sperm progressivity was also better in G5 with 26.9±3.6, compared to 20.9±2.7 for G2 and 20.4±1.6 for the control. HOS test results were similar in all groups. For bovine semen, supplementation with 2% and 5% of AED maintained, significantly, higher sperm viability at 3 hours post-thaw, with rates of 39.4±3.3 for G2 and 44.1±2.7 for G5, compared to 27.5±1.9 for the control. No significant effect was observed on sperm membrane integrity. These promising results need further trials to refine the optimal doses and determine the most effective extract type for enhancing the quality of refrigerated and frozen semen.

Key words: *cryopreservation, ovine semen, bovine semen, plant extract, Urtica Dioica.*

INTRODUCTION

Cryopreservation is the best tool to adapt animal species to global changes (reproductive accidents, epidemics) and to protect agricultural animal genetic resources. It also ensures the storage of genetics of endangered breeds or species (Woolliams et al., 2008).
Cryopreservation of sheep and bovine semen plays an important role in preserving genetic diversity, facilitating artificial insemination, and improving reproductive efficiency. Currently, there are mainly two methods for sperm preservation. Liquid storage at 5 °C or 15 °C and cryopreservation in liquid nitrogen at -196 °C, (Rahim A. et al. (2023). However, the freezing and thawing process of sperm cryopreservation can damage sperm and reduce fertility (Saramon et Maxwell 2000).
Cryoprotectants are essential for maintaining sperm quality during cryopreservation and reducing the deleterious effects of cold and osmotic shock (Huang et al., 2022). In an other way plant extracts are a natural and inexpensive source of additives used to preserve and improve sperm function during semen preservation (Ros-Santaella et Pintus, 2021) such as *Urtica Dioica L* . This study aims to provide the effects of using aqueous extract of *Urtica doica* as an additive in the cryoprotectant to preserve ram and bull spermatozoa during sperm freezing.

MATERIALS AND METHODS

The experimental work was carried out at the Laboratory " Appui à la durabilité des systèmes de production agricoles du Nord Ouest" in Tunisia and the laboratory of AGRIS (Regional Agency for Agricultural Research Sardaigne)

Preparation of plant extracts:

The aerial parts of the plant were collected from the second week of January, dried in the shade at room temperature and then ground to a fine particle size.

The plant extract was obtained by maceration of dried herbs ground to a fine powder in distilled water. To obtain a clear extraction, the mixture was centrifuged and then filtered. The filtered extracts were kept in the freezer until use.

Experimental design

a base medium containing Tris, citric acid, glucose, Milli Q water, penicillin, streptomycin, plant extract, glycerol and egg yolk. In ovine species, fresh semen from 15 adult rams of the Sarda breed was diluted in an egg yolk-based medium supplemented with 2% (G2) and 5% (G5) aqueous extract of nettle. Physiological parameters were evaluated using the CASA system, including motility, progressivity, and morphology, along with the hypo-osmotic swelling (HOS) test for membrane integrity. In bovine species, the effect of adding the same aqueous extracts was tested on the spermatozoa viability of Tarentaise bulls (n=3) after freezing-thawing, with membrane integrity assessed using the HOS test.

Statistical analysis

All collected data were statistically analyzed using the ANOVA model (Proc ANOVA) using SAS Institute Inc., with the following static model:

$Yij = \mu + level_i + \varepsilon_{ij}$

where: Y i j is the observation of the dependent variable, μ is the overall mean, level i is the effect of the doses of Urtica dioica, and εij is the residual experimental error associated with the observation.

RESULTS

CASA system results for ovine frozen semen indicated that AED supplementation improved sperm motility, with values of 38.5±4.5 for G2 and 41.9±3.86 for G5, compared to 33±1.44 for the control (figure1)

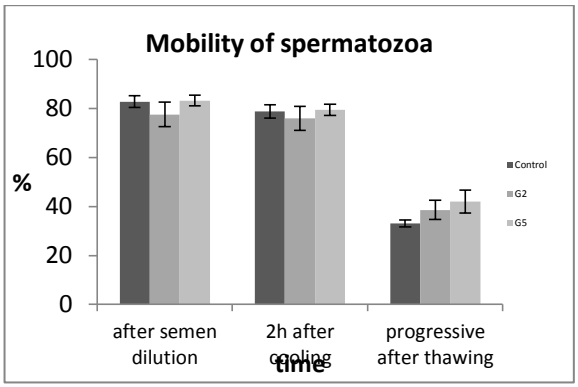


Figure 1. Effects of aqueous Urtica doica extract supplementation on the mobility of ovine spermatozoa after freezing (mean ± S.E.M).

Sperm progressivity was also better in G5 with 26.9±3.6, compared to 20.9±2.7 for G2 and 20.4±1.6 for the control (Figure 2).

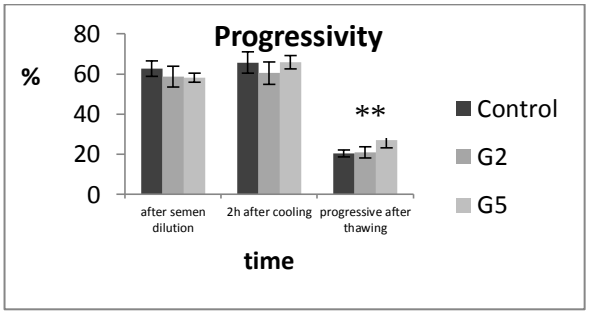


Figure 2. Effects of aqueous Urtica doica extract supplementation on the progressivity of ovine spermatozoa after freezing (mean ± S.E.M).

The HOS test results for the ovine semen, assessing sperm membrane integrity after semen thawing, were similar across all groups. also the rate of normal morphology is similar in the three batches of each species and does not differ significantly (table 1).

Table 1. Effects of aqueous Urtica doica extract supplementation on the membrane integrity and morphology of ovine semen after freezing (mean ± S.E.M).

	HOS+	*Normal morphology*
Control	*49.83 ± 3.15*	*87.68 ± 1.7*
G2	*38.83 ± 5.01*	*83.35 ± 5.71*
G5	*40.08 ±2.88*	*88.46 ± 3.15*
P	*ns*	*Ns*

G2: Group supplemented with 2% (aqueous extract of nettle
G5: Group supplemented with 5 % (aqueous extract of nettle
HOS+: % of endammeged spermatozoa

For bovine semen, supplementation with 2% and 5% of AED maintained, significantly, higher sperm viability at 3 hours post-thaw, with rates of 39.4±3.3 for G2 and 44.1±2.7 for G5, compared to 27.5±1.9 for the control (table 2).

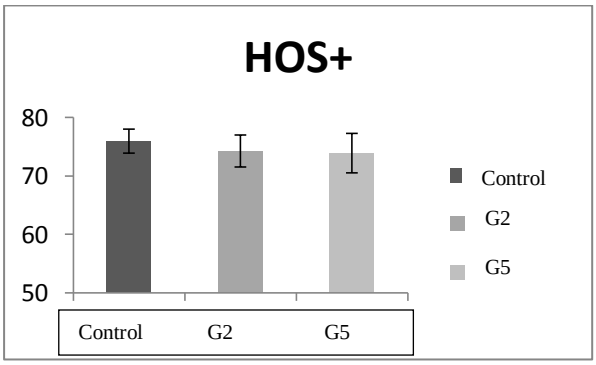
Table 2. Effect of aqueous Urtica doica extract supplementation on the viability of bovine spermatozoa after freezing (mean ± S.E.M).

	IM post-freezing	*IM 30mn*	*IM 60mn*	*IM 1h30*	*IM*	*IM 2h30*	*IM 3h*
control	*48 ± 1.4*	*47.03 ± 1.53*	*43.90 ± 1.42*	*38.4 ± 1.22*	*32.9 ± 0.91*	*30 ± 0.82*	*27.5 ±0.75*
G2	*59.6 ±1.4*	*57 ± 1.21*	*52 ± 1.3*	*50 ± 1.3*	*46 ± 1.10*	*41 ± 1.6*	*39 ± 1.5*
G5	*62 ± 0.85*	*60 ± 0.91*	*56 ± 0.98*	*53 ± 0.96*	*50 ± 1*	*45 ± 0.86*	*44 ±0.8*
P	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*	*<0.05*

G2: Group supplemented with 2% (aqueous extract of nettle
G5: Group supplemented with 5 % (aqueous extract of nettle IM: individual motility

The HOS test results for the ovine semen showed that there is no significant effect was observed on sperm membrane integrity (figure 3).

Figure 3. Effects of aqueous Urtica doica extract supplementation on the membrane integrity of bovine spermatozoa after freezing



DISCUSSION

The study suggests that AED (aqueous extract of Urtica dioica supplementation improves the quality of frozen ovine and bovine semen. Improved sperm motility in treated groups (38.5±4.5 for G2 and 41.9±3.86 for G5 in ovine) is a key indicator of post-thaw quality, as freezing-thawing often impairs motility due to membrane damage and free radicals (Aitken &Curry, 2011; Watson, 2000). Natural antioxidants in Urtica dioica extract can mitigate these effects by neutralizing free radicals and stabilizing cell membranes (Telo et al., 2017). The HOS test results were similar across all groups, indicating no adverse effect on membrane function. However, higher post-thaw sperm viability in bovine semen with 2% and 5% AED (39.4±3.3 for G2 and 44.1±2.7 for G5) suggests better resistance to oxidative stress and cryodamage (Yoon et al., 2016). The lack of a significant effect on membrane integrity suggests additional compounds or mechanisms are needed for improvements in this parameter (Pini et al., 2018).

CONCLUSIONS

Supplementation with AED shows potential in enhancing frozen semen quality in ovine and bovine species by improving motility and viability post-thaw. Further research is needed to refine dosages and explore synergistic effects with other additives for optimal reproductive outcomes in livestock.

ACKNOWLEDGEMENTS

We thank the AGRIS Research Center for Agriculture in Sardinia, Italy, and the Office of Livestock and Pasture in Sidi Thabet, Tunisia, for their invaluable support.

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Estimated breeding values of buffalo bulls- a progeny testing program

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Abstract

Estimated breeding values are commonly used in genetic evaluations and final selection of sires. A data set of lactation milk yields of buffaloes belonging various parities expanding over more than six decades were analysed to estimate breeding values of bulls to be selected as future sires for breed improvement program running under the control of Buffalo Research Institute in Punjab, Pakistan. The progeny data were employed for estimation of breeding values using animal model. Evaluated bulls were categorized as A, B, C grades and only A grade buffalo bulls were recommended to be used for breed improvement.

Key words: *Breed improvement, buffalo bulls, progeny testing program, estimated breeding values, genetic selection*

Myogenic regulatory genes involved in muscle development in farm animals

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Abstract

Farm animals, particularly poultry species, play a vital role in supplying global meat demand and are genetically modified to boost meat production efficiency. Improving muscle growth and development is critical to meeting customer demand for high-quality meat. Fetal skeletal muscle formation includes myogenesis, fibrogenesis, and adipogenesis. Myogenesis is governed by a complex network of intrinsic and extrinsic components, which are often separated into two or three stages and regulated by kinase-encoding and myogenic regulatory factor genes. The fundamental family of helix-loop-helix transcription factors, which includes MYF5, MYOD, myogenin, and MRF4, controls the specification and differentiation of skeletal muscle cells during embryogenesis and postnatal myogenesis. The discovery of the myogenic regulatory factor family, which comprises the transcription factors MYF5, MYOD, Myogenin, and MRF4, has allowed researchers to define the skeletal muscle lineage and better understand the regulation of myogenic differentiation during development. These factors also influence the muscle satellite cell lineage, which develops into the resident stem cell compartment in adult skeletal muscle. MYF5, MYOD, Myogenin, and MRF4 have small functions in mature muscle, but they play a crucial role in regulating satellite cell activity to regenerate skeletal muscle, connecting the genetic regulation of development and regeneration myogenesis. Understanding and identifying these genes is a critical step toward increasing meat yield and quality. This in-depth investigation looks at how myogenic regulatory variables influence satellite cell specification, maturation, and skeletal muscle regeneration.

Key words: *Meat, Myogenesis, Satellite cells, Myogenic differentiation, Regeneration, Skeletal muscle.*

INTRODUCTION

Every livestock breeder strives to produce animals with sufficient body weight and optimal nutritional requirements at the lowest possible cost. More importantly, with the growing world population and the need to fulfill increasing human protein requirements for various age groups, livestock breeds' high growth rate and muscle development have become critical traits for meat farmers and producers. According to some research, myogenic regulatory factors (MRFs) and growth promoters are critical for muscle differentiation, growth, and development in farm animals. It is also commonly acknowledged that these factors influence muscle growth in farm animals at both the embryonic and postnatal phases. As a result, it emphasizes the need of studying and comprehending muscle regulating variables.

MRFs include MRF4, Myogenic Determination Factor 1 (MYOD), Myogenic Factor 5 (MYF5), and Myogenin, also called Herculin or Myf6. These regulatory elements guide myogenesis, or the production of skeletal muscles, and they control the many stages of developmental skeletal muscle formation beginning with embryogenesis. Two of the four MRFs, Myogenic Determination Factor 1 (MYOD) and Myogenic Factor 5 (MYF5), regulate myogenic progenitor specification. MRF4 and Myogenin (MYOG) are expressed considerably later in embryonic development, yet they play an important role in defining and differentiating embryonic stem cells into committed myogenic cells. MYOG is the major determinant of myoblast development, whereas mature myocytes express MRF4 (Nabeshima et al., 1993).

In addition to these, Myostatin (MSTN) is an important growth factor. It is the most strong negative regulator of myogenesis, although it is also produced in adult

muscles, suggesting that it suppresses postnatal muscle growth (McPherron et al., 1997; Lee and McPherron, 2001; Amthor et al., 2004).

On the other hand, understanding the impact of these regulatory variables on skeletal muscle gene expression and its impact on meat quality and production, as well as future implications such as regenerative myogenesis, is critical for successfully changing these genes.

To control farm animals' myogenetic potentials, we need complete information about MRFs, which is why this publication exists.

MUSCLES AND THEIR GENESIS

The skeletal system, consisting of over 600 individual muscles, is the body's most vital tissue mass, playing a crucial role in movement and support. There are two primary types of striated muscle: skeletal and cardiac. Skeletal muscles are voluntary, innervated muscle cells that can experience fatigue and require substantial energy, whereas cardiac muscles are self-exciting, non-fatiguing cells with moderate energy needs. The organism's ability to actively control skeletal muscles sets them apart from cardiac and smooth muscles. Skeletal muscle is a key component of the muscular system due to its complex and heterogeneous nature (Bentzinger et al., 2012). In vertebrates, skeletal muscle is highly abundant and performs essential metabolic functions. The amount of lean skeletal muscle influences the body's metabolic rate (Mifflin et al., 1990; Nelson et al., 1992; Taguchi et al., 2011). It has been suggested that increasing muscle mass and energy expenditure through muscle protein oxidation may help prevent obesity (Wolfe, 2006). Additionally, skeletal muscle plays a significant role in maintaining overall insulin sensitivity by being the primary site for insulin-stimulated glucose uptake (DeFronzo et al., 1981). In farm animals, the development of skeletal muscle is crucial for producing tissue that meets human meat consumption needs. The formation of fetal skeletal muscle, derived from mesenchymal stem cells (MSCs), involves myogenesis (including myoblast proliferation, differentiation, and fusion), fibrogenesis, and adipogenesis (Du et al., 2010). Myogenesis is regulated by a complex network of intrinsic and extrinsic factors, often divided into multiple phases, and is controlled by genes encoding kinases. Meat quality can also be enhanced by promoting the commitment of MSCs to adipocyte formation, leading to increased intramuscular fat. The proliferation and differentiation of myoblasts, the progenitors of muscle cells, are critical for skeletal muscle formation. Growth promoters and myogenic regulatory factors (MRFs) are essential for muscle development in agricultural animals (Parakati & DiMario, 2013).

The position of muscle fiber types can influence muscle growth, an inherited trait particularly linked to metabolism, contraction rate, temperature, and food availability (Leatherland, 1994; Rehfeldt et al., 2011). Skeletal muscle has an extraordinary capacity to renew and rebuild itself in response to growth and injury by activating muscle stem cells, also known as satellite cells (Shi et al., 2006; Meadows et al., 2008).

Myogenesis, the intricate process by which skeletal muscles are developed across various species, including farm animals, primarily aims to produce multinucleated myofibers with contractile abilities. Different species require varying durations for each developmental stage (Knight & Kothary, 2011). During embryogenesis, the basic components and structure of skeletal muscle are established (Buckingham et al., 2014; Bentzinger et al., 2012; Tapscott, 2005). Early in pregnancy, the locations and characteristics of the cells that will form the three germ layers (ectoderm, mesoderm, and endoderm) are determined (Arnold & Robertson, 2009). The mesoderm, depending on its proximity to the midline/neural tube, is morphologically divided into paraxial, intermediate, and lateral mesoderm. The paraxial mesoderm, which forms during the tail bud stage of embryonic axis elongation and subsequently in the primitive streak/blastopore during gastrulation, is the origin of skeletal muscles. The presomitic mesoderm, located at the posterior end of the embryo, consists of the developing paraxial mesoderm. This temporary tissue is further divided into an

immature posterior region and a specialized anterior region, the latter of which segments to form somites. Skeletal myogenesis begins with the determination of premyogenic progenitors and skeletal myoblasts within these somites. Through multiple stages of proliferation and differentiation, mononuclear myocytes fuse to create multinucleated myofibers. Myogenesis is generally regulated by a complex network of internal and external signals (Bentzinger et al., 2012) and is controlled at various stages by MRF genes and those encoding protein kinases (Knight & Kothary, 2011). Nutrition also plays a crucial role in myogenesis. Both undernutrition and overnutrition during pregnancy can inhibit fetal myogenesis, but only overnutrition leads to increased intermuscular fat accumulation (Zhao et al., 2019; Berri et al., 2006). The earliest marker of myogenesis in mouse and chicken embryos is the activation of the myogenic factor MYF5 in cells located in the dorsomedial part of newly formed somites (Ott et al., 1991; Pownall & Emerson, 1992).

Research by Biressi et al. (2007) and Stockdale (1992) indicates that myogenesis occurs in two stages during development: the early embryonic or primary stage (E10.5–E12.5 in mice; E3–7 in chicken) and the later fetal or secondary stage (E14.5–17.5 in mice; E8+ in chicken). The initial myofibers are generated from PAX3+/PAX7+ progenitors in chickens or PAX3+/PAX3+ progenitors in mice (Horst et al., 2006; Hutcheson et al., 2009; Otto et al., 2006). These early myofibers contribute to the formation of early myotomes and limb muscles, which serve as precursors for adult muscles (Murphy & Kardon, 2011). Secondary myogenesis, which predominantly involves the fusion of cells and the addition of myonuclei from dividing PAX7+ progenitors, maintains muscle development (White et al., 2010). Postnatally, satellite cells support muscle growth by proliferating and fusing with pre-existing muscle fibers. In farm animals, muscle formation involves a series of biochemical processes, including protein deposition and muscle cell growth (Du et al., 2010). A small fraction of progenitor cells from the myotome proliferate before differentiating into myoblasts, which then exit the cell cycle to differentiate and fuse, forming primary myofibers and myotubes (Buckingham et al., 2014). Secondary muscle fibers arise from the proliferation and fusion of myoblasts near primary muscle fibers (Beermann et al., 1978). In adult animals, muscle development primarily occurs through secondary myogenesis. Satellite cells emerge when certain myogenic cells enter quiescence during the late fetal period. Consequently, the number of myoblasts affects both the number of muscle fibers and the number of satellite cells present during postnatal development (Zhao et al., 2019). Effective muscle growth in farm animals requires fetal myogenesis, as the number of muscle fibers typically does not change postnatally (Du et al., 2010). Postnatal muscle hypertrophy, or growth in muscle size, results from the differentiation and fusion of satellite cells with existing muscle fibers after their initial proliferation. In the absence of external stimuli such as injury or activity, satellite cells in mature animal muscles remain dormant. When muscle fibers are injured, activated satellite cells repair or replace them. However, some age-related diseases lead to a decline in satellite cells, impairing regeneration and contributing to muscle deterioration (Fukada, 2018).

REGULATORY FACTORS INVOLVED IN MYOGENESIS

Myogenesis is primarily regulated by a group of unique muscle-specific transcription factors, including the Myogenic Regulatory Factors (MRFs)—MYF5, MYOD, myogenin, and MRF4—as well as PAX7 and PAX3. These factors play crucial roles in the final stages of signaling, guiding the production of specific transcripts necessary for each step of muscle development. MYOD, a basic helix-loop-helix (bHLH) factor, was first identified in 1987 through advanced subtractive hybridization studies using myoblast cDNA libraries. These studies demonstrated that MYOD could convert various cell types, such as fibroblasts, into cells capable of fusing into myotubes. The discovery of MYOD and related proteins marked a significant advancement in understanding the molecular mechanisms governing the selection and differentiation of muscle progenitors. Following this, three

additional myogenic bHLH factors—MYF5, myogenin, and MRF4 (also known as Myf6)—were also found to induce myoblast characteristics in non-muscle cell lines (Braun et al., 1989; Edmondson & Olson, 1989; Rhodes & Konieczny, 1989; Braun et al., 1990; Miner & Wold, 1990). One of the most remarkable features of these factors is their ability, when ectopically expressed, to transform various cell types into myogenic lineages (Edmonson & Olson, 1993). Therefore, MRFs, which include MYOD, MYF5, myogenin, and MRF4, are highly conserved genes collectively expressed in the skeletal muscle lineage (Weintraub et al., 1991; Rudnicki & Jaenisch, 1995).

The expression of the four MRF genes begins early in development, coinciding with the commitment of cells to the myogenic lineage in somites and developing limbs, as documented by several studies. These proteins share a highly conserved basic helix-loop-helix (bHLH) domain. The helix-loop-helix motif, found in the promoters of many muscle-specific genes, is essential for heterodimerization with E proteins, facilitating the recognition of genomic E-boxes. Conversely, the basic domain of MRFs aids in DNA binding. The resulting heterodimer binds strongly to the CANNTG DNA motif, known as the E-box. According to Edmonson and Olson (1993), this binding is crucial for the transcriptional activation of genes containing the E-box motif. This motif is present in the promoters of many, though not all, skeletal muscle-specific genes.

MYF5 is the first MRF expressed during embryonic development, briefly upregulated in the paraxial mesoderm before collaborating with other MRFs to establish the myotome (Ott et al., 1991; Buckingham, 1992). The paired homeobox transcription factors PAX3 and PAX7 play a dominant role in the next level of genetic control over myogenesis. PAX7, in particular, is upregulated during myoblast differentiation but downregulated in proliferating myoblasts (Seale et al., 2000). In adult muscles, PAX7 is expressed in both quiescent and proliferating satellite cells (Zammit et al., 2004). Given that all vertebrates seem to possess at least one of these genes, it is suggested that these genes originated from the duplication of a common ancestral gene (Noll, 1993).

PAX3 and PAX7 are expressed in embryonic myogenic progenitors derived from the nuclear region of the somitic dermomyotome (Seale et al., 2000; Relaix et al.). During this stage of embryogenesis, MYF5 and MYOD are fully hierarchically induced, followed by myogenin, MRF4, and MYOD. These three myogenic factors are key regulators of myogenesis. Before the production of MYF5 and MYOD, PAX3 and PAX7 are first expressed in mesodermal cells (Buckingham, 2001). PAX3 promotes MYOD expression during skeletal myogenesis, which is critical for the formation of skeletal muscle. Additionally, PAX7 not only regulates MYF5 expression but also maintains satellite cells in a quiescent state and is necessary for the growth of activated myoblasts (Knight et al.; Ridgeway et al.). A subset of myogenic precursor cells, which do not express MRFs but still express PAX3 and PAX7, is believed to be the precursors of adult satellite cells.

ROLE OF MYOGENIC REGULATORY FACTORS (MRFS) IN MUSCLE DEVELOPMENT AND DIFFERENTIATION

MYF5, MYOD, and MRF4 Overlap in Myogenic Specification, while myogenin is Essential for Myogenic Differentiation. Myogenic differentiation is orchestrated by a hierarchical network of transcriptional regulators, each precisely controlled by key regulatory factors that function at specific developmental stages, both temporally and spatially (Buckingham et al., 2014). The natural gene regulatory mechanisms of a non-muscle cell can be altered to induce a myogenic-like phenotype through the ectopic expression of any of the myogenic regulatory factors (MRFs), which serve as master regulators of muscle development. However, during normal development, the expression, timing, and localization of these MRFs are finely tuned to ensure proper muscle formation. Studies of cultured myogenic cells reveal that the sequential activation of these bHLH myogenic regulators indicates distinct roles in the regulation of myogenesis. Quiescent satellite cells do not express MRFs; Myogenin and MRF4 transcripts are only elevated as cells

initiate differentiation, while MYOD and/or MYF5 are the first MRFs expressed in activated muscle satellite cells (Smith et al., 1994; Yablonka-Reuveni and Rivera, 1994; Cornelison and Wold, 1997).

During mouse embryogenesis, the four MRFs are expressed in specific spatiotemporal patterns (Currie and Ingham, 1998). MYF5 expression is first observed in the dorsomedial cells of the dermomyotome, which give rise to myogenic progenitors that will develop into epaxial muscles. The ventrolateral cells of the dermomyotome, which will form the hypaxial muscles, subsequently begin expressing the MYOD gene. Myogenin and MRF4 are crucial for the differentiation and development of muscle fibers (Rehfeldt et al.). MYOD and MYF5 play significant roles in the development of various muscle cell types, being expressed earlier than myogenin during myotome development. MYF5, MYOD, and MRF4 generally activate genes essential for muscle stem cell proliferation, and they are also critical for myoblast differentiation and fusion into myotubes. Myogenin, along with Myocyte Enhancer Factor 2 (MEF2), is necessary for myoblast differentiation, further promoting the formation of muscle-specific proteins (Shi et al., 2006). MYOD is particularly powerful in activating other MRFs, driving the production of muscle-specific proteins, especially in avian species (Pownall and Emerson).

Expression of MYF5 is notably lower in Wagyu × Angus cattle compared to Angus, with a higher rate of myoblast proliferation observed in in vitro cultures from Angus samples (Coles et al., 2015). Furthermore, the pectoralis major muscle in low-weight selected (LWS) chickens expressed more PAX3, MYOD, and MRF4 than in high-weight selected (HWS) chickens on the day of hatching. By day 28, PAX3, PAX7, MYF5, MYOD1, MYOG, and MRF4 expression was higher in HWS chickens (Yin et al., 2014). Similar findings were reported in Dzhalginsky Merino sheep, where MYOD1 showed the highest expression among 17 genes studied in the loin muscle (Trukhachev et al., 2016). In pigs and cattle, MYF5, another key regulator of myogenesis, has been linked to traits affecting meat quality (Ujan et al., 2011; Liu et al., 2008). Regulatory transcription factors like MYOD1 have been identified in specific muscles of purebred and hybrid pigs (Ayuso et al., 2016), indicating that MYOD1 is critical during both birth and growth, influencing muscle-building phenotypes.

Gene disruption studies in mice have provided insights into the roles of bHLH myogenic regulators. In the absence of both MYF5 and MYOD, MRF4 can only initiate limited myogenesis during embryonic development (Kassar-Duchossoy et al., 2004). When MYF5, MYOD, and MRF4 are all absent, there is a complete failure of myoblast differentiation and muscle formation (Rudnicki et al., 1993). These factors work together in partially redundant transcriptional networks to guide myoblast cell fate during development. While MRF4 and MYOD can support some differentiation during embryogenesis, myogenesis largely fails in Myogenin-null animals, with only a few differentiated myofibers forming (Hasty et al., 1993; Nabeshima et al., 1993; Venuti et al., 1995). Myogenin plays a unique role in fetal muscle development, while muscle growth in MYOD or MYF5 null mutants remains mostly normal. However, early muscle development is delayed in MYOD null embryos and trunk muscle development is delayed in MYF5 null embryos. The absence of skeletal myocytes or myofibers in MYOD and MYF5 double-null mice suggests that either MYF5 or MYOD is necessary for myoblast development or survival. The myogenin null mutation results in a significant reduction in skeletal muscle tissue due to interference with myogenic cell development. In contrast, targeted silencing of the MRF4 gene has minimal impact on muscle development, highlighting that MRF4 is not essential for the development or maintenance of differentiated skeletal muscle. However, MRF4/MYOD double mutants exhibit severe muscle deficiencies, comparable to those seen in Myogenin-null mice, suggesting that myogenin alone cannot sustain proper muscle development when MYOD is inactivated. These studies show that while MRFs have overlapping roles, each also has unique functions.

MRFs play roles in the intricate signaling pathways that initiate myogenesis but do not exclusively regulate the process. Protein

kinases, a family of enzymes essential for phosphorylation, play a crucial role in myogenesis, where their activation or inhibition can significantly affect muscle cell activity (Knight and Kothary, 2011). Protein kinase A (PKA) is particularly important for the formation of myogenic precursors in the dermomyotome at different stages of muscle development. PKA works alongside myogenic factors like PAX3, MYOD, and MYF5 to form myotomes (Chen et al., 2005). Wnt signaling, particularly Wnt1 and Wnt7a, from the dorsal neural tubes is also involved in this process. The Wnt/β-catenin pathway regulates myogenesis and adipogenesis (Du et al., 2010). PKA promotes myogenic factor production and increases cell proliferation by phosphorylating and inhibiting MEF2 (Knight and Kothary, 2011). Additionally, cell cycle progression is regulated by cyclin-dependent kinases (CDK2, 4), which phosphorylate retinoblastoma protein (Rb) to prevent its binding to E2F, allowing cell cycle progression and suppression of differentiation (Skapek et al., 1996; Gu et al., 1993). Growth factors like fibroblast growth factor (FGF) and insulin-like growth factor (IGF) activate extracellular signal-regulated kinase (ERK), essential for myoblast proliferation and differentiation during early myogenesis (Knight and Kothary, 2011). Akt1 promotes proliferation and inhibits cell cycle exit by phosphorylating FOXO1 (Nagata et al., 1998; Morooka et al., 1998; Bhat et al., 2007). These signaling elements, along with phosphorylated RNA polymerase II activated by MYOD and CDK9, work together to induce myogenic differentiation.

FUNCTION OF MYOGENIC REGULATORY FACTORS IN MATURE MUSCLE: MRF4 DOMINATES AS THE MAIN MRF IN ADULT MUSCLE

In postnatal muscle, satellite cells, identified by PAX3 and PAX7 proteins located beneath the basal lamina of adult myofibers, express PAX7 universally, though not all express PAX3 (Kassar-Duchossoy et al., 2005). Gene expression analysis in primary myoblasts and ChIP-seq studies on PAX7 and PAX3 have shown that PAX7 has a stronger affinity for homeodomain binding motifs than PAX3, although both recognize the same DNA sequences. PAX7 primarily activates genes involved in maintaining adult satellite cell phenotype, from proliferation regulation to differentiation inhibition. Conversely, PAX3 binds a subset of PAX7 target genes involved in embryonic functions and maintaining an undifferentiated state (Soleimani et al., 2012).

Research has focused on the regulation of MYF5 and MYOD expression in satellite cells and their influence on myogenic lineage commitment. Recent studies indicate that adult satellite cells do not express MYOD in a quiescent state, but lineage-tracing in MYOD-iCre mouse models shows that all satellite cell-derived progenitors express MYOD prenatally (Kanisicak et al., 2009). The histone methyltransferase complex Wdr5-Ash2l-Mll2 (Kmt2) is necessary for MYF5 expression, promoting its transcription through asymmetric muscle stem cell divisions (McKinnell et al., 2008). Satellite cells produce MYF5, but the transcript is sequestered in mRNP granules by miR31, keeping these cells in a quiescent state. Upon satellite cell activation, the separation of mRNP granules releases MYF5 mRNA, allowing rapid translation (Crist et al., 2012). The transcription factors FoxO3, Six1/4, PAX3, and PAX7 drive MYOD expression in proliferating myoblasts (Grifone et al., 2005; Hu et alend of fattening had a higher MyoG gene expression fold change than those with low body weight ($P<0.05$).

REGULATION OF MRFS BY SIGNALING MOLECULES

The expression of myogenic regulatory factors (MRFs) is modulated synergistically by a variety of signaling molecules released from the neural tube and adjacent tissues, which play a critical role in controlling vertebrate myogenesis during embryonic development. These molecules help in identifying myogenic progenitors within somites and driving their differentiation (Bryson-Richardson et al., 2008). Key signaling molecules involved in myogenic specification include Wnt proteins, Sonic hedgehog (Shh), the Notch receptor, and bone morphogenetic proteins (BMPs) (Bentzinger et al., 2012; Marcelle et al., 1997). Wnt proteins, a large family of glycoproteins, are crucial for early myogenesis in somites (Rudnicki et al.,

2015). Alongside Wnts, Shh, produced by the notochord and dorsal neural tube, plays a role in promoting myogenesis in somitic tissues (Münsterberg, 1995). Shh signaling is essential for maintaining the expression of MYF5 and MYOD in mouse limb buds during the formation of hypaxial muscles, with a notable deficiency in these muscles observed in Shh knockout animals (Krüger et al., 2001). Shh acts on the MYF5 enhancer to identify myogenic progenitor cells, with Gli proteins directly stimulating MYF5 expression through specific binding sites (Anderson et al., 2012). These observations indicate that Wnts and Shh collaboratively influence the myogenic potential of cells.

In contrast, BMPs and the Notch receptor inhibit MRF production, while Wnt and Shh proteins positively regulate the properties of myogenic progenitors (Hirsinger et al., 2001; Hirsinger et al., 1997; Schuster-Gossler et al., 2007). BMPs, which are part of the Transforming Growth Factor (TGF) superfamily, use serine-threonine kinase receptors to activate SMAD proteins, leading to their translocation into the nucleus where they can either activate or repress target genes (Hinck, 2012).

FUNCTİON OF MYOGENİC REGULATORY FACTORS İN SATELLİTE CELLS DURİNG REGENERATİVE MYOGENESİS

To evaluate the functionality of satellite cells in vivo, researchers use either acute or chronic regeneration models. Acute regeneration is typically studied through intramuscular injections of myotoxins such as cardiotoxin or notexin, or through methods like freezing or crushing, which are synchronous but more traumatic (Hardy et al., 2016). Chronic regeneration, on the other hand, is often assessed using models of muscle diseases that undergo repeated cycles of regeneration and degeneration, such as the mdx mouse model (Bulfield et al., 1984). Various signals, including hepatocyte growth factor, sphingolipids, and nitric oxide, can activate satellite cells (Comai et al., 2014; Dumont et al., 2015a; Dumont et al., 2015b).

In quiescent satellite cells, MYF5 mRNA is released from mRNP granules, facilitating rapid translation alongside MYF5 protein (Crist et al., 2012). The expression of MYOD and Myogenin can be detected in mononuclear cells shortly after DNA synthesis begins, typically 4–8 hours following an acute crush injury in mice. Myotube expression levels of these factors decrease after about 8 days, returning to baseline levels (Grounds et al., 1992; Rantanen et al., 1995). When muscle damage is combined with denervation, MYOD expression increases and persists longer, detectable as early as 12 hours in vivo, though it is only transiently present in some nuclei of newly formed myotubes (Koishi et al., 1995; Rantanen et al., 1995). Acute injuries, such as muscle excision followed by marcaine HCl immersion and regrafting, also induce MYOD expression in mononuclear cells and newly formed myotube nuclei (Fuchtbauer et al., 1992).

Myogenin appears in mononuclear cells around 12 hours post-injury and later in myotubes (Fuchtbauer et al., 1992; Rantanen et al., 1995). In mature muscle, MRF4 is localized in myonuclei and its expression increases following muscle injury (Zhou et al., 2001). Although MRF4 does not play a significant role in establishing the myogenic lineage during embryogenesis, it is expressed in adult muscle only after myoblasts have fused and matured into myotubes (Kassar-Duchossoy et al., 2004). MRF4 transcripts and protein are not present during the activation and proliferation of satellite cells or during early myogenic differentiation and fusion (Hinterberger et al., 1991; Zhou et al., 2001; Pavlath et al., 2003).

Growth factors such as transforming growth factor-beta (Vaidya et al., 1989; Heino et al., 1990), fibroblast growth factor (Vaidya et al., 1989; Brunetti et al., 1990), and insulin-like growth factor (Florini et al., 1991) have been implicated in myogenic determination and differentiation by modulating myogenic factor expression in in vitro systems. Although the precise mechanisms by which these factors regulate muscle growth are not fully understood, existing research indicates that innervation affects muscle development. For instance, denervation of neonatal chicken breast muscle prevents the transition of myofibrillar protein isoforms from neonatal to adult types (Obinata et al., 1984). Denervated adult muscle re-expresses

neonatal isoforms, including slow C-protein, muscle-type f-tropomyosin, and neonatal forms of troponin T (Obinata et al., 1984; Obinata et al., 1986). This suggests that variations in the expression patterns of myogenic factors may be crucial for muscle development from embryonic stages to adult fast or slow muscle types, with innervation potentially playing a critical role in regulating this process.

ROLE OF GROWTH FACTORS (GFS) İN SKELETAL MUSCLE GROWTH

Growth factors (GFs) significantly influence the differentiation and proliferation of skeletal muscle. Hepatocyte growth factor (HGF) has been found to enhance the surface elasticity of bovine satellite cells in vitro and to promote the proliferation and migration of myogenic cells (Lapin et al., 2013; Bandow et al., 2004). In poultry, the fibroblast growth factor FGF2 has been shown to inhibit muscle cell differentiation while promoting the proliferation of satellite cells and myoblasts, crucial for muscle precursor development (Velleman, 2007). Despite its role in stimulating proliferation, FGF2 can hinder myotube formation by suppressing myogenin transcription (Brunetti et al., 1990). Insulin-like growth factors (IGFs) are vital for regulating cell proliferation, differentiation, hypertrophy, and protein synthesis associated with myogenesis (Knight and Kothary, 2011; Kamanga-Sollo et al., 2003). Transforming growth factor (TGF) and myostatin (GDF-8) have contrasting effects on muscle differentiation, necessitating careful regulation of their expression in livestock used for meat production (Shahjahan, 2015). For instance, IGF-1 mRNA levels in chicken muscle decline during development, rise after hatching, and then decrease again after seven days post-hatching, with higher expression in embryonic muscle compared to embryonic liver (Wu et al., 2011). In pigs, IGF-I and IGF-II levels increase during satellite cell differentiation (Theil et al., 2006). Notably, IGF-II mRNA peaks at gestational day 85 in fetal sheep, underlining its importance in leg muscle fiber development during this phase (Fahey et al., 2005). Conversely, double-muscled Gerrard (DM) cattle exhibit delayed IGF-II expression but develop more muscle fibers due to a MSTN gene mutation (Gerrard et al., 1994). Growth hormone (GH), which is central to the GH-IGF axis, also plays a crucial role in influencing skeletal muscle development in farm animals through both genetic and environmental factors (Rehfeldt et al., 2011).

CONCLUSION

Future research should focus on understanding why adult skeletal muscle stem cells and various muscle lineages persist throughout embryonic development. There remain unresolved questions regarding the functions of myogenin and MRF4 in adult muscle and in muscle regeneration. In developmental biology, emerging single-cell techniques and lineage tracing studies are being utilized to reveal new mechanistic insights into upstream regulatory networks during embryogenesis and connect these findings with our current biochemical knowledge of muscle differentiation. Such advancements are crucial for developing effective treatments for skeletal muscle disorders, including muscular dystrophies and age-related regeneration issues. In agricultural settings, selection processes can enhance muscle growth, and identifying relevant candidate genes can further this improvement. Recent progress has significantly advanced our understanding of muscle growth and development, highlighting the roles of key regulators like transcription factors and growth factors (GFs). Identifying these regulators and genes aids in marker-assisted selection, which is vital for increasing meat yield and quality. Additionally, gene sets associated with muscle growth are valuable for applied research in mammalian muscle development. Nevertheless, further investigation is needed to fully comprehend the principles governing muscle growth and development in livestock species such as sheep and cattle.

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The use of regression models in method comparison

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Abstract

Type I and Type II regression analysis methods are used according to the assumption whether the variables in the regression model contain measurement error or not. Type I regression analysis causes the results to be obtained and interpreted at the desired level in some studies. Type II regression analysis methods are used when it is assumed that the observation values of the independent variables may be inaccurate and have measurement error. Method comparisons, which are frequently made in clinical studies, maintain their importance and various regression methods, especially Passing-Bablok and Deming, are used extensively. Bland-Altman analysis is used to determine the difference of measurement values between the two methods and Passing-Bablok analysis is used to determine the relationship between the methods. In this study, Passing-Bablok and Deming regression analyses from TYPE II regression models are focused on.

Key words: *Passing-Block Regression, Deming regression, Method Comparison, Type II Regression*

Application of genomic selection in species derived from fisheries of interest in aquaculture within RAS systems: The case of Seriola lalandi

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Abstract

The yellowtail kingfish, also known as Seriola lalandi, is a carnivorous fish that is mainly found in the Southern Hemisphere in tropical and temperate waters of the Pacific. Due to the high demand for yellowtail kingfish and the depletion of fishery quotas, commercial aquaculture production has been successfully implemented in countries like Australia, the Netherlands, Germany, and Denmark. To develop effective breeding programs, genomic selection is expected to play a crucial role in increasing profitability while minimizing inbreeding rates. However, genomic selection has been primarily implemented in well-structured populations like salmonids and less for other marine species. This is mainly due to the lack of genomic resources for many of these species and the challenges posed by natural spawnings, which make it difficult to account for genetic bottlenecks. In this study, we implemented genomic selection to improve the harvest weight of S. lalandi farmed in recirculating aquaculture systems in the north of Chile. We developed a reference genome, a genotyping array, and a marker genotyping by sequencing (GBS) panel for this species for evaluating different strategies when implementing genomic selection. Our results show that cost-effective genomic selection can be easily implemented in practice considering a low density GBS panel, without increasing costs over conventional breeding programs based on predicted paternity. Increases in accuracy can be as large as 20%, when compared with breeding values predicted on paternity data. These findings will provide guidelines for designing efficient and cost-effective breeding programs for yellowtail kingfish in RAS, considering the biological constraints of the species.

Key words: *Seriola genomics*

Longevity risk levels by fore udder attachment scores and its relationship with 305-day milk yield in Holstein cows

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Abstract

The study was carried out to determine longevity risk levels (LR_L) by fore udder attachment scores (FUA_S) and the associations of this trait with 305-day milk yield (305-dmy) in dairy cows. A total of 85 Holstein cows raised in Bafra district of Türkiye was constituted the study material. The cows were scored by FUA_S using a 1 to 9 scale and the data were evaluated in three risk groups (FUAs1-3= high risk, FUA4-6= moderate risk and FUAs7-9=without risk) by longevity. Before the analysis, all FUA_S values were converted to total longevity risk points in 100 p base (FUA_S1=100, FUA_S2-3= 75; FUA_S4-6=50; FUA_S7-8=25 and FUA_S9=0 p). To reveal the effects of non-genetic factors on LR_L, two stage of lactation (SL1= <100 d and SL2= ≥100 d), and three parity (P1=1, P2=2-3 and P4=≥4) and four calving season (CS1=winter; CS2=spring; CS3= summer and CS4=autumn) groups were divided. Effect of parity on LR_L were found as statistically significant (P<0.05). 305-dmy means for three LR_L groups were calculated as 4040.19 kg; 4130.12 kg and 4602.05 kg, respectively. The overall mean of LR_L was 44.41±1.471 p (FUA_S=5.82±0.134 p), and the correlation coefficient of LR_L with 305-dmy was r=-0.13.

Key words: *Dairy, Longevity, Milk yield, Udder traits, Type scoring*

INTRODUCTION

In any dairy farms, the length of the life of a milking cow has a major effect on economic actions. Traits of longevity, lifetime milk and obtaining healthy calf are the main goals of dairy owners. Such that, higher longevity minimizes the cost of herd replacements, raises the number of animals available for marketing, and elevates the ratio of the high-producing, mature animals in the herd (Effa et al., 2013). The selection for high-level milk production shortened the productive life of cows. Hu et al. (2021) emphasized that longevity is a comparatively difficult character to select for dairy cow breeding because of low heritability and numerous non-genetic factors of the longevity in dairy cows. For this reason, eliminating longevity risk factors is an essential action in animal selection process and dairy sector.

Sawa et al. (2013) informed the relationships of longevity and some body traits. The authors reported that dairy cows with well-attached fore udder, high attached rear udder, strong central ligament, close front teat placement, and with moderately long teats presented the longest productive life. Thusly, animal selection based on the type traits is a practice tool that supports the true decision on the longevity risks in dairy animals (Marinov et al., 2015).

Udder depth, udder cleft, teat placement and fore udder attachment are the most important traits to reveal longevity risk of a dairy cow (Stamschror et al., 2000). For instance, moderate amount of bulge to the fore udder is assumed to be favorable for a high yielding cow.

Finally, separately determination of the associations of the mentioned traits with milk production will give an important information to dairy farmers.

The aims of the present study were to reveal longevity risk levels (LR_L) by fore udder attachment scores (FUA_S) and to determine the relationship of this trait with 305-day milk yield (305-dmy) in Holstein cows.

MATERIALS AND METHODS

Holstein cows (n=85) raised in Bafra district of Türkiye were constituted as the study material. The cows were scored by FUA_S using a 1 to 9 scale (Figure 1) and the data were evaluated in three risk groups (FUA*1-*

3= high risk, FUA*4-6= moderate risk and* FUA*7-9=without risk*) according to risk guide for longevity that provided by Sahin (2011). Before the analysis, all FUAs values were converted to total longevity risk points in 100 p base (FUAs*1=100,* FUAs*2-3= 75;* FUAs*4-6=50;* FUAs*7-8=25 and* FUAs*9=0 p*).

To reveal the effects of non-genetic factors on LR_L; two stage of lactation (*SL1= <100 d and SL2= ≥100 d*), and three parity (*P1=1, P2=2-3 and P4=≥4*) and four calving season (*CS1=winter; CS2=spring; CS3= summer and CS4=autumn*) groups were divided.

The 305-dmy values of cows were used as the milk production trait.

To evaluate the associations of LR_L with 305-dmy, Kendall's tau-b correlation method was applied.

SPSS-17.0 windows program was performed for all statistical processes at the significance of 0.05 level.

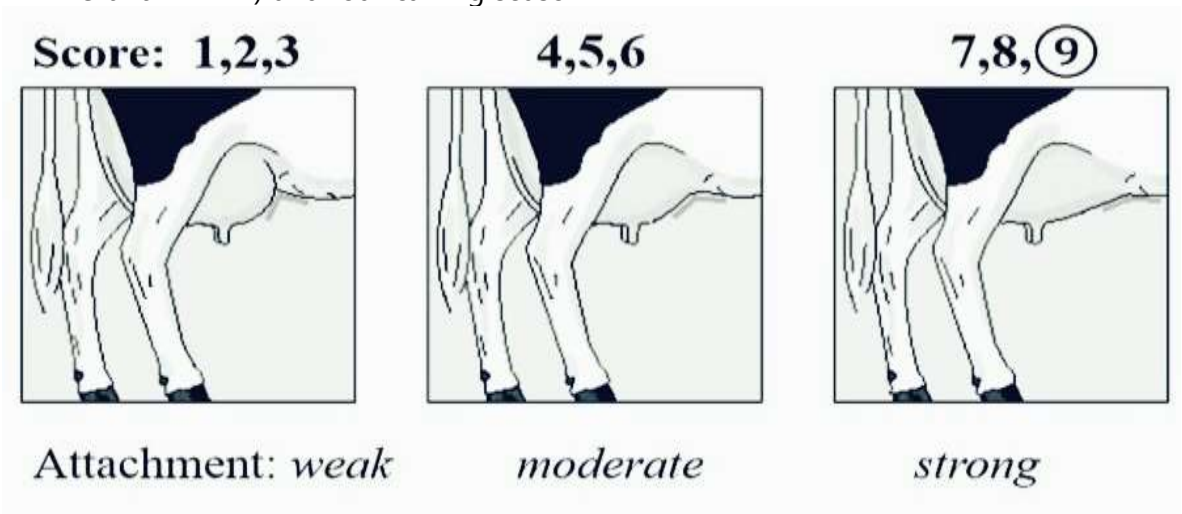

Figure 1. Longevity risk scale by FUA values (Sahin, 2011; ICAR, 2023)

RESULTS AND DISCUSSION

Effects of different non-genetic factors on LR_L were tested in the present study (Table 1). In normal conditions, early lactation stage may be expected as the a risky period for lactating cows because of this phase is referred as stressful for new-calved cows in terms of calving process. Additionally, new calved cows are highly exposed to peak milk production during this period. Thusly, udder type traits may adversely be affected by these cases. However, effect of SL was found as insignificant in the current study.

Adversely, LR_L means differed ($P<0.05$) by parity groups (Table 1). As seen, primiparous cows had the highest LR_L and this value was different from the mean of cows with second parity group. Effect of fully undeveloped physiologic structure of udder gland in the primiparous cows may be a major factor in this case. Also, gained experience for milk production with later lactations may be causative on the dropping the risk points in cows with 2nd or 3rd lactation. Fully developed udder glands in the advanced parities might show itself with moderate risk points those close to the overall mean of the herd.

Table 1. LR_L means (±SEM) by non-genetic factors

Factors	n	LR_L
Stage of lactation		
<100 d	36	42.36±1.946
≥100 d	49	45.91±2.106
Parity		
1	34	48.52±2.572[b]
2-3	22	38.63±2.716[a]
≥4	29	43.96±1.471[ab]
Calving season		
Winter	36	36.88±2.099[a]
Spring	23	45.65±2.020[ab]
Summer	13	55.76±5.027[b]
Autumn	13	46.15±2.603[ab]
Overall	85	44.41±1.471

[a,b]: P<0.05

In some initial studies, winter and summer have initially been reported as the stressful seasons (Duru, 2018; Liu et al., 2019; Aksu and Atasever, 2024). Similarly, CS was an affective factor, statistically (Table 1). As seen, the highest and the lowest LR_L means were obtained in the summer and winter CS, respectively. In other words, effect of high ambient temperature had adverse impact on FUA and thusly, LRL mean reached to relatively higher points in cows calved in this season.

Table 2. 305-dmy means (±SEM) by LR_L subgroups

Risk Level		305-dmy (kg)
High	22	4049.19±334.664
Moderate	53	4130.12±137.867
No	10	4602.05±248.614
Overall	85	4242.75±115.292

In this study, 305-dmy means according to LR_L subgroups are given in Table 2. Although no statistical difference was noted among the subgroups, higher than 0.5 ton milk yield in cows with high and no risk was found as attractive. Such that, this amount is equal to 50 tons higher milk in a herd with no risk that including 100 milking cows.

In this context, preventing LR_L in dairy herds can be seen one of the useful husbandry applications to collect more amount milk from the milking cows.

Associations of LR_L groups with 305-dmy is presented in Figure 2.

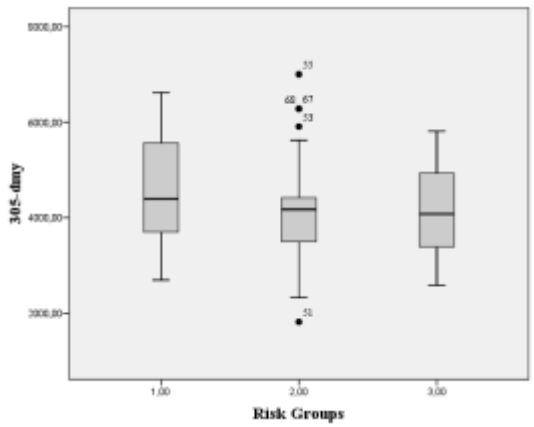

Figure 2. Interrelationships of LR_L groups with 305-dmy *(1=high risk; 2=moderate risk and 3=no risk)*

As seen, the major portion of the evaluated cows here had a moderate risk. However, the overall mean of 305-dmy of the cows was found as 4242.75±115.292 kg. This amount is lower than the results of some studies (Koç, 2006; Keskin and Boztepe, 2011; Karaağaç and Genç, 2019) those conducted in Türkiye conditions. Also, the overall mean of LR_L and FUAs were calculated as 44.41±1.471 p and 5.82±0.134 p, repectively. These values might be assumed in the moderate risk classes. Besides, the correlation coefficient of LR_L with 305-dmy was estimated to be negative and weak (*r=-0.13*).

CONCLUSIONS

In the view of the obtained findings of the present study, taking some severe measures to elevate milk production via reducing LRL may be seen as an important issue in the evaluated dairy farms.

Selecting cows according to FUAs and other udder type traits related to milk production may firstly be suggested.

ACKNOWLEDGEMENTS

The authors thank to the dairy farm owners for allowing the data collection for the current study.

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Effect of live yeast culture (Saccharomyces cerevisiae NCYC R-625 and NCYC R-732) added to the ration on fattening performance, milk yield and quality in dairy cattle

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Abstract

The study was carried out to investigate the effects of live yeast culture added to beef cattle and dairy cattle rations on the following parameters; feed conversion, daily body weight gain and carcass yield during fattening and milk yield and quality (protein, fat) in dairy cattle. In the fattening trial, 60 male Simmental cattle were divided into 3 groups and the research was designed in a random plots experimental design for the control and yeast-added groups. The concentrate feed containing 15% crude protein and 3000 kcal/kg ME was used as the basic ration in all groups. In trial group 1, 7.5 g/head live yeast culture (Saccharomyces cerevisiae NCYC R-625, 1 x 1010 CFU/g, Sorbitan monostrate) was added to the basic ration, and in trial group 2, 7.5 g/head live yeast culture (Saccharomyces cerevisiae NCYC R-732, 1 x 1010 CFU/g) was added to the basic ration. The total ration of both groups was balanced in terms of protein and energy. Fattening study, after 15 days of adaptation feeding, animals were fattened for 235 days and at the end of the period, animals were slaughtered. As a result of slaughtering, live weight at the end of 235 days of fattening, net carcass weight, carcass yield and daily live weight gain were 582±2,50 kg, 626±2,57 kg, 632±4,43 kg, 307±2,52 kg, 362±2,58 kg, 372±3,19 kg, 53%, 58%, 59%, 1,36±0,033 kg, 1,47±0,015 kg, 1,45±0,014 kg in control, R-625 experimental group and R-732 experimental group, respectively. The difference between the control and experimental groups in the parameters of end of fattening body weight, net carcass weight, carcass yield and daily body weight gain was found to be significant (P<0.001), while the difference between R-625 and R-732 experimental groups was statistically insignificant (P>0.05). In the study conducted in dairy cattle, 15 days of adapdation feeding was done and the trial lasted 90 days. At the end of the experiment period, average daily milk yield, milk protein content (%) and milk fat content (%) were 30.16 kg, 30.68 kg, 31.03 kg, 3.23±0.017, 3.31±0.013, 3.35±0.025, 3.56±0.078, 3.82±0.032 and 3.91±0.033 in control, R-625 group and R-732 group, respectively. According to the data obtained in the experiment, there was no statistical difference between the control and experimental groups in daily average milk yield (P>0.05). The difference between control and experimental groups in terms of milk protein and milk fat content was found statistically significant (P<0.05). The difference between R-625 and R-732 groups was statistically insignificant (P>0.05). As a result of the experiment, it was determined that the use of live yeast in cattle fattening had a positive effect on live weight gain, carcass yield and carcass yield. While there was no effect on daily milk yield in dairy animals, a significant difference was found in terms of milk fat and protein content.

Key words: *beef fattening, nutrition, carcas, yeasacc, Saccharomyces cerevisiae NCYC R-625*

Factors affecting growth during the grazing and development periods in central Anatolian Merino

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Abstract

Türkiye holds a significant position in the region and globally in small ruminant farming with a population of 56 million small ruminants (79.4% sheep, 20.6% goats). In sheep farming, there are different geographic and climatic conditions, with the breeding of 91.14% native sheep breeds and 8.86% Merino crossbreeds adapted to these conditions. This study aims to determine characteristics such as initial turnout weight, return-from-pasture weight, and average daily live weight gain in Central Anatolian Merino sheep raised in the vicinity of Ankara province, as well as to identify the effects of certain environmental factors on these traits. For this purpose, a linear mixed model was used to reveal the impact of factors, and the least squares means for the desired traits were calculated. Additionally, a multiple comparison test was applied to determine the differences between groups. In the study, the influence of factors on first time grazing weight (FTG), return period from grazing weight (RPG), and average daily weight gain (ADWG) was found to be significant for most factors. In the obtained results, the initial turnout weight, return-from-pasture weight, and average daily live weight gain are 22.77 kg, 34.01 kg, and 269.27 g, respectively. In the study, the time spent in the farm until turnout, the time spent on grazing, and the time spent from birth to grazing end are 65.99, 45.94, and 111.92 days, respectively. Additionally, the most significant proposal made in our study is to evaluate the heritability of these variables using full pedigree and to improve the breed genetically in addition to improving environmental conditions in order to analyse the grazing time attributes of Central Anatolian Merino sheep in more detail.

Key words: *Grazing performance, Environmental factors, Central Anatolian Merino*

Determination of the relationship between live weight and cellular properties of Pectoralis Major skeletal muscle in Peking duck

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Abstract

The DNA, RNA, and protein content form the fundamental genetic materials at the cellular level. The content of these molecules is critically essential for the functionality and growth of cells. Therefore, this study aims to determine the relationship between cellular characteristics such as protein, DNA, and RNA content, and protein/DNA, protein/RNA, and RNA/DNA ratios in the Pectoralis major (PM) muscles of Pekin Ducks with different slaughter weights. In this study, 60 Pekin duck chicks (30 female and 30 male) were fed for eight weeks. At the end of the feeding period, the live weights of female and male Pekin ducks were determined, and they were divided into two experimental groups, low and high, based on the average live weight within each gender. A total of 20 ducks (10 males and 10 females) from each experimental group were randomly selected and subjected to standard slaughter. Immediately after slaughter, the Pectoralis major (PM; breast muscle) muscle on the right side of the carcass was isolated and used as the study material. Genomic DNA was isolated from the muscle samples using the manual DNA isolation protocol. RNA was isolated using the FavorPrep Tissue Total RNA Purification Mini commercial kit from FAVORGEN, following the manufacturer's recommendations. The Bradford (Coomassie brilliant blue) method determined the total protein amount. The total amounts of DNA, RNA, and protein were determined using spectrophotometric methods. Total genomic DNA and protein amounts were similar among Peking ducks of different genders and slaughter weights. However, the total RNA amount of male and female Peking ducks with high slaughter weight was found to be higher than that of male and female Peking ducks with low slaughter weight ($P<0.05$). The protein/DNA ratio was higher in low-body-weight male Peking ducks than in low-body-weight female and high-body-weight male and female ducks ($P<0.05$). The protein/RNA ratio was higher in male and female Peking ducks with high slaughter weight than in male and female ducks with low slaughter weight ($P<0.05$). As a result, while slaughter weight does not affect the amount of DNA and protein in PM muscle, the high amount of RNA and RNA/DNA, Protein/DNA, and Protein/RNA ratios in ducks with high slaughter weight indicate that these ducks have more significant and more developed muscle mass than ducks with low slaughter weight.

Key words: *Pekin ducks, Pectoralis major muscle, Slaughter weight, DNA, RNA, Protein*

INTRODUCTION

Duck farming is quite common around the world. It is grown on a large scale, especially in the Asian and European continents. These animals are valued for their meat and eggs. Duck availability varies across countries and regions, but some regions, such as China, Vietnam, India, the USA, and European countries are prominent in duck breeding. Exact numbers are subject to constant change, but it is estimated that millions of ducks are raised worldwide. Duck production and consumption play an important role, especially in Asia, with limited but significant consumption in other regions as well.

In animal husbandry, live weight and body condition are important factors affecting the productivity of animals (Robinson, 1990). Animals try to maintain their live weight by using their bodies' fat and protein reserves against the environmental conditions they are exposed to during the production season and physiological effects such as growth and egg laying (Butler-Hogg, 1984). Cellular developmental features such as

protein, DNA, and RNA content and protein/DNA, protein/RNA, and RNA/DNA ratios in skeletal muscles are directly related to growth and development. Therefore, quantitative analysis of protein, DNA, and RNA in muscle tissue provides an excellent way to estimate farm animals' growth and development potential (Greenwood et al., 2006a; Greenwood et al., 2006b). In addition, the cellular properties of muscle tissue can be used as an essential indicator for estimating the number of muscle fibers in muscle tissue, the size of muscle fibers, and the transcriptional and translational capacities of muscle fibers (Greenwood et al., 2006a; Greenwood et al., 2006b; Kuran et al., 2012; Sen et al., 2021). Therefore, this study aims to determine the relationship between body weight and cellular characteristics such as protein, DNA, RNA content, protein/DNA, protein/RNA, and RNA/DNA ratios of the pectoralis major (PM) in the Pekin duck.

MATERIALS AND METHODS

The study was conducted at the Ondokuz Mayıs University Agricultural Faculty's Farm. The study used Pekin ducks, bought from a commercial hatchery, as animal material. All ducklings were transferred to a production house at daily age. Each animal was sexed from the cloaca, the wing numbers were attached to each individual. Ducks were reared with a feeding program to standard commercial practices. Ducks were fed a diet containing 22% crude protein (CP) and 2950 kcal/kg ME for the first four weeks and 16% CP and 3100 kcal/kg ME from the 5th to 10th weeks. Sawdust + straw were used as bedding material. The lighting schedule was 24 h lights (L): 0 h dark (D) during the first week, 22L: 2D for 2-4 weeks and 18L: 6D for 5-10 weeks. The temperature was between 28-35 °C during the first week and maintained between 20-32 °C from the 2nd week onwards [8]. Ducks were reared at a stocking density of 5 birds/m^2. In this study, 60 Pekin duck chicks (30 female and 30 male) were fed for eight weeks. At the end of the feeding period, the live weights of female and male Pekin ducks were determined, and they were divided into two experimental groups, low and high, based on the average live weight within each gender. A total of 20 ducks (10 males and 10 females) from each experimental group were randomly selected and subjected to standard slaughter.

At the end of the feeding period, all ducks were transported to an abattoir, and a standard commercial slaughter procedure was carried out. Immediately after slaughter, the Pectoralis major (PM; chest muscle) muscle on the right side of the carcass was isolated and cleaned of fat and connective tissues. Then, pieces of 5 (length) × 2 (width) × 2 (height) cm were taken from the PM muscle, frozen in liquid nitrogen, and stored at -80 °C until analysis. After removing all internal organs and abdominal fat, warm carcass weights were determined by adding PM muscle weight. The eviscerated carcasses were chilled for 24 h at four °C and reweighed to determine cold carcass weight.

Genomic DNA isolation in PM muscle samples was performed according to the DNA isolation protocol reported by Miller et al., (1988). In the study, RNA isolation was performed by a commercial RNA (PureLink ™, RNA Mini Kit, Invitrogen™, 12183018A) purification kit as suggested by the manufacturer. The purity and quantity of isolated DNA and RNA were measured by using NanoDrop™ 2000/2000c spectrophotometers (Thermo Fisher Scientific), and 1 % w/v agarose gel was used to check the DNA and RNA quality.

Total protein of muscle samples was isolated by radioimmunoprecipitation assay (RIPA) with some modifications (Valkova et al., 2005). Total protein in the extracts was determined in triplicate in suitable dilutions of both fractions by the method of Bradford (1976) using bovine serum albumin as a standard. The quality of proteins was checked in 12 % w/v SDS-PAGE gel electrophoresis

The data obtained at the end of the study were conducted using the Shapiro-Wilk test, Levene test, and one-way analysis of variance using the SPSS 20.0 version OMU license. According to the Shapiro-Wilk test results, it was determined that the data were suitable for normal distribution ($P<0.05$) and according to the Levene test results, the variances were homogeneous ($P<0.05$). One-way analysis of variance was used to compare the trial groups.

RESULTS

Live weight and carcass characteristics of male and female Peking ducks with low and high slaughter weights are presented in Table 1. In the study, significant differences were observed between trial groups and sex in terms of live weight at the end of fattening ($P<0.05$), and the same trend was detected in hot, cold carcass, and chilling loss ($P<0.05$). Male and female Peking ducks with low fattening body weight had lighter hot and cold carcass weights than male and female Peking ducks with high fattening body weight ($P<0.05$). However, no statistical difference was observed between male Peking ducks with low fattening body weight and female ducks with high fattening body weight regarding slaughter weight and hot and cold carcass weights. Interestingly, male Peking ducks with low body weight at the end of fattening had more chilling losses than females with low body weight and male and female Peking ducks with high live weight ($P<0.05$). While no statistical difference is observed between male and female Peking ducks with low and high slaughter weight in terms of hot and cold carcass yield, male Peking ducks with low live weight at the end of fattening have higher cooling yield than low live weight females and male and female Peking ducks with high live weight ($P<0.05$).

Table 1. Live weight and carcass characteristics of male and female Peking ducks with low and high slaughter weights

Traits	Sex	Groups	
		Low	High
Slaughter Weight (g)	M	4027.20 ± 59.81^{b}	4577.00 ± 134.48^{a}
	F	3317.60 ± 51.11^{c}	3820.00 ± 109.43^{b}
Hot Carcass Weight (g)	M	2824.90 ± 53.34^{b}	3170.30 ± 82.93^{a}
	F	2325.20 ± 59.76^{c}	2654.30 ± 70.99^{b}
Cold Carcass Weight (g)	M	2677.70 ± 45.80^{b}	3085.50 ± 71.85^{a}
	F	2255.60 ± 48.78^{c}	2569.92 ± 67.01^{b}
Chilling Loss (g)	M	147.20 ± 14.29^{a}	84.80 ± 17.49^{b}
	F	69.60 ± 12.39^{b}	84.38 ± 13.08^{b}
Hot Carcass Yield (%)	M	70.13 ± 0.52	69.29 ± 0.29
	F	70.07 ± 1.15	69.50 ± 0.34
Cold Carcass Yield (%)	M	66.49 ± 0.65	67.46 ± 0.53
	F	67.99 ± 0.97	67.30 ± 0.49
Yield Loss (%)	M	3.64 ± 0.31^{a}	1.83 ± 0.34^{b}
	F	2.08 ± 0.35^{b}	2.20 ± 0.34^{b}

^{a,b} Mean values with different superscripts in the same row and column indicate a significant difference ($p \leq 0.05$). M= male, F= female.

Total DNA, RNA, and protein amounts in the Pectoralis major (PM) muscle in the chest region of Peking ducks with low and high slaughter weights are presented in Table 2. In the study, the total genomic DNA and protein amounts in the PM muscle were similar between Peking ducks of different genders and slaughter weights. However, the total RNA amount in the PM muscle of male and female Peking ducks with high slaughter weight was found to be higher than that of male and female Peking ducks with low slaughter weight ($P<0.05$).

Table 2. Total DNA, RNA, and protein amounts in the Pectoralis major (PM) muscle in the chest region of Peking ducks with low and high slaughter weights

Traits	Sex	Groups	
		Low	High
DNA (µg/g)	M	309.80 ± 26.19	380.20 ± 46.49
	F	370.60 ± 61.79	418.20 ± 50.22
RNA (µg/g)	M	186.20 ± 12.49[b]	400.80 ± 35.01[a]
	F	220.20 ± 22.43[b]	357.40 ± 46.91[a]
Protein (g/100g)	M	24.81 ± 0.20	24.84 ± 0.06
	F	24.99 ± 0.04	25.00 ± 0.04

[a,b] Mean values with different superscripts in the same row indicate a significant difference (P<0.05). M= male, F= female.

RNA/DNA, Protein/DNA, and Protein/RNA ratios in the Pectoralis major (PM) muscle in the chest area of Peking ducks with low and high slaughter weights are presented in Table 3. The study observed no statistical difference between Peking ducks of different genders and slaughter weights regarding RNA/DNA ratio. However, it was determined that the protein/DNA ratio was higher in low-live-weight male Peking ducks than in low-live-weight females and high-live-weight male and female Peking ducks (P<0.05). The protein/RNA ratio was higher in high slaughter-weight male and female Peking ducks. It was determined that it was higher in male and female Peking ducks than in male and female Peking ducks with low slaughter weight (P<0.05).

Table 3. RNA/DNA, Protein/DNA, and Protein/RNA ratios in the Pectoralis major (PM) muscle in the chest area of Peking ducks with low and high slaughter weights

Traits	Sex	Groups	
		Low	Low
RNA/DNA	E	0.60±0.09	0.74±0.10
	D	0.59±0.08	0.73±0.11
Protein/DNA	E	80.08±9.67[a]	65.33±11.24[b]
	D	67.43±13.48[b]	59.78±11.63[b]
Protein/RNA	E	133.24±20.48[a]	88.46±14.48[b]
	D	113.49±18.54[a]	81.86±13.65[b]

[a,b] Mean values with different superscripts in the same row indicate a significant difference (P<0.05). M= male, F= female.

DISCUSSION

In this study, the live weight and carcass characteristics of male and female Peking ducks with low and high slaughter weights were determined, and these characteristics are essential in evaluating some of the production characteristics of male and female Peking ducks with different slaughter weights. When the carcass characteristics of male and female Peking ducks were evaluated, it was determined that male ducks had higher carcass weight than females. As in all farm animals, the slaughter weights of males in Peking ducks were higher than those of females. The results of our study are consistent with those of Omojola (2007). However, hot and cold carcass yields were similar for male and female ducks. Observations of male Peking duck carcass weight and yield in this study were higher than those reported by Omojola (2007) and Ahaotu and Agbasu (2015) for the same duck breed. However, cooling is one of the environmental factors that cause a significant increase in meat hardness and does not decrease with maturation (Mushi et al., 2008); in this study, male Peking ducks with low body weight at the end of fattening, females with low body weight and male and female Peking ducks with high live weight. They had more significant chilling loss than their ducks.

The current study examined the relationship between skeletal muscle development, molecular characteristics, and fattening performance of male and female Peking ducks with low and high slaughter weights. Skeletal muscle mass is an important parameter associated with the growth process of vertebrate animals. In the study, total genomic DNA and total protein amounts in the PM skeletal muscle of Peking ducks were similar in Peking ducks of different genders and slaughter weights. However, it was determined that the total RNA amount in the PM muscle of male and female Peking ducks with high slaughter weight was higher than that of male and female Peking ducks with low slaughter weight. Similarly, Greenwood et al. (2000) found that low birth-weight lambs had lower amounts of RNA in their skeletal muscles. In the study, although the amounts of DNA and total protein in the PM muscle were similar in body weight groups and genders, the amount of RNA was higher in male and female Peking ducks with high live weight, which may be a cellular indicator of the weight difference. Although genome content remains constant, more giant cells produce and maintain higher concentrations of RNA to maintain biomass and functions (Marguerat and Bahler, 2012). Therefore, the current study's findings suggest that cellular processes essential for the growth and development of muscle mass may be less in samples with low slaughter weight.

RNA/DNA and protein/DNA ratios differ as a function of muscle cell size (Carpenter et al., 1996). Therefore, cellular properties of muscle tissue, such as transcriptional and translational capacity, indicate muscle fiber size, which is essential for growth and development (Greenwood et al., 2000; Greenwood et al., 2006a). The current study determined that the RNA/DNA ratio in PM skeletal muscle was higher in ducks with high slaughter weight than in ducks with low slaughter weight. The RNA/DNA ratio indicates cellular activity and higher ratios may mean faster growth and cell division (Marguerat and Bahler, 2012). This finding indicates that the skeletal muscles of ducks with high slaughter weight grow and develop faster. However, no statistically significant difference was detected between ducks of different gender and slaughter weights in terms of DNA, RNA, protein amounts, and RNA/DNA ratios in the PM skeletal muscle. However, it was determined that the protein/DNA ratio was higher in male Peking ducks with low live weight at the end of fattening than in females with low live weight and male and female Peking ducks with high slaughter weight. The protein/RNA ratio was higher in male and female Peking ducks with high slaughter weight than in male and female Peking ducks with low slaughter weight.

CONCLUSIONS

In conclusion, this study revealed that Peking ducks with higher slaughter weights had better skeletal muscle development and fattening performance. While slaughter weight does not affect the amount of DNA and protein in PM skeletal muscle, the high RNA amount and RNA/DNA, Protein/DNA, and Protein/RNA ratios in ducks with high slaughter weight show that these ducks have more significant and more developed muscle mass than ducks with low slaughter weight.

ACKNOWLEDGEMENTS

This study was supported within the scope of TUBITAK 2209/A University Students Research Projects Support Program.

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The effect of external egg quality traits on hatching results in geese

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Abstract

*The aim of this study was to determine the effect of egg weight, shape index, egg shell opacity and shell thickness, which are egg external quality characteristics, on hatching results in native geese reared in Yozgat province, Türkiye. In the study, a total of 400 eggs were collected from parent geese at the age of 44 weeks during 3 weeks. The external quality characteristics of the collected eggs were determined and incubation was carried out. After incubation, weight and quality characteristics were determined. The study was evaluated according to the random plots experimental design. Data were subjected to variance analysis using one-way ANOVA procedure. Discrete data were analysed using the Generalized Linear Model procedure with binomial logit-link function for two levels and multinomial cumulative logit-link function for more than two levels. Higher egg width and length, egg weight, length, shank length and diameter were determined in the heavy egg group obtained from local genotype geese. In addition, a positive phenotypic correlation was determined between egg weight and body weight, length, shank diameter and length. Eggs with an egg shape index of less than 65% had a higher gosling weight. Hatching egg weight loss was higher in eggs with shell thickness less than 55 mm. There was a low level of positive phenotypic correlation between shell opacity and hatchability. The data obtained as a result of the study provided important information about egg quality characteristics, hatching results and gosling quality characteristics of native geese in Yozgat Region. * This work has been supported by Yozgat Bozok University Scientific Research Projects Coordination Unit under grant number "FYL-2024-1428".*

Key words: *Geese, Egg, External quality, Internal quality, Hatchability*

Determination of the relationship between expression levels of MyoD and MyoG myomarker genes and body weight in Peking ducks

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Abstract

MyoD and MyoG genes, essential biomarkers, are the primary regulators of muscle mass in the embryonic and fetal periods and are among the genes that regulate the differentiation and subsequent development of skeletal muscle cells. Expression levels of MyoD and MyoG genes can be used as a molecular technique that can help to determine the fattening potential of Pekin duck kids, which are widely cultivated in duck meat production. Therefore, this study aimed to determine the expression levels of MyoD and MyoG genes in the pectoral major (PM) skeletal muscles of the Pekin ducks with different slaughter weights. Total RNA was isolated from muscle samples using a commercial RNA extraction kit as recommended by the manufacturer. The isolated RNA was converted into cDNA using a commercial cDNA kit in a Thermal Cycler. MyoD and MyoG gene expression levels in PM muscle were determined by real-time quantitative polymerase chain reaction. While there were no differences in terms of MyoD gene expression fold change between low and high-body-weight male Peking ducks, the MyoD gene expression fold change was more significant in high-body-weight female Peking ducks than in low-body-weight female ducks (P<0.05). The MyoD gene expression folds change of low and high body weight of female Peking ducks was lower than that of male Peking ducks with low and high body weight (P<0.05). In both male and female Peking ducks, MyoG gene expression fold change was higher in ducks with high body weight than in ducks with low body weight (P<0.05). In addition, while the MyoG gene expression fold change of female Peking ducks with low weight was lower than that of male Peking ducks, the MyoG gene expression fold change of female Peking ducks with high body weight was higher than that of male Peking ducks (P<0.05). The study results showed that the difference in live weight at the end of fattening in Peking ducks may be due to the difference in the expression level of the MyoD and MyoG genes, and there may be a positive relationship between fattening performances.

Key words: *Pekin ducks, Pectoralis major muscle, Slaughter weight, DNA, RNA, Protein*

INTRODUCTION

As with other poultry, ducks were initially classified according to their morphological structure. However, over time, it became clear that it was more appropriate to classify them according to their productivity characteristics. Considering their productivity characteristics, it is possible to separate some world-renowned duck breeds according to meat, egg, and feather yield. However, it should not be forgotten that new breeds continue to be developed depending on differences in geographical regions and local needs. A similar situation exists in Turkey, and the creation of new breeds by taking into account the geographical conditions of the area and the economic efficiency levels of the enterprises will make duck breeding more effective and sustainable.

Skeletal muscle development is controlled by myogenic regulatory factors (MRFs), a family of muscle-specific basic helix-loop-helix (bHLH) transcription factors. Skeletal muscle differentiation occurs when MRF genes of the MyoD family are activated in muscle progenitors, and this genetic program operates in both the trunk and head regions. MRFs: myogenic factor 5 (Myf5), myogenic differentiation (MyoD), myogenic regulatory factor 6 (Mrf4), and myogenin are essential components of the

myogenic pathway (Zhong et al., 2013). The main functions of myogenic regulatory factors are as follows;

The MyoD gene is responsible for the growth and proliferation phase of the early formation process of muscle fibers (Braun and Arnold, 1995). The MyoG gene regulates postnatal muscle maturation and differentiation (Braun and Arnold, 1995). The Mfy5 gene is considered the first expressed MDF and is controlled by a 140 kb enhancer complex in its regulatory region (Carvajal et al. 2008). The Myf5 gene is mainly involved in the physiological process of muscle fiber elongation and development. The Myf6 gene is responsible for postnatal muscle maturation and differentiation. It has been shown that Mfy6 can also be effective in the growth and proliferation phase of muscle fibers during their early formation (Kassar-Duchossoy et al. 2004).

By changing the skeletal muscle development profiles (muscle fiber type, diameter, intramuscular fat ratio) of the animals to be fattened by gene expression organizations in farm animals, the feed conversion ratio can be affected for higher amounts of meat production (Kassar-Duchossoy et al., 2004). In addition, determining the cellular or molecular characteristics in the skeletal muscle tissue of farm animals to be used in meat production is of great importance in predetermining the meat production potential of fattening animals, increasing meat production, reducing production costs, and reducing the price.

Peking ducks exhibit rapid growth and high meat yield, and only phenotypic values were taken into account in studies on this breed's development, meat yield, and fattening performance. The underlying mechanism of the differences observed in the studies has not been fully revealed and has been attributed to environmental factors.

Due to all these facts, in the current study, we aimed to determine the expression levels of MyoD and MyoG genes, which serve as the primary regulators of skeletal muscle myogenesis (formation of muscle tissue) in the pectoralis major (PM) skeletal muscles of Peking ducks of different slaughter weights, and to determine the gene expression level and live results at the end of fattening.

MATERIALS AND METHODS

The study was conducted at the Ondokuz Mayıs University Agricultural Faculty's Farm. The study used Pekin ducks, bought from a commercial hatchery, as animal material. All ducklings were transferred to a production house at daily age. Each animal was sexed from the cloaca, the wing numbers were attached to each individual. Ducks were reared with a feeding program to standard commercial practices. Ducks were fed a diet containing 22% crude protein (CP) and 2950 kcal/kg ME for the first four weeks and 16% CP and 3100 kcal/kg ME from the 5th to 10th weeks. Sawdust + straw were used as bedding material. The lighting schedule was 24 h lights (L): 0 h dark (D) during the first week, 22L: 2D for 2-4 weeks and 18L: 6D for 5-10 weeks. The temperature was between 28-35 °C during the first week and maintained between 20-32 °C from the 2nd week onwards (Thinh et al., 2021). Ducks were reared at a stocking density of 5 birds/m^2. In this study, 60 Pekin duck chicks (30 female and 30 male) were fed for eight weeks. At the end of the feeding period, the live weights of female and male Pekin ducks were determined, and they were divided into two experimental groups, low and high, based on the average live weight within each gender. A total of 20 ducks (10 males and 10 females) from each experimental group were randomly selected and subjected to standard slaughter.

At the end of the feeding period, all ducks were transported to an abattoir, and a standard commercial slaughter procedure was carried out. Immediately after slaughter, the Pectoralis major (PM; chest muscle) muscle on the right side of the carcass was isolated and cleaned of fat and connective tissues. Then, pieces of 5 (length) × 2 (width) × 2 (height) cm were taken from the PM muscle, frozen in liquid nitrogen, and stored at -80 ºC until analysis. After removing all internal organs and abdominal fat, warm carcass weights were determined by adding PM muscle weight. The eviscerated carcasses were chilled for 24 h at four °C and reweighed to determine cold carcass weight.

Total RNA of PM muscle samples were isolated by a commercial RNA (PureLink ™, RNA Mini Kit, Invitrogen™, 12183018A) purification kit using the TRIzol Reagent

(Thermo Fisher Scientific, US) as suggested by the manufacturer. Genomic DNA was eliminated by digestion with DNase I (Thermo Fisher Scientific Inc., Waltham, MA, USA). The purity and concentration of isolated RNA were evaluated by the A260/A280 ratio using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and all RNA samples showed A260/A280 values within the range of 2.01 to 2.08 and A260/ A230 values above 2. The integrity of collected RNA was checked with 1 % w/v agarose gel electrophoresis. Total RNA was converted to cDNA using a commercial cDNA kit (BIORAD iScript cDNA, 1708890) following the manufacturer's instructions in the Thermal Cycler (BIORAD) device. For GADPH the prepared cDNA samples were further purified, quantified, diluted to the same initial concentration, and stored at −20 °C until subsequent quantitative real-time PCR analysis.

qRT-PCR Analyses

Primers used for the amplification of genes were designed using online tools (https://www.ncbi.nlm.nih.gov/tools/primerblast/) (accessed on 12 April 2022) based on the related gene sequences of duck (Table 1).

Table 1. Primer Sequences for the mRNA expression analysis of genes

Genes	Primer sequence (5′-3′)		PS (bp)
	Forward	Reverse	
MyoD	AAGGCGTGCAAGAGGAAGAC	TGGTTGGGGTTGGTGGA	131
MyoG	CGGATCACCTCCTGCCTGA	CGTCCTCTACGGCGATGCT	87
GAPDH	AAGGCTGAGAATGGGAAAC	TTCAGGGACTTGTCATACTTC	118

PS= Product size

GADPH was selected as housekeeping gene to normalize the expression of target genes. All primers were synthesized by Sentebiolab (Ankara, Türkiye). The specificity of each of the designed primers was checked via online Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and melt curve analysis was carried out during qRT-PCR. Relative quantification of all transcripts was performed by qRT-PCR using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Real-time quantitative PCR were run with EvaGreen mastermix (5× HOT FIREPol EvaGreen qPCR Mix Plus, Solis BioDyne, Tartu, Estonya). The reaction mix was in a total volume of 10 µL comprising 5 µL of 5X HOT FIREPol mix, 0.5 µL of forward primer (10 µmol/L), 0.5 µL of reverse primer (10 µmol/L), Dye, 2 µL of DEPC treated water, and 2 µL of template cDNA. PCR amplification was carried out as follows: denaturation of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, specific annealing temperature of 60 °C for 30 s. The relative mRNA expression levels of the genes were calculated by the $2^{-\Delta\Delta Ct}$ method.

The data obtained at the end of the study were conducted using the Shapiro-Wilk test, Levene test, and one-way analysis of variance using the SPSS 20.0 version OMU license. According to the Shapiro-Wilk test results, it was determined that the data were suitable for normal distribution ($P<0.05$) and according to the Levene test results, the variances were homogeneous ($P<0.05$). One-way analysis of variance was used to compare the trial groups.

RESULTS

The expression fold changes of myogenic differentiation factor (MyoD) and myogenin (MyoG) genes in the Pectoralis major (PM) skeletal muscle of male and female Peking ducks with low and high slaughter weight are presented in Table 2. In the study, significant differences were observed between experimental groups (except MyoD gene expression fold change in low and high-body-weight male Peking ducks) and sex in terms of expression fold changes of MyoD and MyoG genes ($P<0.05$). While there were no differences in terms of MyoD gene expression fold change between low and high-body-weight male Peking ducks, the

MyoD gene expression fold change was more significant in high-body-weight female Peking ducks than in low-body-weight female ducks (P<0.05). The MyoD gene expression fold change of low and fattening body weight of female Peking ducks was lower than that of male Peking ducks (P<0.05).

In both male and female Peking ducks, MyoG gene expression fold change was higher in ducks with high body weight than in ducks with low body weight (P<0.05). In addition, while the MyoG gene expression fold change of female Peking ducks with low weight was lower than that of male Peking ducks, the MyoG gene expression fold change of female Peking ducks with high body weight was higher than that of male Peking ducks (P<0.05).

Table 2. The expression fold changes of myogenic differentiation factor (MyoD) and myogenin (MyoG) genes in the Pectoralis major (PM) skeletal muscle of male and female Peking ducks with low and high slaughter weight

Traits	Sex	Groups	
		Low	Low
MyoD	M	246.94±41.38^{A}	201.96±32.51^{A}
	F	35.79±9.10^{Bb}	117.19±18.60^{Ba}
MyoG	M	40.85±6.14^{Ab}	69.09±6.59^{Ba}
	F	26.20±7.35^{Bb}	131.74±20.57^{Aa}

^{a,b} Mean values with different superscripts in the same row indicate a significant difference (P<0.05)
^{A,B} Mean values with different superscripts in the same column indicate a significant difference, (P<0.05). M= male, F= female.

The expression levels of the myogenic differentiation factor (MyoD) gene in the Pectoralis major (PM) skeletal muscle of low and high-slaughter-weight female Peking ducks are presented in Figure 1. The study determined that female ducks with high body weight at the end of fattening had a higher MyoD gene expression fold change than those with low live weight (P<0.05).

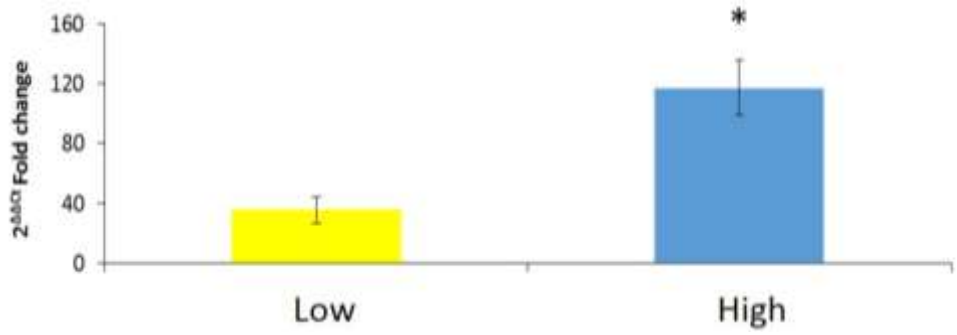

Figure 1. The expression levels of the myogenic differentiation factor (MyoD) gene in the Pectoralis major (PM) skeletal muscle of low and high-slaughter-weight female Peking ducks. *P<0,05.

The expression levels of the myogenic differentiation factor (MyoD) gene in the Pectoralis major (PM) skeletal muscle of male Peking ducks with low and high slaughter weights are presented in Figure 2. The study detected no statistically significant difference regarding MyoD gene expression fold change between low and high-fattening body-weight male ducks.

The expression levels of the myogenin (MyoG) gene in the Pectoralis major (PM) skeletal muscle of male Peking ducks with low and high slaughter weights are presented in Figure 3. The study determined that female ducks with high live weight at the end of fattening had a higher MyoG gene expression fold change than those with low live weight (P<0.05).

The expression levels of the myogenin (MyoG) gene in the Pectoralis major (PM) skeletal muscle of male Peking ducks with low and high slaughter weights are presented in Figure 4. The study determined that male ducks with high live weight at the end of fattening had a higher MyoG gene expression fold change than those with low body weight (P<0.05).

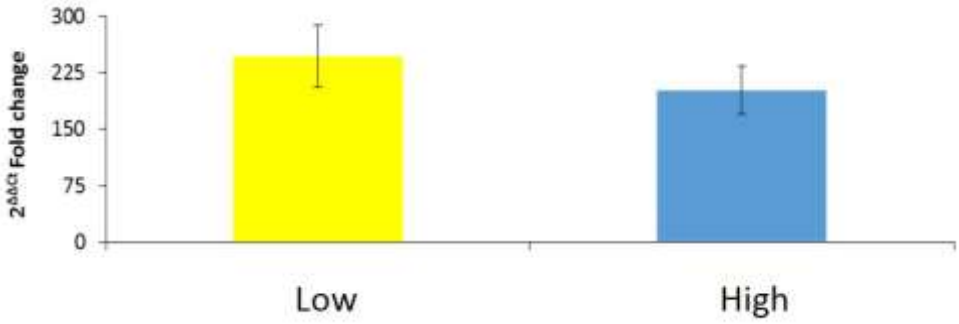

Figure 2. The expression levels of the myogenic differentiation factor (MyoD) gene in the Pectoralis major (PM) skeletal muscle of low and high-slaughter-weight male Peking ducks.

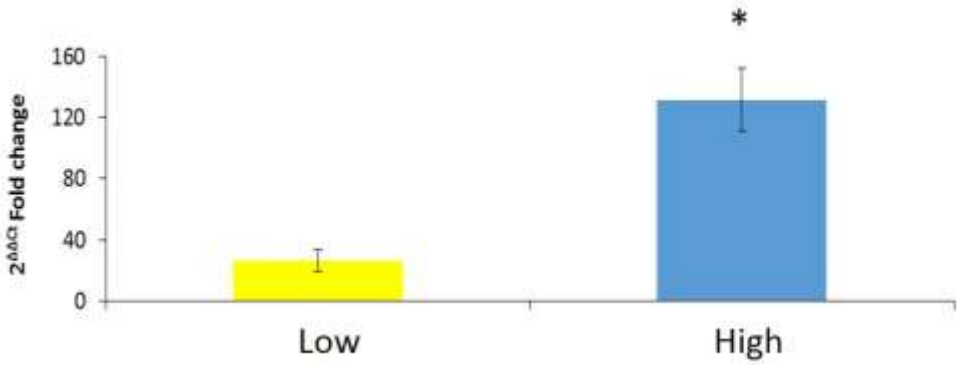

Figure 3. The expression levels of the myogenic differentiation factor (MyoG) gene in the Pectoralis major (PM) skeletal muscle of low and high-slaughter-weight female Peking ducks. *P<0,05.

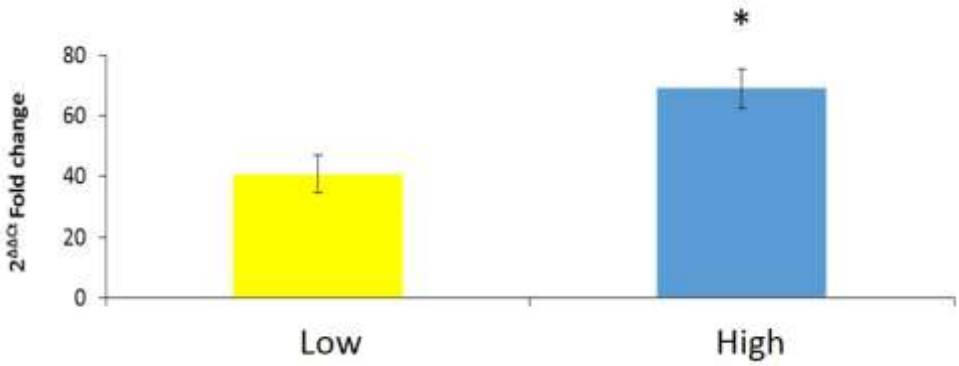

Figure 4. The expression levels of the myogenic differentiation factor (MyoG) gene in the Pectoralis major (PM) skeletal muscle of low and high-slaughter-weight male Peking ducks. *P<0,05.

DISCUSSION

Many genetic and environmental factors affect meat quality and yield in farm animals. The development and growth of muscle fibers in vivo are regulated by four conserved basic helix-loop-helix (bHLH) transcription factors of the MDF gene family

(Myf5, Myf6, MyoD, and MyoG) (Zhong et al., 2013).

MyoD is a transcription factor mainly involved in the development and differentiation of muscle cells. The MyoD gene enables the formation of muscle fibers by triggering the differentiation of muscle cell precursors called myoblasts. MyoD is activated by the action of cellular signals that regulate gene expression. When it becomes active, it initiates the expression of other genes, enabling myoblasts to turn into muscle cells. It also stops the proliferation of myoblasts and directs them to differentiate into muscle fibers. In this process, MyoD activates myogenic (muscle-building) factors and promotes the synthesis of muscle-specific proteins.

MyoG is also a transcription factor and has similar functions to MyoD. The MyoG gene enables myoblasts to differentiate into muscle fibers at later stages. MyoG is engaged in maintaining and completing myoblast differentiation, which MyoD initiated. MyoG changes gene expression in the nuclei of myoblasts, allowing them to acquire the typical properties of muscle cells. It also helps myoblasts come together and form muscle fibers called myotubules. MyoG is also vital in regulating muscle fibers' size and contraction abilities.

MyoD and MyoG genes play critical roles in the development and differentiation of muscle cells. MyoD triggers the differentiation of muscle cell precursors, while MyoG promotes further differentiation of myoblasts and the formation of muscle fibers. The interaction of these two genes determines the structural and functional properties of muscles by ensuring the correct development of muscle cells.

Live weight gain and carcass weight in farm animals are related to growth characteristics, and these characteristics are closely related to the increase in the number or diameter of the animal's muscle fibers and the development of muscle mass. Growth, which is related to carcass weight, the most significant indicator of meat yield, is divided into two parts: prenatal and postnatal growth. Muscle development is a multidimensional chain of events that includes cellular increase and specialization in the embryo during growth, maturation, and development of functions (Kassar-Duchossoy et al. 2004; Siqin et al., 2017). The MDF gene family mainly controls this event. MDFs regulate myogenesis, from the formation, development, and proliferation of muscle fibers to postnatal muscle maturation, differentiation, and functions (Patel et al., 2014; Siqin et al., 2017; Zhong et al., 2013). The growth and development of muscle mass cells in farm animals are regulated by MDF gene family members MyoG, MyoD, Myf5, and Myf6. These genes control the formation of muscle cells during the embryonic period and the maturation and functions of muscle fibers after birth (Hughes and Schiaffino, 1999). Extensive studies are needed to more precisely determine the relationships between MyoD and MyoG genes and meat yield and quality parameters. Muscle expression of specific genes, such as myogenic transcription factors, can significantly affect meat content in the carcass and meat quality.

In the current study, significant differences were found between groups regarding the expression level of MyoG and MyoD genes in the PM muscle of Peking ducks with different slaughter weights. This study will illuminate the definitive solution to the question marks that may arise regarding live weight in future breeding studies. Using the genetic information obtained in this research can accelerate genetic advancement in livestock farming and improve future research. In other words, since the Peking duck is a breed widely used in our country in terms of meat yield, such studies will allow it to increase meat yield. In addition, this study was conducted on Peking ducks, and determining the expression levels of the MyoG and MyoD genes we focused on for the first time has a great potential to eliminate a significant deficiency in this field. If progress is made in this direction, it can contribute to competition with highly developed countries. In addition, while the skeletal muscle fiber defined during development in the embryonic period occurs in two separate stages, postnatal growth is limited by hypertrophic muscle fiber growth. Skeletal muscle fibers are the primary cell type of the meat mass obtained after slaughter. Therefore, differences in the activity of the

MDF gene family may be crucial for the amount of meat stored in these animals, which is of great economic importance. Therefore, MDF genes, especially MyoG and MyoD, can be considered potential candidate genes to investigate the relationship between genomic variation and skeletal muscle mass and meat mass.

In the current study, while there was no difference in terms of MyoD gene expression fold change between low and high-fattening body weight male Peking ducks, it was determined that the MyoD gene expression fold change in high-fattening body weight female Peking ducks was higher than that of low fattening body weight female ducks. Although skeletal muscle mass cells or muscle fibers have a multinucleated structure, the amount of DNA in the muscle mass can be directly related to the number of muscle fibers. It may indicate that ducks with higher body weight have more skeletal muscle fibers than ducks with lower body weight, and ducks with higher body weight may indicate higher fattening potential.

CONCLUSIONS

This study presents a significantly different expression of MyoG and MyoD genes in duck skeletal muscles. This study has shown that high-body-weight ducks may have higher muscle growth due to higher MyoG and MyoD gene expression levels and that this duck breed can be recommended for a more efficient fattening application.

ACKNOWLEDGEMENTS

This study was supported within the scope of TUBITAK 2209/A University Students Research Projects Support Program.

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Hormonal changes during the transition period

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Abstract

The transition period is a critical period that is very important for the cow and the offspring, covering the three weeks before birth and the three weeks after birth. In this process, there is special emphasis on the nutritional needs, milk yield, health status and general well-being of the cattle. It is important for herd productivity. Many changes occur during the transition period. The effects of these changes on the reproductive system, digestive system, immune system and mammary glands are obvious and rapid. If the negative energy balance during the transition period is excessive, the animal is at risk of developing conditions such as ketosis, fatty liver, hypocalcaemia and retention secundinarum. In addition to metabolic changes, all kinds of stress factors that accompany labor and delivery cause significant changes in estrogen, progesterone, oxytocin, cortisol, FSH, LH and many hormones. In this presentation, the hormonal changes that occur during the transition period in cows will be discussed.

Key words: *Cortisol, Estrogen, Ketosis, Progesterone, Transition period,*

Economic evaluation of broiler diets supplemented with either selected herbs or their associated essential oils

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Abstract

In recent years, there has been a growing interest in incorporating phytogenic feed additives (PFAs) into broiler chicken diets as potential alternatives to traditional growth-promoting additives.
This study evaluated the economics of individually incorporating either six different dried herbs or their essential oils into broiler diets: chamomile, rosemary, lavender, oregano, thyme and St. John's wort. A total of 390 day-old male broiler chicks (Ross 308) were randomly divided into 13 groups of 30 chicks with three replicates (10 chicks/replicate). The control group received a basal diet, while the other groups received a basal diet supplemented with 2% of each dried herb (E1-E6 groups) or 0.02% of their essential oils (E7-E12 groups) for 39 days. The parameters measured were feed intake, body weight gain, feed conversion ratio, feed costs, economic efficiency and European Broiler Index (EBI).
The results showed better economic efficiency with 2% dried St. John's wort herb, as well as 0.02% St. John's wort, rosemary, thyme or lavender essential oils compared to the other treatments ($P<0.05$), but not compared to the control group ($P>0.05$). Unsatisfactory results were observed with dry lavender herb and essential oils of chamomile or oregano, which resulted in a significant decrease in net income and economic efficiency due to higher feed costs per kilogram live weight ($P<0.05$). EBI values were not significantly increased in any of the treated groups compared to the control group ($P>0.05$). These results suggest that while certain PFAs can improve economic efficiency, their overall effect is variable and some may not outperform traditional growth promoters

Key words: *broiler performance; phytogenic feed additives; herbs, essential oils; economic efficiency;*

INTRODUCTION

The global poultry industry is continuously searching for sustainable and economically viable strategies to enhance the efficiency of broiler production. Over the years, synthetic products, including antibiotic growth promoters, have been extensively used in broiler production to improve growth rate, feed efficiency, prevent diseases, and reduce mortality (Abd El-Hack et al., 2022; Mohamed and Hassan, 2023). However, increasing concerns about the emergence of antibiotic-resistant pathogenic bacteria, as well as antibiotic residues in poultry products, have led to the exploration of alternative, safe, and cost-effective additives that can maintain or even improve production performance without compromising the birds' health, the quality of poultry products, human health, and the environment (Alagawany et al., 2021).

Phytogenic feed additives (PFAs), derived from plant sources with bioactive compounds like aromatic and medicinal plants, their extracts, or essential oils, have gained attention due to their numerous biological and beneficial properties (Puvaĉa et al., 2015; Hassan and Awad, 2017; Giannenas et al., 2018; Kadhim, 2018; Singh et al., 2018; Jin et al., 2020). Previous studies indicate that the inclusion of PFAs in diets significantly impact various aspects of broiler performance. It has been reported to stimulate appetite and feed intake, promote the release of digestive enzymes, enhance nutrient utilization, improve gut morphology, modulate the immune system, as well as enhance resilience to heat stress (Omar et al., 2016; Galli et. al., 2020; El-Ashram and Abdelhafez, 2020; Moustafa et al., 2020; Alagawany et al., 2021; Ayalew et al., 2022; Phillips et al., 2023; Señas-Cuesta et al., 2023; Mahasneh et al., 2024). Some essential oils (EOs), such as oregano and thyme, have demonstrated potential in reducing the incidence of common poultry diseases, including coccidiosis and necrotic

enteritis (Adhikari et al., 2020). Taken together, these findings suggest a range of potential benefits for maximizing the genetic potential of chickens and reducing mortality, thus increasing profitability (Puvaĉa et al., 2022). Additionally, some additives enhance nitrogen absorption, control excreta odor, and reduce ammonia concentration, thereby decreasing nitrogen excretion into the environment (Chowdhury et al., 2018). Moreover, phytogenic feed additives have shown potential in improving meat quality (fatty acid profile, flavor and shelf-life) which can positively impact consumer preference and marketability (Giannenas et al., 2013; Galli et. al., 2020; Mohamed and Hassan, 2023).

The economic efficiency of diets with these supplements is critical in determining their viability and practicality for broiler production. Therefore, the objective of this study is to evaluate the cost-effectiveness of including six herbs or their essential oils as phytogenic feed additives in broiler chicken diets. The findings of this study will contribute to the existing knowledge on alternative feed additives and provide valuable information to poultry producers, feed manufacturers, and other stakeholders in making informed decisions about their inclusion in broiler chicken diets.

MATERIALS AND METHODS

The present study was conducted at the Poultry farm of the Agricultural Institute, Stara Zagora, Bulgaria. A total of 390 day-old Ross 308 male broiler chicks, with an initial body weight of 49.78 ± 0.2 g, were randomly assigned to thirteen treatment groups. Each group, comprising 30 chicks, was further divided into three replicates (10 chicks/replicate). Subsequently, each replicate was allocated to a floor pen equipped with one feeder and drinker. Additionally, a continuous lighting program was implemented. The temperature was initially set at 33°C during the first week and gradually decreased by 3°C per week until stabilizing at 21°C in the fourth week.

The dietary treatments included a control group fed basal diets without supplementation and twelve experimental groups. The experimental groups received basal diets supplemented with either 2% herbs or 0.02% essential oils (EOs) derived from the same herbs. The following herbs, in dried powdered form, were used separately: chamomile (*Matricaria chamomilla*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula angustifolia*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and St. John's wort (*Hypericum perforatum*). The essential oils were obtained from commercial companies (Nature Energies LTD; ALTEYA ORGANICS LTD, Bulgaria).

Chicks were fed a starter diet (1-10 days of age), a grower diet (11-28 days of age) and a finisher diet (29-39 days of age). The diets were formulated to meet the nutritional recommendations of the National Research Council (NRC, 1994). The composition and calculated nutritional value are shown in Table 1. Feed (in mash form) and water were provided ad libitum to the chicks throughout the experiment.

Table 1. Ingredients and calculated nutrient composition of diets

Ingredients, %	Starter (1-10 days)	Grower (11-28 days)	Finisher (29-39 days)
Soybean meal	35.00	31.00	25.00
Wheat	30.00	29.69	35.87
Maize	21.22	25.00	24.00
DDGS	5.00	5.00	5.00
Sunflower oil	5.00	6.00	7.00
Dicalcium phosphate	2.05	1.80	1.65
Limestone	0.60	0.50	0.55
Salt	0.20	0.20	0.20
Premix*	0.20	0.20	0.20
Lysine	0.25	0.17	0.12
Methionine	0.18	0.14	0.11
Salgard	0.20	0.20	0.20
Optizim	0.10	0.10	0.10
Calculated nutrient composition, %			
Metabolizable energy, kcal/kg	2912.80	3042.19	3111.17
Crude protein	22.47	21.01	19.02
Ether extract	7.03	8.10	9.08
Crude fiber	3.99	3.77	3.48
Calcium	1.03	0.90	0.85
Av.phosphorus	0.50	0.45	0.43
Lysine	1.44	1.25	1.05
Methionine	0.50	0.45	0.40
Met + cys	0.85	0.77	0.68

*Composition/kg of premix: Vit. A: 6 000 000 IU; Vit D3: 2 500 000 IU; Vit. E: 45 000 mg; Vit B1: 2 000 mg; Vit B2: 4 500 mg; Vit B6: 2 500 mg; Pantothenic acid: 10 000 mg; Biotin: 125 mg; Vit. K3: 2 000 mg; Folic acid: 1 100 mg; Nicotinic acid: 32 500 mg; Vit. B12: 10 mg; Selenium: 150 mg; Manganese: 60 000 mg; Iron: 12 500 mg; Zinc: 45 000 mg; Copper: 7 500 mg; Iodine: 500 mg

Performance parameters, including body weight and the feed consumed, were measured at the end of the starter, grower, and finisher periods. Then body weight gain, feed intake, and feed conversion ratio were calculated for specified periods, as well as for the entire fattening period of the chickens. Additionally, mortality rates were also recorded daily.

Input-output analysis was used to assess the economic efficiency of the experimental diets, assuming other costs remained constant, as suggested by Hassan and Awad (2017), as follows:

(1) Total feed cost = feed intake per bird x cost of 1 kg diet
(2) Feed cost/kg weight gain = feed conversion x cost of 1 kg diet
(3) Net revenue/kg gain = revenue/kg gain – feed cost/kg gain
(4) Economic efficiency = net revenue/feed cost per kg gain

Input costs data were collected by calculating the prices of feed ingredients available on the market at the time of the experiment. The additional costs of the tested dried herbs and essential oils were included in the feed price.

In the economic assessment, the total feed cost, feed cost per kg of weight gain for each feed period, as well as for the entire experimental period were taken into account. Total revenue per bird sell was also considered assuming 4.80 BGN/kg live body weight constant for all treatment birds.

The economic efficiency of growth was determined through the calculations of European Broiler Index (EBI) based on the following formula (Marcu et al., 2013):

$$EBI = \frac{\text{Viability (\%) x ADG (g/chick/day)}}{\text{FCR (kg feed/kg gain) x 10}} \text{ x } 100$$

where: ADG- average daily gain; FCR- feed conversion ratio;

Statistical analysis

The data were analyzed using General Linear Model procedure of SPSS (version 19.0). Means were compared using Duncan's Multiple Range Test, with the level of significance set at $P < 0.05$. The replicate pens served as experimental units for all parameters.

RESULTS

Table 2 presents the economics of cost in relation to the supplementation of dried herbs in broiler diets during the starter, grower, and finisher periods. The inclusion of the tested herbs in the basal diet resulted in an increase in the price of the dietary mixtures for all three phases, ranging from 0.10 to 0.40 BGN/kg. However, no significant differences in feed consumption and feed conversion were found during these periods ($P>0.05$). As a result, most of the groups given herbal diets had higher total feed costs and feed costs per kilogram of gain compared to the control group ($P<0.05$), except for those receiving rosemary (E2), thyme (E5) or St. John's wort herb (E6) during the starter and grower phases, which were comparable to the control birds ($P>0.05$).

On the other hand, the starter diet containing lavender herb (E3) resulted in the highest total feed cost (34.48%) and feed cost per kilogram of gain (45.45%), a trend that continued in subsequent periods ($P<0.05$), followed by the group fed a diet supplemented with chamomile (E1) during both the grower and finisher periods compared to the control group.

Table 2. Cost effectiveness of broiler diets supplemented with 2% dried herbs

Parameters	Groups							SEM	*P*-value
	C	E_1	E_2	E_3	E_4	E_5	E_6		
Starter period (1-10 d)									
Feed price, BGN/kg	0.93	1.17	1.12	1.31	1.07	1.03	1.03	-	-
Feed intake, kg/bird	0.32	0.29	0.28	0.30	0.33	0.30	0.30	0.01	0.177
Total feed cost, BGN	0.29^{b}	0.34^{ac}	0.32^{bc}	0.39^{a}	0.36^{ac}	0.31^{bc}	0.31^{bc}	0.02	0.004
Feed conversion, kg/kg	1.78	1.71	1.94	1.83	1.85	1.89	1.86	0.09	0.672
Feed cost/kg gain, BGN	1.65^{c}	2.00^{b}	2.18^{ab}	2.40^{a}	1.99^{b}	1.94^{bc}	1.91^{bc}	0.10	0.004
Grower period (11-28 d)									
Feed price, BGN/kg	0.90	1.14	1.09	1.29	1.05	1.00	1.00	-	-
Feed intake, kg	1.57	1.56	1.47	1.58	1.58	1.64	1.48	0.07	0.610
Total feed cost, BGN	1.42^{c}	1.78^{b}	1.61^{bc}	2.03^{a}	1.66^{bc}	1.64^{bc}	1.48^{c}	0.08	0.002
Feed conversion , kg/kg	1.70	1.56	1.83	1.58	1.59	1.70	1.63	0.08	0.304
Feed cost/kg gain, BGN	1.53^{b}	1.78^{ab}	2.00^{a}	2.03^{a}	1.66^{b}	1.70^{b}	1.62^{b}	0.09	0.011

Finisher period (29-39 d)									
Feed price, BGN/kg	0.86	1.11	1.06	1.26	1.02	0.97	0.97	-	-
Feed intake, kg	1.10	1.05	0.88	0.97	1.06	1.08	0.95	0.05	0.080
Total feed cost, BGN	0.95^{b}	1.16^{a}	0.94^{b}	1.21^{a}	1.08^{ab}	1.04^{ab}	0.92^{b}	0.06	0.011
Feed conversion, kg/kg	2.04	2.06	1.74	2.12	2.19	2.14	1.71	0.13	0.114
Feed cost/kg gain, BGN	1.77^{b}	2.29^{ad}	1.86^{bc}	2.66^{a}	2.22^{cd}	2.07^{bcd}	1.65^{b}	0.13	0.001

[a-d] -Means in the same row with different superscripts are significantly different P<0.05; C – Control group; E1 – 2% *Matricaria*; E2 – 2% *Rosmarinus officinalis*; E3 – 2% *Lavandula*; E4 – 2% *Origanum vulgare*; E5 – 2% *Thymus*; E6 – 2% *Hypericum perforatum*

Data on the economic efficiency of feeding different experimental diets over a period of 39 days, influenced by dried herbs, are presented in Table 3. There were no significant (P>0.05) differences in total feed consumption and feed efficiency between the groups. However, the total feed cost showed a significant (P<0.05) increase of 36% in the diet supplemented with lavender herb (E3), followed by the diets supplemented with either chamomile herb (E1) by 23.5% or oregano herb (E4) by 16 % compared to the control group. This indicates that the choice of herb supplementation in the diets had a notable impact on the overall feed cost. In terms of feed cost per kilogram of gain, data analysis revealed the most significant (P<0.05) increase of 39 % in the lavender supplemented group (E3). In contrast, the St. John's wort supplemented group (P<0.05), demonstrated the lowest value (10-26%) compared to the other treated group, which were comparable to those of the control group (P>0.05).

The economic efficiency values observed in the study were not influenced by the specific properties of each herb, including its potential effects on feed consumption and broiler growth, but rather by the different market prices of the herbs used. According to the input-output analysis (Table 3), the economic efficiency (EE) values varied among the treatments, with the highest value of 1.96 observed for broilers fed the control diet, followed by 1.88 for chicks fed diets supplemented with St. John's wort, while the lowest value of 1.13 was recorded for the lavender supplementation (P<0.05).

Table. 3. Economic efficiency of broiler diets supplemented with 2% dried herbs

Parameters	Groups							SEM	*P*-value
	C	E_1	E_2	E_3	E_4	E_5	E_6		
Average feed price, BGN/kg	0.90	1.14	1.09	1.29	1.05	1.00	1.00	-	-
Feed intake, kg	2.99	2.90	2.63	2.84	2.98	3.02	2.73	0.09	0.069
Total feed cost, BGN	2.68^{d}	3.31^{b}	2.88^{cd}	3.65^{a}	3.11^{bc}	3.01^{bcd}	2.72^{d}	0.10	0.000
Feed conversion, kg/kg	1.80	1.75	1.81	1.75	1.79	1.85	1.67	0.05	0.251
Feed cost / kg gain, BGN	1.62^{c}	2.00^{b}	1.97^{b}	2.25^{a}	1.87^{b}	1.85^{b}	1.67^{c}	0.05	0.000
Net revenue/ kg gain, BGN	3.18^{a}	2.80^{b}	2.83^{b}	2.55^{c}	2.93^{b}	2.95^{b}	3.14^{a}	0.05	0.000
Economic efficiency (EE)	1.96^{a}	1.40^{b}	1.44^{b}	1.13^{c}	1.57^{b}	1.59^{b}	1.88^{a}	0.07	0.000
Relative EE	100^{a}	71.43^{b}	73.47^{b}	57.65^{c}	80.10^{b}	81.12^{b}	95.92^{a}	3.31	0.000

[a-d] -Means in the same row with different superscripts are significantly different P<0.05; C – Control Group; E1 – 2% *Matricaria chamomilla*; E2 – 2% *Rosmarinus officinalis*; E3 – 2% *Lavandula angustifolia*; E4 – 2% *Origanum vulgare*; E5 – 2% *Thymus vulgaris;* E6 – 2% *Hypericum perforatum*

The cost economics related to dietary supplementation of essential oils (EOs) in broiler diets during the starter, grower, and finisher periods are shown in Table 4. The inclusion of essential oils in broiler diets during the starter period had a significant effect on all the parameters studied (P<0.05). Although feed intake was reduced in the groups supplemented with rosemary (E8) or lavender (E9) essential oil (P<0.05), no significant difference in feed utilization was observed compared to the control group (P>0.05). However, there was an improvement in feed conversion compared to the chamomile oil treated group (P < 0.05). The corresponding total feed cost was significantly lower for the diet supplemented with lavender oil (E9) compared to the other groups, which was attributed to reduced feed intake (P<0.05). However, there was a significant increase (P<0.05) in total feed cost in the chamomile oil diet (E7) due to the higher cost of chamomile oil. No significant difference (P>0.05) was observed between the control group and the rosemary (E8) or St. John's wort (E12) dietary supplement groups. Similar trends were observed for feed cost per kg gain. During

the next two fattening periods, feed consumption and feed conversion ratio remained unaffected by the addition of essential oils (P>0.05). However, due to the higher prices of chamomile and oregano oil, total feed costs and feed costs per kg of gain were highest in these groups compared to the other groups (P<0.05). Specifically, they were between 30-35% higher during the grower phase and 30-41% higher during the finisher phase compared to the control group.

Table 4. Cost effectiveness of broiler diets supplemented with 0.02% essential oils

Parameters	Groups							SEM	*P*-value
	C	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}		
Starter (1-10 d)									
Feed price, BGN/kg	0.93	1.41	0.97	0.97	1.37	1.12	0.97	-	-
Feed intake, kg	0.32^{a}	0.32^{a}	0.27^{bc}	0.25^{c}	0.28^{abc}	0.31^{ab}	0.30^{ab}	0.01	0.010
Total feed cost, BGN	0.29^{d}	0.45^{a}	0.26^{de}	0.24^{e}	0.39^{b}	0.34^{c}	0.29^{d}	0.01	0.000
Feed conversion, kg/kg	1.78^{ab}	2.03^{a}	1.59^{b}	1.63^{b}	1.63^{b}	1.85^{ab}	1.76^{ab}	0.09	0.048
Feed cost/kg gain, BGN	1.65^{c}	2.85^{a}	1.55^{c}	1.58^{c}	2.13^{b}	2.06^{b}	1.71^{c}	0.10	0.000
Grower (11-28 d)									
Feed price, BGN/kg	0.90	1.38	0.95	0.95	1.35	1.09	0.95	-	-
Feed intake, kg	1.57	1.55	1.61	1.67	1.63	1.39	1.46	0.10	0.443
Total feed cost, BGN	1.42^{b}	2.14^{a}	1.53^{b}	1.59^{b}	2.19^{a}	1.52^{b}	1.38^{b}	0.11	0.000
Feed conversion , kg/kg	1.70	1.64	1.60	1.65	1.61	1.46	1.50	0.10	0.600
Feed cost/kg gain, BGN	1.53^{b}	2.26^{a}	1.52^{b}	1.57^{b}	2.17^{a}	1.60^{b}	1.42^{b}	0.11	0.000
Finisher (29-39 d)									
Feed price, BGN/kg	0.86	1.35	0.91	0.91	1.31	1.05	0.91	-	-
Feed intake, kg	1.10	1.00	1.03	1.03	1.19	1.05	1.10	0.06	0.412
Total feed cost, BGN	0.95^{c}	1.35^{b}	0.94^{c}	0.91^{c}	1.55^{a}	1.10^{c}	1.00^{c}	0.06	0.000
Feed conversion, kg/kg	2.04	1.86	1.77	1.93	2.31	1.95	2.18	0.20	0.530
Feed cost/kg gain, BGN	1.77^{c}	2.50^{ab}	1.61^{c}	1.76^{c}	3.02^{a}	2.06^{bc}	1.98^{bc}	0.22	0.005

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05; C – Control group ; E7 – 0.02% *Matricaria chamomilla* oil; E8 – 0.02% *Rosmarinus officinalis* oil; E9 – 0.02% *Lavandula angustifolia* oil; E10 – 0.02*%; Origanum vulgare* oil; E11 – 0.02% *Thymus vulgaris* oil; E12 – 0.02% *Hypericum perforatum* oil

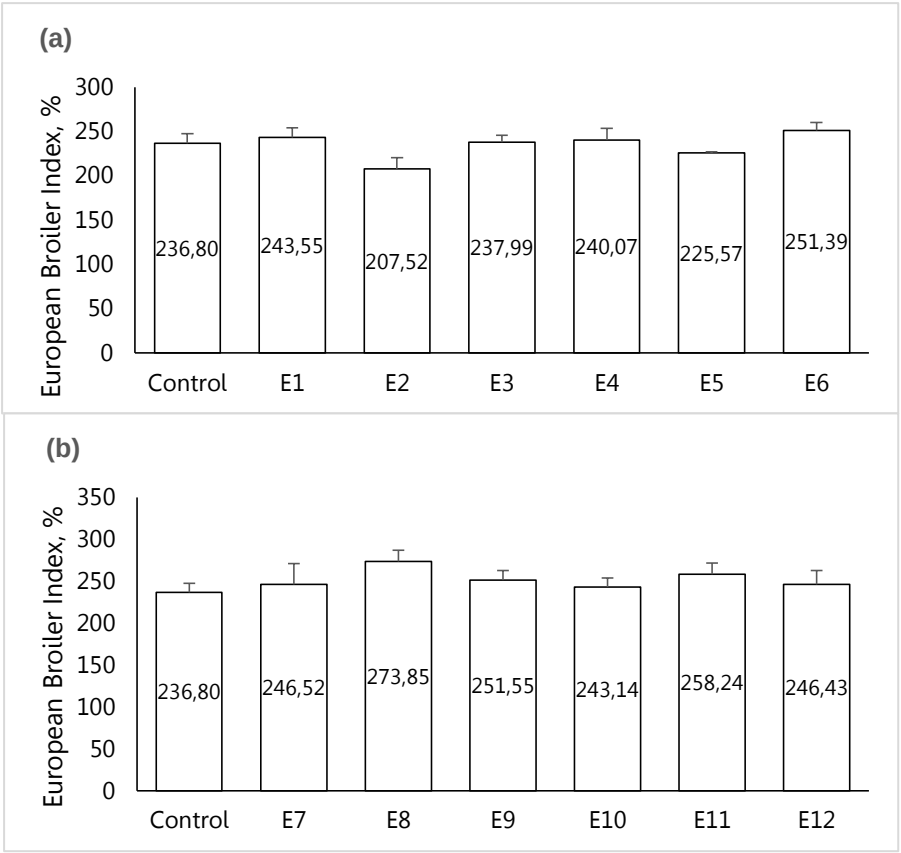
Table 5 shows the results of the economic efficiency of the dietary supplementation of essential oils in broiler diets. The total feed consumption of the treated groups was comparable to that of the control group (P>0.05). Throughout the experimental period, the broilers fed the diets supplemented with essential oils did not utilize the feed more efficiently than those in the control group (P>0.05). However, there was a highly significant difference between treatments in total feed cost, feed cost per kg gain, net revenue per kg gain and economic efficiency (P<0.001). It is evident that the inclusion of either chamomile or oregano oil in the diet increased the total feed cost by 47-55% and the feed cost per kg gain by 48-55% compared to the control group, resulting in the lowest economic and relative efficiency values, which were 0.98-1.00 and 50-51%, respectively. On the other hand, no significant (P>0.05) differences were found between the control group and the other supplemented groups.

Table 5. Economic efficiency of broiler diets supplemented with 0.02% essential oils

Parameters	Groups							SEM	*P*-value
	C	E_7	E_8	E_9	E_{10}	E_{11}	E_{12}		
Average feed cost, BGN/kg	0.90	1.38	0.95	0.95	1.34	1.09	0.94	-	-
Feed intake, kg	2.99	2.87	2.91	2.95	3.09	2.75	2.86	0.14	0.694
Total feed cost, BGN	2.68^{b}	3.95^{a}	2.75^{b}	2.79^{b}	4.15^{a}	2.99^{b}	2.69^{b}	0.16	0.000
Feed conversion, kg/kg	1.80	1.74	1.65	1.74	1.81	1.66	1.73	0.08	0.752
Feed cost / kg gain, BGN	1.62 ^{b}	2.40^{a}	1.56^{b}	1.64 ^{b}	2.43^{a}	1.80 ^{b}	1.63^{b}	0.10	0.000
Net revenue/ kg gain, BGN	3.18 ^{a}	2.40^{b}	3.24^{a}	3.16^{a}	2.37^{b}	3.00^{a}	3.17^{a}	0.10	0.000
Economic efficiency (EE)	1.96^{ab}	1.00^{c}	2.08^{a}	1.93^{ab}	0.98^{c}	1.67^{b}	1.94^{ab}	0.12	0.000
Relative EE	100^{ab}	51.02^{c}	106.12 ^{a}	98.47^{ab}	50.00^{c}	85.20^{b}	98.98^{ab}	5.93	0.000

$^{a-d}$ -Means in the same row with different superscripts are significantly different P<0.05; C – Control Group; E7 – 0.02% *Matricaria chamomilla* oil; E8 – 0.02% *Rosmarinus officinalis* oil; E9 – 0.02% *Lavandula angustifolia* oil; E10 – 0.02*%; Origanum vulgare* oil; E11 – 0.02% *Thymus vulgaris* oil; E12 – 0.02% *Hypericum perforatum* oil

The values for the economic efficiency of growth were obtained by calculating the European Broiler Index (EBI) and are shown in Figures 1 (a,b). Typically, improvements in the European Broiler Index (EBI) result from better body weight gain, liveability and a lower feed conversion ratio. A high EBI value indicates good overall technical efficiency of the broiler operation and is desirable for optimal returns (Samarakoon and Samarasinghe, 2012). In our study, the European Broiler Index was not significantly affected by dietary supplementation with either dried herbs or their essential oils, with values ranging from 207.52 to 251.39 for dried herbs and 236.80 to 273.85 for essential oils, respectively. Based on our results, a trend can be observed that supplementing the birds' diet with either St. John's wort herb or rosemary essential oil was more economical than the other treatment groups. Contrary to our results, several authors have stated that the addition of dried herbs or essential oils to broiler diets may have a beneficial effect on EBI (Arczewska-Wlosek and Swiatkiewicz, 2012; Salama et al., 2023).



C – Control Group; E1 – 2% *Matricaria chamomilla*; E2 – 2% *Rosmarinus officinalis*; E3 – 2% *Lavandula angustifolia*; E4 – 2% *Origanum vulgare*; E5 – 2% *Thymus vulgaris;* E6 – 2% *Hypericum perforatum; E7 – 0.02% Matricaria chamomilla oil; E8 – 0.02% Rosmarinus officinalis oil; E9 – 0.02% Lavandula angustifolia oil; E10 – 0.02%; Origanum vulgare oil; E11 – 0.02% Thymus vulgaris oil; E12 – 0.02% Hypericum perforatum oil*

Figure 1. European Broiler Index in relation to dietary supplementation with dried herbs (a) and essential oils (b).

DISCUSSION

In broiler rearing, feed is the major component of input costs, accounting for up to 70% of the total production costs (Shahin et al., 2020). Consequently, the value of the end product is directly influenced by the price of raw materials and the efficiency of feed utilization. Therefore, it is essential to optimize broiler diets, taking into account both biological performance and economic

feasibility without any adverse effects. In theliterature, available reports suggest that the financial benefits of supplementation with some herbs and their derivatives are not always consistent. The use of phytogenic feed additives (PFAs) in broiler diets has demonstrated an economic advantage, particularly when considering the cost of feed (Mohamed and Hassan, 2023). For instance, the inclusion of thyme feed additives in broiler diets showed the lowest cost per kilogram of gain and the highest percentage of economic efficiency compared to the unsupplemented diet (Osman et al., 2010).

Similarly, Omar et al. (2016) indicated that diets containing herbal extracts were more economical than the control diet, possibly due to enhanced feed conversion efficiency or a reduction in the amount of feed required to produce a unit of meat. Furthermore, improved economic efficiency through PFA supplementation, leading to increased returns and gross margins, has been documented in other studies as well (EL-Faham et al., 2014; Oleforuh-Okoleh et al., 2014; Shahin et al., 2020; Salama et al., 2023). However, these findings are not consistent with the results obtained in the present study. On the other hand, according to Puvaĉa et al. (2020), the cost per treatment increases depending on the natural additive used. Some researchers found a significant reduction in net revenue and economic efficiency when thyme powder was added at the level of 8 g/kg, while statistically similar values were observed in birds fed diets supplemented with 2 and 5 g/kg thyme compared to the group fed the control diet (Hassan and Awad, 2017). This was due to the fact that the improvement in growth occurred along with a significant concurrent increase in total feed cost. Similarly, Singh et al. (2018) concluded that supplementing broiler diets with phytogenic feed additives did not improve economic efficiency. The inconsistency in response to either dried herbs or essential oils (EOs) in the above studies may be due to the variability of active components. Chemical composition is highly dependent on variables such as plant species, harvest time, drying technology, storage time, and extraction process (Hippenstiel et al., 2011). Another important aspect include dosage used, variations in dietary formulations, interactions with other dietary components, and environmental and management factors (Behboodi et al., 2021). Moreover, it has been demonstrated that using a mixture of properly chosen herbs or essential oil blends would yield better results due to synergistic effects than using the same herbs or essential oils individually (Hippenstiel et al., 2011).

CONCLUSIONS

The economic evaluation indicated better economic efficiency with the dietary supplementation of 2% dried St. John's wort herb, as well as 0.02% St. John's wort, rosemary, thyme, or lavender essential oil supplemented group compared to the corresponding other treated groups ($P<0.05$), but not when compared to the control group ($P>0.05$). Unsatisfactory results were observed when using dry herb lavender or essential oil of chamomile or oregano, expressing in a significant decrease in net revenue and economic efficiency due to the highest feed cost to produce 1 kg of live weight compared to other treatments ($P<0.05$). The European Broiler Index (EBI) values in the groups treated with dry herbs or corresponding essential oils did not show a significant increase compared to the control group ($P>0.05$). These results underline the need for a comprehensive evaluation of the economic implications associated with the inclusion of phytogenic feed additives in broiler diets

ACKNOWLEDGEMENTS

We are highly grateful to Natinal Science Fund for Financial Support of the project "Opportunities for obtaining functional foods from poultry"

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Relationship between body condition scores and lactation performance in Polish Holstein-Friesian dairy cattle

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Abstract

Body Condition Score (BCS) is a widely utilized metric for assessing subcutaneous adipose tissue reserves in bovine subjects. This study investigated the relationship between body condition and lactational output in dairy cattle. A large-scale observational study was conducted on Holstein-Friesian cows (n = 12,510) maintained under commercial dairy production conditions. BCS was measured using linear conformation scoring, performed once within the first 120 days of the cows´ initial lactation. To characterize the relationships between BCS and milk yield parameters (based on 29,243 lactations), we employed a dual analytical approach. Statistical analyses were conducted using multifactorial analysis of variance (ANOVA). In the first stage, the significance of environmental factors on BCS (Body Condition Score) was determined. In the next stage, the effect of BCS class on lactation performance was examined. The classification model included BCS class as well as other environmental factors. A multifactorial analysis of variance examined the effects of age at first calving (AFC) (<24; 24-<26; 26-28; >28 months), herd (6), year (2012-2021), calving season (4 seasons), and 4 complete lactations. Initial data analysis revealed a mean BCS of 5.43 (±0.85) across the study population. During the study period, the average lactation length was 357 days, with milk yield at 13,054±3,274 kg, and fat, protein, and lactose contents at 3.87±0.58, 3.34±0.22, and 4.86±0.14 percentage points, respectively. Primiparous cows with the highest AFC (>28 months) exhibited the highest BCS (5.65±1.01). A positive correlation was observed between AFC and mean BCS. Herd-dependent BCS ranged from 5.11 (±0.63) to 5.80 (±1.15). A temporal decline in BCS was noted, with primiparous cows calving in 2012 scoring 5.59 (±1.05) compared to 5.35 (±0.74) in 2021, indicating a year-on-year reduction in body condition. Cows completing four lactations demonstrated the highest BCS, while the lowest scores were observed in the first lactation (5.43±0.85). A highly significant effect of BCS class on controlled milk production traits was demonstrated. Optimal milk production was associated with cows exhibiting moderate BCS values. In conclusion, the findings from the research studies underscore the intricate link between the body condition of dairy cows and their milk production performance. The obtained results suggest the need to include information on the condition of cows in management systems that helps to maximize milk production.

Key words: *Body Condition Score, Holstein-Friesian, lactation performance, age at first calving, dairy cattle*

Estimation of breast meat quality in Pekin Ducks using the color and Ph values with MARS algorithm

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Abstract

Estimating the meat quality is a common approach for animal scientists. There are many traits to evaluate the meat quality. In this study, we aimed to determine the some meat quality traits (SG and RG) using multi trait MARS algorithm. The SG (Springiness Breast) and RG (Resilience Breast) variables were used as multi response variables. SoLG (Cold L Breast), SoaG (Cold a Breast), SobG (Cold b Breast), SoPhG (Cold pH Breast) were used as explanatory variables. The results showed that R^2 values for SG and RG response variables were determined to be 0.543 and 0.394, respectively. When Pearson's correlation coefficients for the same variables were examined, it was seen that they were between 0.74 and 0.63. It is understood that the AIC values obtained as a result of the analysis are -116.514 for SG and -98.128 for the RG variable.

Key words: *Pekin duck, Meat quality, MARS, Multi response estimation*

INTRODUCTION

Ducks can be raised in a wide range of climate zones and nutritional conditions and are considered to be one of the commercially important poultry species. (Chang et al., 2003). In Europe, the ducks most commonly used for the meat production are the Pekin ducks (Michalczuk et al., 2016). Pekin ducks are characterized by high viability, relatively low nutritional requirements compared to broiler and young fattening turkeys, high resistance to a harsh rearing environment, and considerable immunity to disease. (Kokoszyński et al., 2019).

Meat quality of livestock are critical factors in the meat industry. The main methods for determining meat quality are costly, time-consuming and labour intensive because they require laboratory work (Cortez et al., 2006). The use of statistical methods in animal husbandry studies has been going on for many years (Festing and Nevalainen, 2014). In this context, statistical methods such as regression analysis are highly preferred in many areas of animal studies to obtain cheap and reliable results in a shorter time. Some assumptions needed in classical statistical approaches have led to the suggestion of new methods (Ekiz et al., 2020).

Although model estimation using multiple data points generally yields successful predictions, it can also lead to erroneous results (Alkan et al., 2013). In this case, it is more appropriate to prefer nonlinear models. MARS model is among the methods to be used to build up nonlinear models (Eyduran et al., 2017). With the MARS algorithm, the response variables can be used to explain the relationship within the framework of nonlinear effects and interaction effect for explanatory variables. The MARS algorithm can separate multiple slopes in the training set into separate linear segments called splines (Aksoy et al., 2019). The splines are efficiently connected to each other, and the connection point between the splines is also called the 'node' (Muñoz-Osorio et al., 2024).

The aim of this study was to determine the some meat quality traits (SG and RG) using multi trait MARS algorithm.

MATERIALS AND METHODS

The material of this study was consist of 20 male and 20 female Pekin ducks taken from a project conducted on commercially raised for 10 weeks of age. Standard commercial slaughter procedure was carried out. Only breast (pectoralis major muscle) was used in this study. The SG (Springiness Breast) and RG (Resilience Breast) variables were used as multi response variables. SoLG (Cold L Breast), SoaG (Cold a Breast), SobG (Cold b Breast), SoPhG (Cold pH Breast) were used as explanatory variables.

The MARS procedure is based on two procedures such as forward and backward pass stage (Arthur et al., 2020). Model fitting begins with a forward pass phase where many nodes are defined, followed by a backward trimming procedure to chasten the model, in a very fast procedure (Elith and Leathwick, 2007). The MARS algorithm procedure was performed by using equation which is given below.

$$y = \beta_0 + \sum_{M=1}^{M} \beta_m \prod_{k=1}^{k_m} h_m\left(X_{v(k,m)}\right)$$

where; y is the estimated dependent variable, βm is an intercept, $h_m(X_{v(k,\ m)})$ is the basis functions of the MARS model, where $v(k,\ m)$ is predictor of index, k is the parameter regulating the order of interaction. In addition, these additions and eliminations are evaluated for changes in residual squared errors using the generalized cross validation (GCV) method (Elith and Leathwick, 2007).

$$GCV(\lambda) = \frac{\sum_{i=1}^{n}(y_i - y_{ip})^2}{\left(1 - \frac{M(\lambda)}{n}\right)^2}$$

where n is training data's sample size, y_i is observed dependent variable, y_{ip} is the estimated dependent variable, and $M(\lambda)$ is called penalty function with λ terms (Eyduran et al., 2019).

MARS models using single response variables will be insufficient in cases with multiple response variables. In this context, it is possible to estimate the relationships between multiple response variables and explanatory variables more reliably with the same basis function and different coefficients with the multi-response MARS algorithm, which can be used in cases with many response variables (Aguilar-Quiñonez, 2023).

Multi response models go through the same processes as the single response MARS model; however, the squared errors are now averaged across all response variables when the common basis functions that provide the highest average in performance are selected (Hastie et al., 1994; Elith and Leatwick, 2007). The latest estimated MARS model uses a set of fundamental functions common to all variables and predicts a different set of coefficients for each basis function to obtain the optimal model.

In the evaluation of the model within the scope of model comparison criteria, the obtained model results require a lower standard deviation ratio (SDratio), root mean square error (RMSE), mean absolute percentage error (MAPE), relative root mean square error (rRMSE), global relative approximation error (RAE), Akaike's information criterion (AIC) and high determination coefficient (R^2) and Pearson's correlation coefficient (r) values are required for each response variable (Tatliyer, 2020).

All statistical processes were completed by using R software (R Core Team, 2022). To get an information about the data, descriptive statistic was provided by using "psych" package in R software (Revelle, 2022). The "earth" package was used to provide the results of multi response MARS algorithm (Milborrow, 2021). To show and assessment of the model performances, the "ehaGoF" package was used (Eyduran, 2020).

RESULTS

Descriptive statistics for the data obtained as a result of the analyses are given in Table 1 below as mean, standard deviation, maximum and minimum values. In Table 1, The SG and RG variables are relatively stable and low variance variables with low standard deviations and are in a narrow range. While the SoLG and SoaG variables show a medium level of variation, SobG stands out as the most variable variable with the highest standard deviation. On the other

hand, SoPhG exhibits a fairly stable distribution with a low standart deviation. This table summarizes the distribution characteristics of each variable, allowing us to understand which variable are more stable and which are more variable in the data set.

Table 1. Descriptive statistics for the data

	Mean	Standard Deviation	Min	Max
SG	0,58	0,1	0,31	0,69
RG	0,34	0,07	0,25	0,53
SoLG	42,75	3,33	34,89	51,47
SoaG	19,33	1,3	17,2	22,05
SobG	5,9	1,98	3,69	11,64
SoPhG	5,79	0,25	5,59	6,73

SG: Springiness Breast, RG: Resilience Breast, SoLG: Cold L Breast, SoaG: Cold a Breast, SobG: Cold b Breast, SoPhG: Cold pH Breast

In Figure 1, these graphs are visualizations of various analyses used to evaluate the performance and fit of the Multi response MARS (Multivariate Adaptive Regression Splines) model. The model selection graph shows that the model selects the optimal number of terms with the lowest error value and that the model's performance is optimized at this point. The cumulative distribution graph indicates that most of the prediction errors are concentrated at low values, thus the model generally produces small errors and makes successful predictions. The graph showing the distribution of residuals against predictions reveals that the errors are randomly distributed and the model does not contain any systematic errors. The Q-Q plot shows that the error terms of the model are normally distributed, supporting the robustness and reliability of the model.

When all these analyses come together, it can be concluded that the Multi response MARS model performs well against multiple response variables and provides reliable predictions. These results reveal that the model is suitable for use in further analyses and provides successful prediction performance.

In Figure 2, these graphs evaluate the performance of the Multiresponse MARS model for the RG response variable. The model selection graph determined the optimal number of terms with the lowest error value and this model was selected as the most suitable option for RG. The cumulative distribution graph shows that the majority of the prediction errors are concentrated at low values and the model generally produces small errors. The random distribution of the residuals against the predictions shows that the model does not contain any systematic error and provides a good fit to the data. The Q-Q plot shows that the error terms are normally distributed, thus the model is robust and reliable. In general, the MARS model shows that it provides reliable predictions by exhibiting high performance for RG.

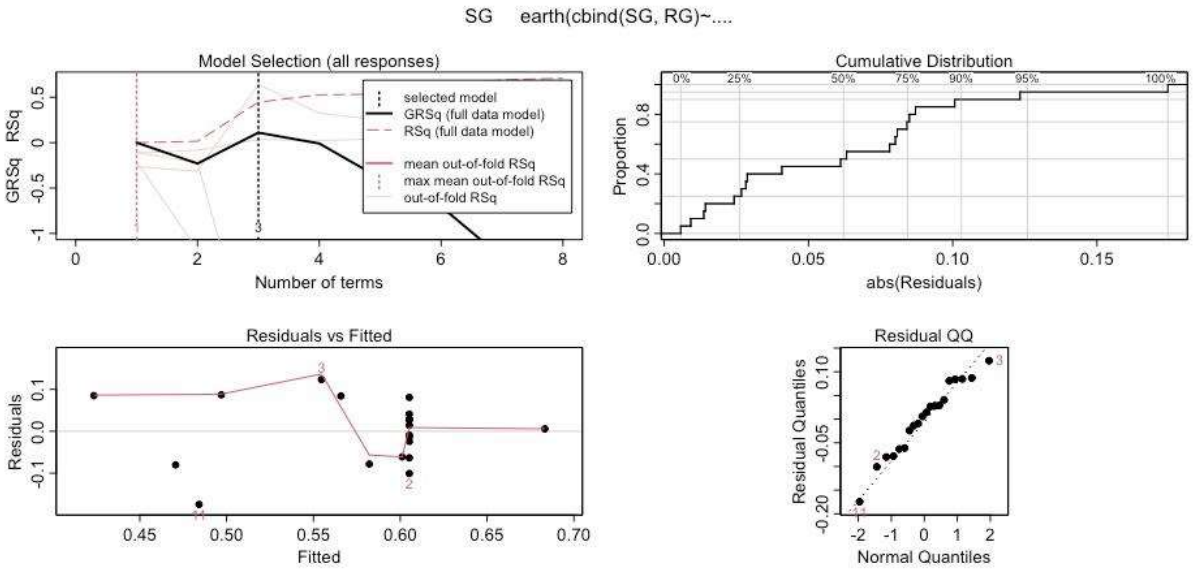

Figure1. Performance Evaluation and Residual Distribution of the MARS Model for SG

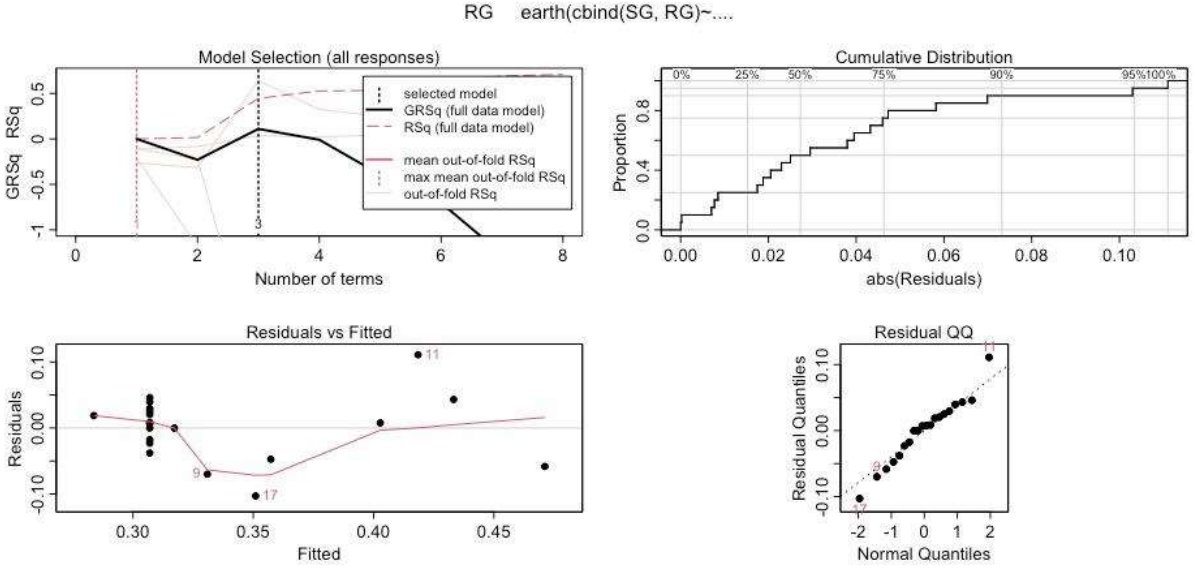

Figure2. Performance Evaluation and Residual Distribution of the MARS Model for RG

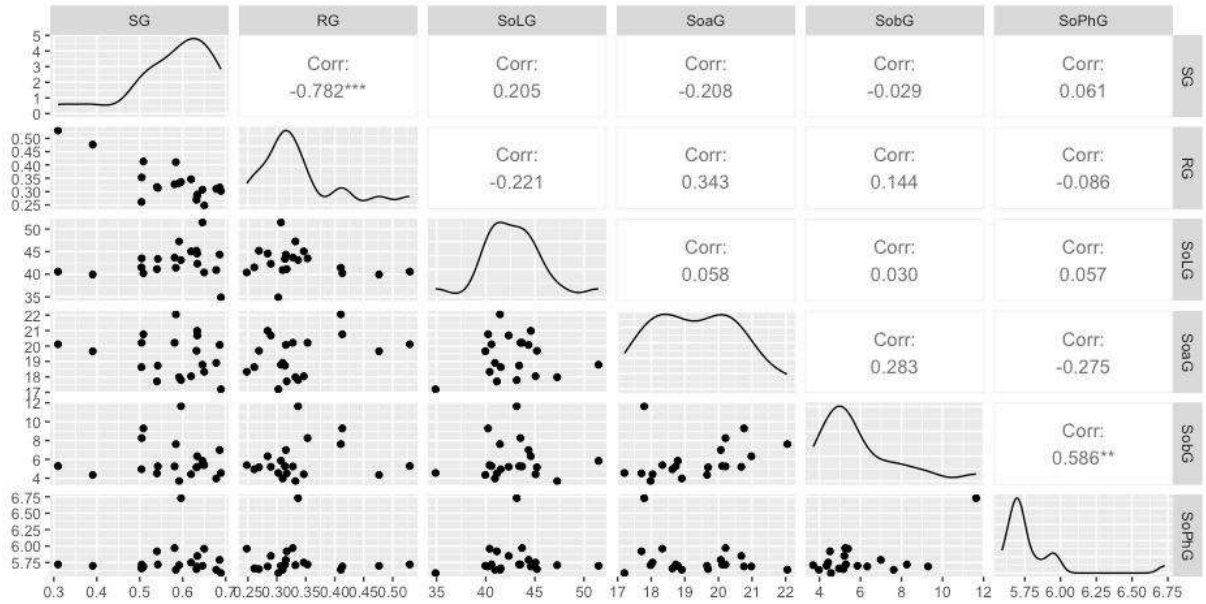

Figure 3. Correlation matrix**.**

Looking at Figure 3, it is understood that the relationship between SG and RG variables is statistically significant (P<0.05) and there is a high and negative correlation between them (-0.782). It is observed that there is a positive correlation between SobG and SoPhG variables and the relationship is statistically significant. It was determined that there was generally weak correlation between other variables.

Table 2. Model of the multi response MARS model

Function	Description	SG	RG
Intercept	(Intercept)	0,605	0,307
Basis function 1	h(42.33-SoLG)	0,479	-0,396
Basis function 2	h(42.33-SoLG) * SoaG	-0,027	0,023

According to Table 2, it was observed that the first term of the MARS model, the intercept, was 0.605 and 0.307 for SG and RG, respectively. In the model, the coefficients for SoLG with the first common basis function value of 42.33 were determined to be 0.479 and -0.396, respectively. It was understood that there was an interaction between SoL and SaG, which is the second common basis function of the model, with a value of 42.33. According to the results obtained, there is a change of -0.027 units in the SG variable and 0.023 units in the RG variable for the SoaG value and SoLG value.

Table 3. Goodness-of-fit criteria.

Criterion	RG	SG
Root mean square error (RMSE)	0,047	0,074
Relative root mean square error (rRMSE)	13,869	12,854
Standard deviation ratio (SDR)	0,676	0,779
Coefficient of variation (CV)	14,23	13,19
Pearson's correlation coefficients (r)	0,737	0,627
Performance index (PI)	7,984	7,898
Mean error (ME)	0	0
Relative approximation error (RAE)	0,018	0,016
Mean relative approximation error (MRAE)	0,03	0,028
Mean absolute percentage error (MAPE)	10,803	12,033
Mean absolute deviation (MAD)	0,036	0,061
Coefficient of determination (R^2)	0,543	0,394
Adjusted coefficient of determination (ARsq)	0,457	0,28
Akaike's information Criterion (AIC)	-116,514	-98,128
Corrected Akaike's information criterion (CAIC)	-115,014	-96,628

Looking at Table 3, it is understood that the R^2 values for the SG and RG response variables of the MARS model are 0.543 and 0.394, respectively. When we look at the Pearson's correlation coefficients, it is observed that the highest value belongs to the SG variable with 0.737 and the correlation coefficient for the RG variable is 0.627. It is understood that the AIC values determined in the MARS model are -116.514 for SG and -98.128 for the RG variable.

DISCUSSION

Germani Adrian ´ Munoz-Osorio et al. (2024) aimed to predict carcass tissue composition using real-time ultrasound measurements (USM) of fat thickness and longissimus thoracis (LT) traits in Black Belly sheep lambs through multi-response multivariate adaptive regression splines (MARS) algorithms. Sixty Black Belly ewe lambs with a body weight (BW) of 26.40 ± 7.01 kg were used. According to the results obtained, TCB (Total carcass bone), TCM (Total carcass muscle), TCF (Total carcass fat) provided the best results for the multi-response MARS model. R^2 values were found as 0.763, 0.953, 0.650, respectively. It was determined that Pearson's correlation coefficients for the same variables ranged between 0.806 and 0.976.

Jos´e Antonio Aguilar-Quinonez et al. (2023) aimed to predict the carcass tissue composition of hair sheep lambs using a multi-response multivariate adaptive regression spline algorithm. The study was conducted on 66 hair lambs (39 Pelibuey and 27 Katahdin sheep breeds). According to the findings obtained from the MARS algorithm, R^2 values for HCW (Hot carcass weight), CCW (Cold carcass weight) CSTW (carcass soft tissue weight) CBWE (Carcass bone weight) variables vary between 0.945 and 0.987. It was determined that there were high correlation values between the variables. These values were between 0.993 and 0.972.

CONCLUSIONS

The aim was to demonstrate the applicability of the MARS algorithm in the prediction of Peking duck breast meat quality using pH and color data. As a result, the data structure being different from other studies may be the reason why similar results were not obtained. The model may not have been a perfect fit because the analyzed sample size was lower than similar studies. Studies can be continued to demonstrate its applicability in different data structures using the MARS algorithm.

ACKNOWLEDGEMENTS

The authors acknowledges that this study was supported by Ondokuz Mayıs University Scientific Research Office with project number of PYO.ZRT.1901.23.005.

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Effectiveness of model comparison criteria

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Abstract

In this study, the effectiveness of the comparison criteria used in modeling growth curves, lactation curves and egg production curves was investigated. For this purpose, two models commonly used in the literature were considered for three different curves. The mean square error, coefficient of determination, corrected coefficient of determination, Durbin-Watson autocorrelation coefficient, Akaike information criterion, Bayesian information criterion, bias factor and accuracy factor values were calculated on the models considered. The effectiveness of the values obtained as a result of the calculation in model comparison and defining model adequacy was examined. On the other hand, it has been determined which criteria should be mandatory in model comparison and model adequacy and which criteria are supportive. As a result of the study, it is aimed to guide researchers in modeling studies in different fields and to conduct more accurate and effective modeling studies.

Key words: Modelling, lactation, growth, egg yield, comparison criteria.

INTRODUCTION

Curve modeling is widely used in the field of poultry and ruminant animal breeding. These studies are concentrated in the field of growth curves (Hojjati and Zadeh, 2018, Tahtal, at al. 2020), daily and cumulative egg yield curves in poultry farming, and growth (Brody, 1945), lactation (Ferris at al., 1985) and gas production curves in ruminant animal nutrition. Especially in breeding studies, selection and culling processes, individual curves have become among the determining criteria. In other words, it provides an important indicator for researchers working in the relevant field to determine the individuals with the highest milk yield, the best live weight gain or the best egg yield in animals (Yavuz at al.,2019). In breeding studies that take many years, the selection or selection of the right individuals at the beginning is extremely important. In the field of animal nutrition, the correct determination and interpretation of gas production curves obtained from in vitro or in vivo studies will lead to a more accurate evaluation of feed resources and therefore a positive reflection on yield. Many different models are used in curve modeling and new ones are added every day in parallel with the developments in the field of computers and software. There are many criteria used in determining the statistical adequacy of the equations used in curve modeling in the field of poultry and ruminant animal breeding and in comparing the equations. The most commonly used among these are the mean square error, coefficient of determination, corrected coefficient of determination, Durbin-Watson autocorrelation coefficient, Akaike information criterion, Bayesian information criterion, bias factor and accuracy factor values. While some of these criteria focus on how much of the existing variation the obtained equations can explain, some are comparison criteria that take into account the relationships between error terms or are calculated over error terms. In most of the existing studies, the coefficient of determination and the mean square error are generally seen. The relationships between the error terms of the model are

generally ignored. This situation actually causes inadequate models to be selected and incorrect interpretations to be made. The presence of as many evaluation criteria as possible in modeling studies will result in the selection of statistically correct models. In this study, growth, lactation and egg yield curves were addressed with two models commonly used in the literature and the effectiveness of the evaluation criteria was investigated. At the end of the study, issues such as which criteria must be included in the studies and which criteria support each other will be addressed and an important resource will be provided to researchers.

MATERIALS AND METHODS

In the study, as material, live weight gains of broiler chickens for 9 different days for growth curves, milk yields of Holstein dairy cattle for 10 different control milk days for lactation curves and 71-day egg yield averages of Lohman Brown breed for egg yields were used. For growth curves (Anthony at al., 1986), lactation curves (Landete-Castillejos and Gallego, 2000)) and egg yield curves (Gavora at al., 1982, McMillan, 1981, Prasad and Singh, 2008), the two most commonly used models in the literature were considered in the study. For this purpose, Gompertz and Logistic equations were used in modeling growth curves, Wood and Wilmink (Motulsky and Ransnas, 1987, Perochon at al., 1996) in modeling lactation curves, and Mcnally and Modified Compartmental equations were used in modeling egg yield curves (Ganesan at al., 2017, McMillan at al., 1986). The equations used are given in Table 1. In the evaluation of model adequacy and comparison of models, mean square error, coefficient of determination, corrected coefficient of determination, accuracy factor, deviation factor, Durbin-Watson autocorrelation test, Akaike information criterion and Bayesian information criterion values were used. In the created models, it is desired that mean square error is low, determination and corrected coefficient of determination are high, accuracy and deviation factor are around 1 and Durbin-Watson autocorrelation value is around 2. The model with the lowest values of Akaike information criterion and Bayesian information criterion is preferred. The models of growth, lactation and egg yield curves used in the study are given in Table 1, and model evaluation and comparison criteria are given in Table 2.

Table 1. Equations used in modeling growth, lactation and egg yield curves.

Curve Type	Models	Equations
Growth	Gompertz	$Y_t = \beta_0 exp(-\beta_1 exp(-\beta_2 t))$
	Logistics	$Y_t = \beta_0 (1 + \beta_1 exp(-\beta_2 t))^{-1}$
	Wood	$Yt = \beta_0 t^{\beta 1}.\exp(-\beta_2 t)$
Lactation	Wilmink	$Yt = \beta_0 + \beta_1 t + \beta_2 exp -0{,}05t$
	McNally	$Y_t = \beta_0 t^{\beta_1} \exp(-\beta_2 t + \beta_3 t^{0.5})$
Egg Yield	Modified Compartmental	$Y_t = \beta_0 exp(-\beta_1 t)/(1 + \exp((-\beta_3 (t - \beta_4))$

** Here, Y_t: observed value on day t, β, β_0, β_1, β_2, β_3 and β_4: the constants defined for the models and t; represents time.

Table 2. Model Comparison Criteria

Criteria	Equations
Mean square error	$MSE = ESS/DFE$
Coefficient of determination	$R^2 = 1 - (SSE/S_y^2)$
Corrected coefficient of determination	$\bar{R}^2 = 1 - (1 - R^2)(n - 1/(n - p - 1))$
Accuracy factor	$AF = 10^{\sum_{i=1}^{n} \lvert \log(\hat{Y}_i - Y_i) \rvert / n}$
Bias Factor	$BF = 10^{\sum_{i=1}^{n} \log(\hat{Y}_i - Y_i)/n}$
Durbin-Watson	$DW = \frac{\sum_{i=2}^{n} (e_1 - e_2)^2}{\sum_{i=1}^{n} e_1^2}$
Akaike information critteria	$AIC = nxln\left(\frac{SSE}{n}\right) + 2k$
Bayesian information critteria	$BIC = nxln\left(\frac{SSE}{n}\right) + kln(n)$

** MSE: mean square error, ESS: error sum of squares, DFE: degrees of freedom for error, S_y^2 ;the partial variance of the y-variable, $\hat{Y}_i$: estimated value, Y_i: observation value, n: simple size, p: number of indipendent variable e_i: error term, k: number of parameters.

RESULTS

The mean square error, coefficient of determination, corrected coefficient of determination, Durbin-Watson autocorrelation coefficient, Akaike information criterion, Bayesian information criterion, bias factor and accuracy factor values of the models created for growth, lactation and daily egg yields were obtained

as in Table 1. Growth curves, lactation curves and daily egg yield curves are given in Figure 1, Figure 2 and Figure 3. When Table 3 is examined, it is seen that the mean square error, coefficient of determination, and corrected coefficient of determination values are better in the Logistic model. It is seen that the accuracy factor value is the same, and the bias factor value is better in the Logistic model. It is seen that the Durbin-Watson autocorrelation value is closer to the value of 2 in the logistic model and there is no autocorrelation problem in both models. The logistic model has smaller values in terms of Akaike information criterion and Bayesian information criterion and brings the logistic model, which is the model indicated by the previous criteria, to the fore. When the lactation curves are examined in Table 3, it is seen that the Wilmik model has a lower degree in terms of mean square error, both models have equal values in terms of coefficient of determination, and the Wilmink model is again better in terms of corrected coefficient of determination value. It is seen that the accuracy factor value is the same, and the bias factor value is better in the Wilmink model. It is seen that the Durbin-Watson autocorrelation value is equal in both models. It is seen that the Wilmink model has smaller values in terms of Akaike information criterion and Bayesian information criterion. Similarly, when the daily egg yield curves are examined in Table 3, it is seen that the Modified Compartmental model has a lower value in terms of mean square error. It is seen that the coefficient of determination, corrected coefficient of determination value, accuracy factor and bias factor values are equal in both models. It is seen that the Durbin-Watson autocorrelation value is equidistant from the value of 2 in both models. In terms of Akaike information criterion and Bayesian information criterion, it is seen that the Modified Compartmental model has smaller values. As a result, when the comparison criteria of the models created for growth, lactation and daily egg yields are evaluated as a whole, it is possible to say that the logistic model has better results in growth curves, the Wilmink model in lactation curves and the Modified Compartmental model in egg yield curves.

Table 3. Comparison criteria for growth, lactation and egg production curves.

Curve Type		MSE	R^2	$\bar{R}^2$	AF	BF	DW	AIC	BIC
Growth	1	15423,12	0,988	0,981	1,2	0,8	1,5	91,73	92,32
	2	5018,18	0,994	0,991	1,0	1,0	2,3	79,03	79,63
Lactation	3	0,271	0,999	0,994	1,0	0,9	1,0	-10,68	-9,72
	4	0,187	0,999	0,997	1,0	1,0	1,0	-14,35	-13,44
Egg Yield	5	1,106	0,997	0,997	1,0	0,9	1,9	11,04	20,09
	6	0,972	0,997	0,997	1,0	0,9	2,1	-0,31	8,73

**1: Gompertz, 2: Logistics, 3: Wood, 4: Wilmink, 5:McNally, 6: Modified Compartmental.

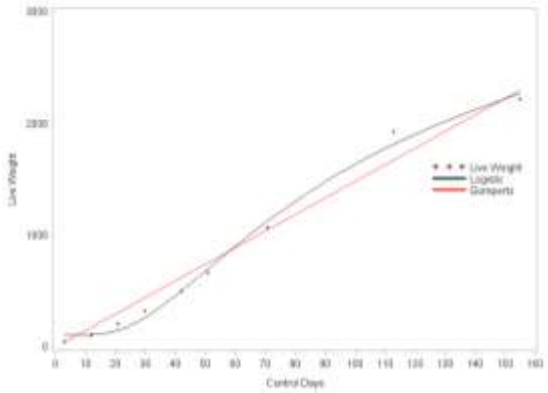

Figure 1. Growth curves.

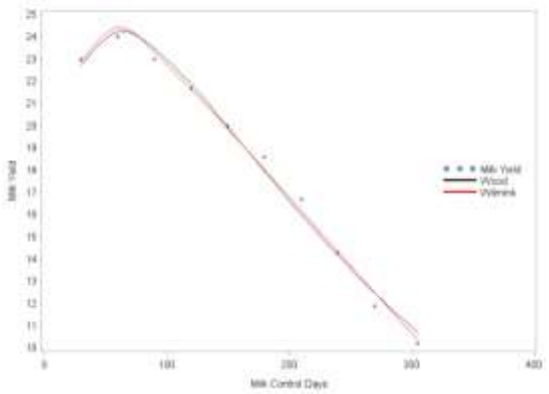

Figure 2. Lactation curves.

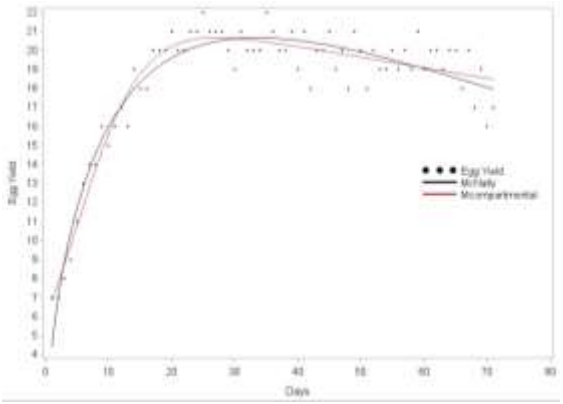

Figure 3. Daily egg yield curves.

DISCUSSION

As a result of the study, it was observed that as the difference between the models in terms of mean square error, coefficient of determination and corrected coefficient of determination increased, the Durbin-Watson autocorrelation coefficient, Akaike information criterion, Bayesian information criterion, bias factor and accuracy factor values calculated on the error terms gave results that supported this result. This situation is clearly seen in the modeling of growth curves. This situation is clearly seen even when models that are widely used in the literature and give similar results are included in the modeling. This situation will become even more apparent if other models used in the relevant field are included in the study. Positive or negative autocorrelation is an undesirable situation in a selected model. In this respect, it is possible to say that the Durbin-Watson autocorrelation value is the most effective determination criterion after the mean square error and coefficients of determination in the results obtained. If the coefficient of determination, corrected coefficient of determination, Durbin-Watson autocorrelation coefficient, bias factor and accuracy factor values are equal or very close, the Akaike information criterion and Bayesian information criteria become the most effective criteria. This situation was clearly seen in the modeling of both lactation curves and egg yield curves. It has been observed that when the mean square error, coefficient of determination and corrected coefficient of determination values are very close, the bias factor and accuracy factor values are equal or very close. This makes the bias factor and accuracy factor values the weakest model selection criterion. On the other hand, in the presence of the corrected coefficient of determination, it was observed that the coefficient of determination was not very effective. As a result, it is possible to say that it would be statistically appropriate to include mean square error, corrected coefficient of determination, Durbin-Watson test and at least one of the information criteria (Akaike or Bayesian) in the study for the purpose of comparing and evaluating the models.

AUTHOR CONTRIBUTIONS

The authors declare that they have contributed equally to the article.

CONFLICT OF INTEREST

The authors of the article declare that there is no conflict of interest between them.

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Genetic evaluation for some reproductive and productive traits in zaraibi goats

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Abstract

The aim of the present work was to estimate phenotypic and genetic parameters for productive variable (litter size at birth (LSB)), productive variables (birth weight (BW), weaning weight (WW) and average daily gain (ADG)) in 1577 Zaraibi kits born between 2108 to 2023 years. Two multi animal models were used, model 1,includes, the fixed effects of month and year of birth, type of birth and sex and the random effects of animals, permanent environmental effect and errors. Model 2 are similar to model 1 and added maternal genetic effects and covariance between direct and maternal genetic effects. Means of LSB, BW, WW and ADG were 2.32, 2.04 kg, 10.22 kg and 90 g, respectively. The statistical analyses showed that the fixed effects on all variables studied were generally significant (P P<0.01) except the effect of sex on LSB and type of birth on WW and ADG. Estimates of direct heritability (h2d) for LSB, BW, WW and ADG were 0.13, 0.30, 0.38 and 0.30, respectively as estimated from model 1 and the crossponding values were 0.07, 0.22, 0.22 and 0.25, respectively as estimated from model 2. Maternal heritability (h2m) for LSB, BW, WW and ADG were 0.15, 0.15, 0.12 and 0.14, respectively. Phenotypic and genetic correlations among all variables are positive and significant. The results in general showed that maternal effects were very important effect for pre- weaning growth variables. Therefore, maternal genetic effect should be taken into consideration when carrying out genetic progress of pre- weaning growth traits for Zaraibi goats.

Key words: *Phenotypic Genetic Parameters Zaraibi goats*

Genetic bottleneck analysis for conservation of poonchi chicken breed located in hilly areas of Jammu and Kashmir, India

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Abstract

In conservation studies population bottlenecks attributes to the loss of genetic variation in the population in due course of time due to drastic climate changes, predation, limited resources that lead to random fluctuation in the population size. Bottleneck effect within Poonchi chicken of Jammu and Kashmir was evaluated using ten number of microsatellite markers. The microsatellite markers namely ADL0268, MCW026, MCW0081, ADL0278, MCE0069, MCW0111, MCW0222, MCW0016, LEI0094 were analyzed in Poonchi chicken. A total of 72 alleles were observed with maximum alleles (9) contributed by locus MCW069, MCW0016 & and the lowest alleles (6) by ADL0268, ADL0278, MCW022. The average observed heterozygosity was 0.8520 with standard deviation of 0.1331, whereas the average expected heterozygosity was 0.8110 with standard deviation of 0.0471. The expected numbers of loci with heterozygosity excess in Poonchi chicken were 5.97 (P<0.05), 5.93 (P<0.05), 5.90 (P<0.05) for Infinite Allele Model (IAM), Two Phase Model of Mutation (TPM) model and Stepwise Mutation Model (SMM), respectively in Sign test. The IAM, TPM and SMM values for one tail for heterozygosity excess in Wilcoxon rank test revealed significant values of (P<0.05) deviation indicated all the loci deviates from mutation-drift equilibrium. The qualitative test for allele frequency showed a slight shift from normal L-shaped curve suggesting the recent bottleneck effect in the population. The presence of genetic bottleneck might have affected the number of alleles and resulted in loss of several effective alleles. Furthermore, loss of several effective alleles suggests urgent need of designing effective breeding policy to conserve this unique native germ plasm of Jammu and Kashmir. This study on native chicken of Poonch region is the first report indicating genetic bottleneck effect.

Key words: *Genetic bottleneck, Poonchi chicken, microsatellite, heterozygote*

"- OMICS" Based approaches in sheep nutrition

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Abstract

The term '-omics' denotes many biological disciplines in biology characterised by titles ending in the -omics prefix, including genomics, proteomics, metabolomics, transcriptomics,. The purpose of omics is to facilitate the comprehensive identification and measurement of groups of biomolecules that govern the structure, function, and dynamics of organisms. The application of emerging omics technologies is seeing a growing prevalence within the realm of animal agriculture. Omics technology significantly enhances comprehension of the genetic structure of animals that influences important economic characteristics. Utilising these omics technologies enables the investigation of animal metabolism in response to a particular stimulus. This methodology enables the examination of how environmental factors (such as temperature and humidity), diet, sex, and welfare affect the physiological processes of animals at the molecular level. Currently, nutrigenomics is a rapidly advancing field of research that investigates how dietary components affect genome functioning in terms of gene expression patterns and epigenetic alterations, including DNA methylation and histone modifications. The novel RNA-Seq technology for transcriptome studies of animals is acknowledged as a potent method to elucidate molecular processes related to nutrient-gene interactions. The present review centres on the potential applications of '-omics' based methodologies in the field of livestock husbandry, specifically highlighting the domain of sheep nutrition.

Key words: *Sheep, Nutrition, -omics, nutrigenomics, Transgenomics, Metabolomics*

INTRODUCTION

In order to guarantee the availability of nourishing food, the overall health and well-being of humans and animals, and the preservation of the environment, it is imperative to implement livestock production in the most efficient and sustainable manner feasible for a successful food security programme supporting a growing population. Fundamental research will yield innovative and technical advancements that enhance all facets of food production. A significant obstacle confronting the livestock sector is the need to satisfy the increasing demand for products derived from cattle. Diminished fertility, immunological response, feed efficiency, and production efficiency in animals adversely affect the cattle sector. Enhancing animal health, performance, and overall wellbeing is the proposed answer. The application of emerging omics technologies is more prevalent in the realm of animal production. In biology, the term "-omics" encompasses various disciplines with names ending in the -omics prefix, including genomics, proteomics, metabolomics, transcriptomics, and others. The purpose of omics is to facilitate the comprehensive identification and measurement of groups of biomolecules that govern the structure, function, and dynamics of organisms (Subedi et al., 2022). The analysis enables the examination of how environmental factors (such as temperature and humidity), nutrition, sex, and wellbeing affect the physiological processes of farm animals at the molecular level (Ribeiro et al., 2020). The term "-omics" originates from the suffix '-ome', which is derived from a Greek word that signifies 'whole', 'all', or 'complete'. 'The suffix '-omics' commonly denotes a discipline within the biological sciences that focusses on analysing extensive and high-throughput data/information to get insights into life, defined by 'omes' (Yadav, 2007). The past twenty years have seen the development of several omics tools for the collection and analysis of high-throughput data on proteins (proteomics), mRNA

transcripts (transcriptomics), gene sequences (genomics), microbial diversity (metagenomics), epigenetic control of gene expression (epigenomics), metabolic profile (metabolomics), lipid profile (lipidomics), and other aspects of a specific cell, tissue, organ, or whole organism at a certain moment in time. Genomics, metagenomics, metabolomics, proteomics, transcriptomics, epigenomics, translatomics and other omics technologies can facilitate the quick and effective identification of phenotypic alterations, dietary responses, and inherent phenotypic tendencies in animals (Mu et al., 2022; Wang et al., 2022). Therefore, the use of omics tools in animal selection and breeding programmes is expected to provide an accurate estimate of breeding value for early selection, reduce the production interval and increase the rate of genetic gain (Figure 1). Omics technologies are effective tools, especially when used in combination with advanced molecular and breeding methods. In particular, genomic research has the potential to improve the precision and efficiency of traditional breeding and advanced breeding approaches by increasing consistency and predictability (Ahmad et al., 2023).

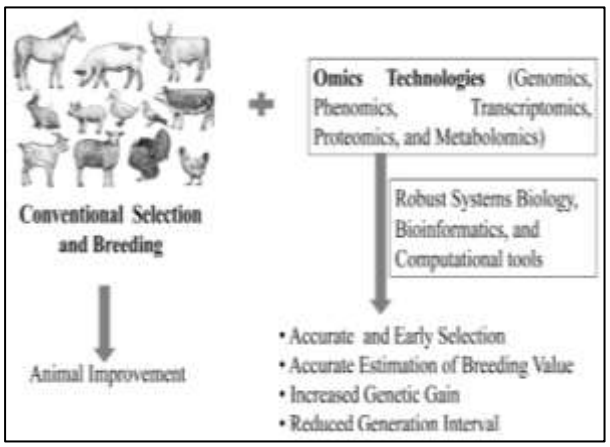

Figure 1. Impact of omics technology in animal improvement (Chakraborty et al., 2023)

The application of -omics technologies (genomics, transcriptomics, proteomics, and metabolomics) has revolutionized our understanding of sheep nutrition by unraveling molecular mechanisms underlying dietary interactions and metabolic pathways. This comprehensive review explores the current applications and future potential of -omics based approaches in advancing sheep nutrition research. By elucidating molecular insights into gene expression, protein synthesis, metabolic profiles, and the interplay with dietary components, -omics technologies offer valuable tools to optimize nutritional strategies for enhanced productivity and health in sheep.

Genomics in Sheep Production

Genomics has significantly advanced our understanding of sheep nutrition by identifying genetic markers associated with important production traits and metabolic pathways. Kijas et al. (2012) conducted a genome-wide association study (GWAS) to explore SNP variation across different sheep breeds, revealing genetic markers linked to traits such as wool production and quality. Their findings underscored the genetic diversity within sheep populations and highlighted genomic regions influencing economically relevant traits, providing a foundation for selective breeding programs aimed at improving productivity and adaptability.

In sheep, the use of GWAS has been facilitated by the development of microarrays or DNA chips, which identify genetic markers associated with traits of interest. Companies like Illumina and Affymetrix have developed various genotyping platforms, with the OvineSNP50 chip being the most commonly used. This chip contains over 54,000 SNPs and has been employed to analyze samples from multiple sheep breeds, including both domestic and wild species (Zhang et al. 2012).

Early GWAS in sheep identified key genetic factors for traits such as horn development. For example, the RXFP2 gene was associated with horn presence and type (Johnston et al., 2011). Additionally, studies on diseases like rickets in Corriedale sheep pinpointed the R145X mutation in the DMP1 gene as a cause (Zhao et al., 2011). Research on meat quality traits linked the Callipyge gene with improved muscle deposition (Nanekarani and Goodarzi, 2014), while other studies identified genes associated with muscle

formation, fatty acid profiles, and stress responses (Guðmundsdóttir, 2015; Pant et al., 2016; Aali et al., 2017).

Recent research has also explored the genetic underpinnings of body size and wool characteristics, revealing several new genes and markers. Notable findings include associations with genes involved in pigmentation, morphology, and milk production (Kominakis et al., 2017; Rochus et al., 2018).

Rovadoscki et al. (2018) investigated the genetic basis of fatty acid composition in Brazilian sheep breeds using GWAS and identified promising genomic regions associated with lipid metabolism. Their study highlighted genetic variants influencing the synthesis and deposition of fatty acids in sheep tissues, offering insights into dietary strategies to modulate meat quality and lipid profiles in sheep production systems.

To further understand the genetic underpinnings of nutritional efficiency in sheep, recent studies have applied advanced genomic techniques such as whole-genome sequencing (WGS) and transcriptomic profiling. In their work, Xiang et al. (2024) performed a genome-wide association analysis (GWAS) including four distinct periods of baby weight (BW) development: birth, weaning, six months, and 12 months. Their investigation revealed the presence of five novel candidate genes, namely MAP3K1, ANKRD55, ABCB1, MEF2C, and TRNAW-CCA-87. Upon additional analysis, these genes were shown to be involved in pathways associated with growth hormone and energy metabolism. The findings suggest that these genes have the ability to impact the growth and development of sheep, so offering meaningful understanding of the genetic processes that underlie characteristics related to body weight and guiding sheep breeding methods. Their study emphasized the role of genomic diversity in shaping dietary responses and metabolic adaptations in sheep, highlighting opportunities for precision breeding to enhance nutritional efficiency and sustainability in sheep production.

Nutrigenomics Approach

Utilising genomic information in ruminant production systems can help alleviate concerns about food safety and sustainability of production. Nutritional genomics (nutrigenomics) is the field of research concerned with any interplay between nutrients and the genomes of organisms, i.e. variable patterns of gene expression and the impact of genetic variations on the nutritional environment (Mathers, 2017; Müller and Kersten, 2003; Sales et al., 2014). Ruminant nutrigenomics, a relatively new field that has developed a revolutionary analytical approach to traditional ruminant nutrition research, has several studies on different aspects of animal production systems (Kızılaslan et al., 2022). Comprehending the precise binary interactions between genes and nutrients will eventually facilitate the tailoring of diets to promote the development of specific traits in individuals or groups. In pursuit of this objective, nutrigenomics, a recently established scientific discipline, applies information from several clearly defined disciplines as outlined in Figure 2 (Kızılaslan et al., 2022).

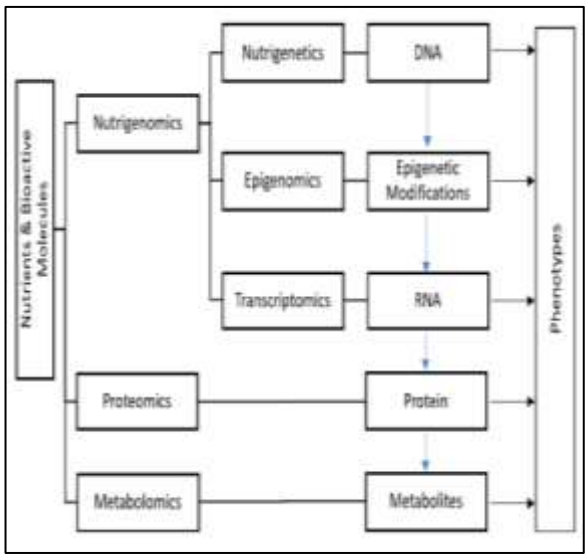

Figure 2. Investigation of phenotypic interactions between nutrigenomics and several other life sciences (Kızılaslan et al., 2022).

Currently, nutrigenomics is a rapidly advancing field of research that investigates how dietary components affect genome functioning in terms of gene expression patterns and epigenetic modifications, including DNA methylation and histone

modifications (Bordoni and Gabbianelli, 2019 Figure 3).

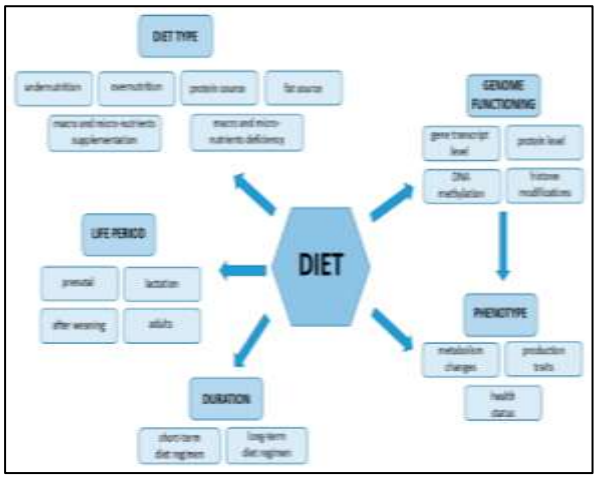

Figure 3. Summary of nutritional factors investigated in nutrigenomic research (Nowacka-Woszuk, 2020).

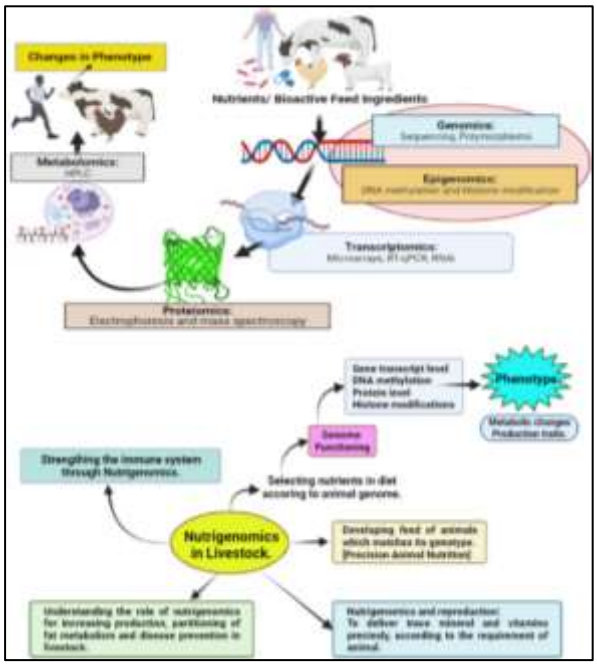

Figure 4. Utilisation and integration of omics technology in nutrigenomics and animal nutrition research (ul Hag et al., 2022).

Transcriptomics Insights

In sheep, transcriptomics provides a dynamic perspective on gene expression patterns in reaction to nutritional treatments and environmental conditions. This field of study is very recent in the realm of ruminant animals, particularly in sheep and goats. Genetic expression is influenced by dietary chemicals either directly or indirectly by their interactions with transcription factors, such as ligand-dependent nuclear receptors. This paper discusses novel findings derived from the application of functional analysis to transcriptome, proteomic, and metabolomic data sets in goats and sheep (Osorio et al. 2017). In their study, Osorio et al. (2017) highlighted the potential of omics and bioinformatics tools to enhance our comprehension of the various degrees of regulation caused in small ruminants by dietary nutrients when used for milk, meat, wool, or reproduction. The initial findings suggest that adopting a nutrigenomic approach may eventually result in more accurate management of goats and sheep, hence facilitating a more efficient use of feed resources.

The study conducted by Wu et al. (2020) employed multi omics approaches to integrate feed nutrient value, sheep microbiome, transcriptome, metabolome, and fatty acid profile in order to examine the mechanisms that influence meat quality in twin ewes that were fed either high fibre low protein (HFLP) roughage (Ceratoides) or low fibre high protein (LFHP) roughage (alfalfa) dishes. The findings indicated that the performance of lamb production and the composition of muscle were notably influenced by the feed. Gas chromatography-mass spectrometry study revealed an increase in the essential fatty acid (linoleic acid and arachidonic acid) content of muscle in lambs fed HFLP. Moreover, the bacteria Bacteroidetes and Firmicutes in the rumen of ewes fed with LFHP were 2.6 times more abundant than in the group fed with HFLP. Comparative transcriptome analysis of muscle showed significant differential expression of genes associated with glucose metabolic pathways and fatty acid production in the two groups. An investigation revealed possible intercommunication across the four omics data layers, elucidating the process by which feed components impact the meat quality of lambs.

Utilising microarrays and RNA arrays, it is now possible to quantify the expression of nearly all transcribed genes in a given sample, hence establishing the

transcriptome. A transcriptome is a comprehensive collection of all transcripts that are currently encoded in a cell (Lowe et al., 2017). Advancements in transcriptome research have enhanced our comprehension of RNA-based gene regulation networks as a cutting-edge high-throughput sequencing method. In 2007, Osorio et al. conducted the first nutrigenomic analysis of goat mammalian transcriptome responses in feed-deprived goats, marking the first instance of such an analysis in small ruminants. The findings of this study indicate that genes associated with the reduction of milk fat, lactose, and protein, as well as genes relevant to the inhibition of cell proliferation and differentiation in mammalian cells, and an elevation in programmed cell death, play a role in the early development of mammals. RNA sequencing has been employed since 2011 and has lately superseded microarray techniques in the field of applied genomics. Previous research has employed this method to investigate the transcriptome and micro RNAome of small ruminants, including goats and sheep (Martyniuk et al., 2020).

High-throughput RNA-seq has emerged as the preferred technique for animal nutritionists to create meal or feed additives and to provide a foundation for enhancing animal growth, health, and productivity. This approach has been employed to examine worldwide patterns of gene expression in tissues associated with economically significant characteristics such as livestock productivity (Alexandre et al. 2015) or to quantitatively identify genes or transcripts that may serve as potential indicators for production characteristics (Han et al., 2015). Thus, the new RNA-Seq technology for transcriptomic studies of living organisms is acknowledged as a potent method to enhance our understanding of molecular processes related to nutrient-gene interactions. However, its implementation encounters certain technical obstacles in both experimental and computational aspects (ul Haq et al. 2022).

Three recent studies explored different aspects of rumen development in lambs and dairy calves in response to varying diets and dietary fiber sources.

Sun et al. (2021) investigated how solid diets influence rumen growth and maturation in Hu lambs. They found that feeding lambs goat milk powder alone or with alfalfa hay or concentrate starter led to increased concentrations of volatile fatty acids (VFA) and microbial crude protein (MCP), along with greater rumen weight and papilla area in the hay and concentrate groups compared to milk alone. Transcriptomic analysis revealed that different diets affected gene expression related to VFA metabolism and immune responses. Specifically, alfalfa hay improved immune function, while concentrate starter enhanced nutrient transport and metabolism.

Nishihara et al. (2023) focused on the weaning transition in dairy calves, examining how short-chain fatty acid (SCFA) levels and associated microbial communities affect the rumen epithelium. Their study showed that SCFA metabolism pathways were up-regulated and apoptosis pathways down-regulated after weaning. They also observed a positive correlation between SCFA levels and genes involved in SCFA absorption and metabolism, with certain microbes like Rikenellaceae and Campylobacter influencing these processes. The findings suggest that SCFA levels and microbial communities play significant roles in rumen epithelial function during weaning.

Liu et al. (2023) compared the effects of different fiber sources-alfalfa hay (forage) versus soybean hull (non-forage)-on rumen development in Hu lambs. They identified that soybean hulls increased immune function and nitrogen utilization, while alfalfa hay supported better rumen morphological development. This study highlighted the differential impact of fiber sources on rumen structure and function.

Together, these studies underscore the importance of diet and fiber source in shaping rumen development, with implications for optimizing feeding strategies in lambs and calves to enhance growth and health.

Antioxidants And Sheep Welfare / Omics Based Approaches

Antioxidants are essential for preserving redox equilibrium, reducing oxidative stress, and improving immunological function in

sheep. Various dietary antioxidants, including vitamins C and E, selenium, and polyphenols, effectively neutralise free radicals and reactive oxygen species (ROS), therefore safeguarding cellular components against oxidative assault. Various field investigations have shown that dietary antioxidant supplementation is effective in enhancing antioxidant status and health outcomes in sheep subjected to different stress situations.

Recent advancements in -omics technologies have facilitated the characterization of antioxidant metabolism and oxidative stress responses in sheep. Genomic studies have identified genetic variants influencing antioxidant enzyme activities and redox regulation in sheep breeds adapted to different environmental conditions. Transcriptomic analyses have elucidated gene expression profiles of antioxidant enzymes and stress-responsive pathways in sheep tissues under oxidative stress conditions. Proteomic and metabolomic approaches have provided insights into protein expression patterns, metabolic biomarkers, and antioxidant metabolites associated with dietary antioxidant interventions in sheep.

Numerous field studies assessing the impact of dietary antioxidants on the health and performance of sheep have yielded significant knowledge regarding their mechanisms of action and practical uses (Ochoa et al., 1992; Lopez-Bote et al., 2001; Lauzurica et al., 2005; Leal et al., 2018; Leal et al., 2019). In a recent study, Leal et al. (2020) examined the effects of adding vitamin E and rosemary extracts to lamb diets on the quality of meat and the ability of sheep to withstand oxidation. They found that the inclusion of these supplements resulted in increased antioxidant capacity and decreased lipid oxidation in the meat samples. Their study emphasised the significance of dietary antioxidants in enhancing the quality and maintaining the shelf life of products in sheep production systems.

Research has indicated that comparable therapies can effectively decrease oxidative stress on proteins and lipids in broiler chickens. Additionally, piglets exhibit reduced omega 6 / omega 3 and PUFA/MUFA ratios compared to the control group. This reduction in oxidative stress may also have a positive impact on the health of ruminants (Skaperda et al., 2019). The aforementioned observations highlight the need of providing natural antioxidants with advantageous characteristics to enhance the redox state of farm animals and further animal welfare. Quantification of oxidative damage in livestock is crucial for comprehending the fundamental processes associated with illnesses and metabolic pathologies. Hence, to prevent the high mortality rates of sheep and other farm animals caused by oxidative diseases and safeguard their tissues from the detrimental oxidation process, it would be beneficial to examine and investigate their redox molecular status by quantifying particular redox markers (Figure 5) (Skaperda et al., 2019).

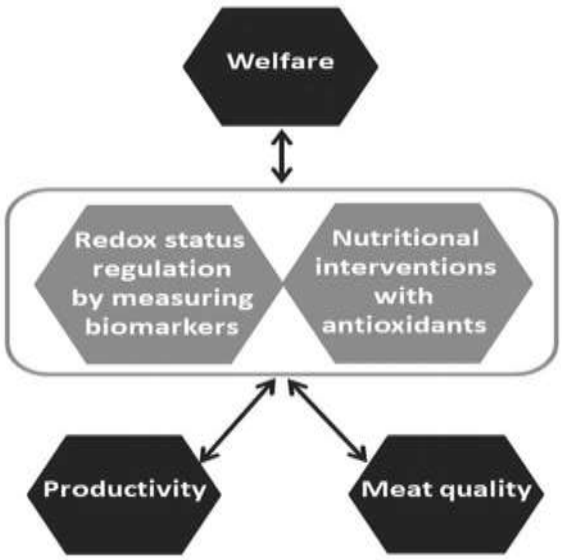

Figure 5. Interactions between welfare of livestock and antioxidant supplementation to diets (Skaperda et al., 2019).

CONCLUSIONS

In conclusion, -omics based approaches have transformed sheep nutrition research by providing molecular insights into gene expression, protein synthesis, metabolic pathways, and the interplay with dietary antioxidants. Genomics, transcriptomics, proteomics, and metabolomics offer powerful tools for identifying genetic markers, gene expression profiles, protein

biomarkers, and metabolite signatures relevant to sheep nutrition and antioxidant metabolism. These technologies enable targeted nutritional interventions tailored to individual sheep genotypes, environmental conditions, and production goals, enhancing productivity, health, and sustainability in sheep production systems.

Future research efforts should focus on integrating multi-omics data to develop precision nutrition strategies, leveraging technological advancements and interdisciplinary collaborations to address emerging challenges and opportunities in sheep nutrition. By harnessing the synergistic potential of -omics technologies and antioxidants, researchers can advance sustainable and resilient sheep production systems that meet global demands for high-quality protein and nutritional security.

Based on current info gathered from various studies, future research directions in -omics based approaches in sheep nutrition may include;

- Epigenetic Modulations: Exploration of epigenetic mechanisms influencing antioxidant gene expression and oxidative stress responses in sheep.
- Gut Microbiome Interactions: Investigation of gut microbiome-host interactions affecting antioxidant metabolism, immune function, and metabolic health in sheep.
- Climate Resilience: Assessment of antioxidant strategies to mitigate heat stress and enhance resilience in sheep exposed to climate variability and extreme weather events.
- Precision Antioxidant Nutrition: Development of personalized antioxidant interventions based on -omics data to optimize metabolic health, immune function, and production outcomes in sheep.
- Data Integration and Interoperability: Standardization of -omics data pipelines, bioinformatics tools, and data sharing platforms to enhance reproducibility and scalability of antioxidant research in sheep nutrition.
- Advancements in Computational Biology: Continued advancements in computational biology, machine learning, and predictive modeling will facilitate the analysis of complex -omics datasets and the development of actionable insights for precision livestock management.

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Use of sudoku experimental design in animal science

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Abstract

In the case of bi-directional heterogeneity, the Latin Square experimental design, which is a two-way blocking process, is widely used. In cases where there are more than two heterogeneities in the experiment, Sudoku experimental design which is developed as an alternative to Latin Square experimental design is used. Thus, it is possible to minimize the experimental error. In this study, the solutions of Type I, Type II, Type III and Type IV Sudoku trial designs are given. Web-based software has been developed to provide solutions for Sudoku experimental design. ASP (Active Server Pages) software language was used in the development of the software. It is thought that the Sudoku experimental design, the source of many factors and heterogeneity are effective in the experiments, can be useful especially the scientists working in fields such as agriculture**.**

Key words: Sudoku, Experimental design, Heterogeneity, Software

Productive portrayal of Marecha Camelid (*Camelus Dromedarius*) in desert biome of Pakistan

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Abstract

The experiments were executed to study the productive capacities of Marecha camel in desert abodes. The camel is a major livestock animal, particularly in desert, semi-arid, and arid areas where it is regarded as a major source of sovereign and food security. About 40 suckling/weaned calves of 8-16 months & 50-62 she-camels of different parities in mid-lactation were kept in semi-half sheds at CBRS and available conditions in adjacent field. They were stall-fed with gram straw, Alfalfa, concentrate of 18% CP & 2.88Mcal energy, allowed grazing/browsing in semi-intensive (SIMS) and extensive management system (EMS) along with ample clean water. Deworming and Vaccination (Trypamedium) was performed against trypanosomes infection. There was about 490 grams daily weight gain (DWG) in open grazing and 560 grams in intensive management system (IMS) while 682 grams in ♂, 660 grams in ♀ under IMS and 460 grams in ♂ and 410 grams in ♀ under SIMS. The calves showed 567 grams in ♂ and 468 grams in ♀ under EMS. In feedlot system, the weaned calves explicit 960-997 grams DWG. The average milk production of Marecha she-camel was found to be 5.78 and 4-9 kg in EMS and milk-fat, milk-protein, milk-lactose, SNF & total-solids as 4.48, 3.44, 4.84, 8.98 and 13.46 %. The average milk production was found to be 6.2 and 5-9 kg under SIMS while milk-fat, milk-protein, milk-lactose, SNF and total-solids were as 4.41, 3.39, 4.77, 8.95 and 13.36 %. Higher daily gains and milk values were achieved which could be used as guidelines for the ideal intensification and prove camel a food security animal of changing climate in this global warming alert.
The financial support from Higher Education Commission (HEC) Islamabad, Pakistan for research and travel grant for oral presentation is gratefully acknowledged.

***Key words**: Camel, Growth, Milk, Meat, Pastoral, Food Security, Pakistan*

Macroscopic lesions and histological changes caused by non-biodegradable foreign bodies in the rumen of cattle

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Abstract

The goal of the current study was to evaluate the gross and histopathological changes caused by the presence of non-biodegradable foreign bodies (plastic bags) in the rumen-reticulum of cattle. To identify this problem we conducted this study at slaughterhouse on a total of 212 cattle without any previous selection. After slaughter and draining of the rumen, foreign bodies and macroscopic lesions were investigated, and rumen samples were taken for histopathological examination. Gross examination of the rumen-reticulum with non-biodegradable foreign bodies revealed congestion, hemorrhage, stunting, sagging, atrophy and thinning of the papillae have been observed. Areas of erosion and ulceration were also observed in the rumen- reticulum of all cattle harboring a large quantity of plastic bags. Ulcerations and nodular formations were also present. The rumen-reticulum wall was thinner than normal and has a light mottled wall and compressed papillae. The histopathological examination revealed a wide variety of lesions. We observed especially lesions of fragmentary or segmental ruptures, destruction, necrosis, degeneration and focal hyperplasia of the keratinized epithelium. The papillae are shortened, enlarged, atrophied, folded and compressed. The length of the taste buds was reduced. These observed histopathological changes can be attributed to mechanical irritation induced by plastic bags or released chemicals by these non-biodegradable foreign bodies.

Key words: *Cattle, Pathology, Plastic Bags, Rumen.*

INTRODUCTION

Plastic is the most common material found in waste, and it is not sent to landfills or incinerated. Plastic waste is harmful to ruminants because it can lead to indigestion and eventually to the death of the animal. This may possibly be one of the major problems affecting cattle health in Algeria. Cattle is more susceptible to ruminal impaction than other small ruminants because they do not use their lips for prehension. Due to a lack of oral discrimination, cattle may ingest non-biodegradable foreign bodies (plastic) that would otherwise be rejected by other species. When ingested, these foreign bodies stay in the rumen of the cattle, compromising ruminal space and interfering with normal physiological functions of the rumen, thereby leading to loss of weight and death of the animal. The main gross lesions encountered in rumen of cattle with non-biodegradable foreign bodies (plastic) observed at slaughter are areas of congestion, hemorrhages, stunting of the papillae, thickening of the wall, erosion, and ulceration. In sparse papillae, there were shortenings. In other areas, complete loss of patches of papillae was evident. Sloughed mucosa was also observed (Bakhiet,2008). Histopathological examinations of sections taken from the rumens with non-biodegradable foreign bodies of animals slaughtered in the abattoirs include hydropic degeneration, cellular vacuolation, submucosal oedema, and disruption of stratified epithelium with dilated lymphatics in the submucosa. Focal hyperplasia of the ruminal epithelium in different regions was also prominent (Hailat et al., 1996; Hailat et al., 1998). This study was undertaken to examine the gross and histopathological changes associated with non-biodegradable foreign bodies (plastic) in the rumen of cattle.

MATERIALS AND METHODS

Animals

The study was conducted on 212 cattle (175 males and 37 females) apparently healthy. The animals were selected by systematic random sampling. The origin of animals slaughtered at Batna Municipal Abattoir is mostly from Batna and other surrounding municipalities. Cattle presented for slaughter were identified by sex, age, and race. Age was determined based on dental eruption.

Postmortem examination

After slaughtering the animals, the rumen and reticulum were examined in the evisceration stage; they were opened, and any foreign bodies were removed and forestomach thoroughly washed for better inspection of the walls, mucosa, the papillae, and the pillars for any abnormalities, and gross lesions were recorded.

Histopathological examination

Samples were collected from areas with gross pathological lesions, and immediately fixed in 10 % neutral buffered formalin. Five micron sections were made and stained by hematoxylin and eosin as previously described by Bancroft and Stevens (1990) and examined by light microscopy (Hailat et al.,1996 ; Bakhiet, 2008), using x 10, x 40 and x 100 objective lenses. The results were recorded and where necessary, photomicrographs were taken using the photomicroscope (Leica DM1000 LED microscope Olympus CXSF1, Olympus Corporation, Tokyo. Japan)(Otsyina et al.,2017a).

RESULTS

Figure 1 and 2. Necropsy finding of affected rumen show stunted and sloughed ruminal papillae, nodular elevation.

Figure 3 and 4. Stunting, bending of papillae, irritation of rumen papillae, squamation of ruminal papillae, and necrotic areas.

Figure 5 and 6: Different undigested foreign bodies found in the rumen of cattle after slaughtering; (5) Plastic bags with, (6) plastic bags and robes.

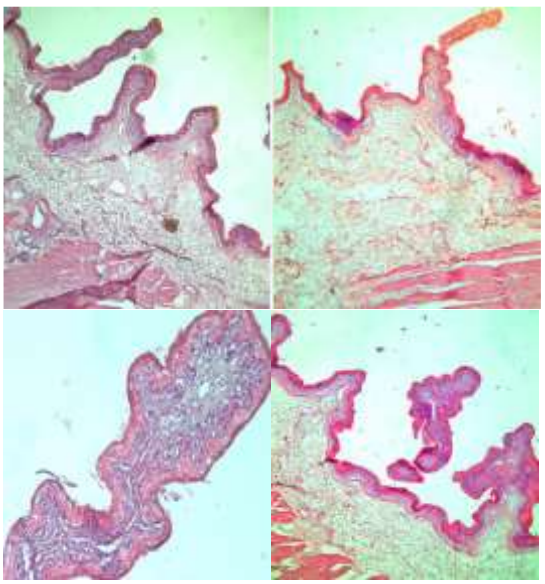

Figure 7. Irregular, disrupted epithelium and flattened papillae atrophied and destroyed papillae

DISCUSSION

This study revealed the gross and histopathological changes associated with the accumulation of plastic bags in the rumen of cattle. The severity of the gross and histopathological changes varied with the amount of plastic masses found in the rumens, ranging from a few small pieces (grams) to 10 kg (Figure 5, 6). In a few cases, the plastic masses were very hard (Hailat et al. 1998). This confirms that the severity of pathological changes depends on the type of foreign body, the duration in the rumen, and the degree of obstruction (Calfee and Manning, 2002; Tesgaye and Chanie, 2012; Otsyin et al., 2017b).

However, the gross examination of the rumens with plastics, which were examined at Batna slaughterhouses, revealed areas of congestion, hemorrhages, stunting of the papillae, thickening of the wall, erosion, ulceration, and scar formation. In sparse papillae, there were shortenings with irregular distribution. In other areas, complete loss of patches of papillae was evident. Mucosal sloughing with focally eroded areas was also observed. Areas of focal thickening of the wall of nodular type or irregularly shaped (focally extensive proliferative areas) were also seen in some cases (Figures 1, 2, 3, and 4) (Hailat et al., 1998; Bakhiet, 2008; Ghurashi et al., 2009; Otsyina et al.,2017a, Otsyina et al.,2017b). These lesions are most likely to be due to the pressure on exerted against the wall of the rumen caused by the non-biodegradable foreign body. This may also be due to the chronic irritation of the forestomach wall by the foreign body, leaving the wall exposed to secondary infection, which resulted in both inflammatory and hyperplasic changes. It is also possible that some of the lesions may result from poisonous substances released from the plastic bags and toxicants absorbed from the rumen contents (EL-maghraby and Hailat,2001). Furthermore, constant irritation of the wall of the rumen by continuous movement of the plastic bags may lead to erosion and excoriations of the rumen papillae, ruminal pillars, and mucosa, resulting in inflammation and hyperplasia of the epithelial mucosa. Stunting, bending of papillae, irritation of rumen papillae, squamation of ruminal papillae, and necrotic areas.

Histopathological examinations (Figure7) revealed a wide variety of changes; the stratified epithelium of the rumen was disrupted with degeneration, necrosis, and patchy hyperplasia in many areas. Papillae were shortened, stunted, sometimes broadened, compressed or flattened, atrophied, and shredded in some areas. There were clefts at the tips of some of the atrophied papillae. Degeneration, necrosis, and hyperplasia of the mucosa with prominent rete ridges of variable length projecting into the submucosa were observed, with both extracellular and intracellular epithelial cell oedema characterized by hydrophic degeneration, cellular vacuolation, and spongiosis (Figure 7). The submucosa appeared widened and oedematous, with dilated lymphatics between the rete ridges. There was degeneration, necrosis, and fibrosis of the connective tissue (Hailat et al.,1996).

CONCLUSIONS

These observed histopathological changes can be attributed to mechanical irritation induced by plastic bags or released chemicals by these non-biodegradable foreign bodies.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Saddek Djaaba, Pathologist, and Professor Omar Benoun for their cooperation in carrying out the study.

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Marker assisted selection of military working dogs

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Abstract

Marker-assisted selection (MAS) is a genetic approach increasingly being utilized in the selection and breeding of military working dogs (MWDs) to enhance their effectiveness in various operational roles such as detection, search and rescue, and patrol duties. Unlike traditional selection methods, which rely heavily on behavioral assessments and physical performance tests, MAS leverages specific genetic markers linked to desirable traits. This method allows for a more objective and efficient selection process by identifying dogs with genetic predispositions that align with the high demands of military operations. Research in MAS for MWDs focuses on identifying genetic markers associated with traits like olfactory sensitivity, stress resilience, cognitive function, and physical endurance. By utilizing MAS, breeding programs can reduce the time and cost associated with training dogs that may not ultimately meet operational requirements. This approach not only improves the success rate of selecting suitable dogs but also minimizes the risk of injury or stress in dogs poorly suited for certain tasks. Furthermore, MAS helps maintain genetic diversity within breeding populations by enabling more precise selection criteria, which can lead to healthier dogs with longer service lives. As a result, MAS is becoming a vital tool in the optimization of MWD breeding programs worldwide, aligning genetic potential with operational needs to enhance both the efficiency and welfare of these crucial military assets. This research provides an overview of how marker-assisted selection is revolutionizing the breeding and selection of military working dogs, based on recent research findings and advancements in genetic technologies.

Key words: *Marker Assisted Selection, Dogs, Military Working Dog, Marker Genes*

Comparison of the ridge and ordinary least square for estimating body weight from body measurements

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Abstract

In this study, Ordinary Least Squares (OLS) and Ridge regression models were compared to predict body weight from body measurements in 89 Gyr x Holstein crossbred heifers. The dataset was divided into 80% training and 20% test. For this aim, OLS model and Ridge model were evaluated in terms of their effectiveness in eliminating the multicollinearity problem among the variables. In this context, the performance of both models was analyzed, and a discussion was presented on how OLS model and Ridge model can be used in different data conditions. According to the results of the present study show that the OLS model has lower error values and higher fit for this data set. However, the Ridge model can be considered as it can be useful in improving the problem of multicollinearity between variables.

Key words: *Ridge, OLS, Gyr x Holstein, crossbreed, multicollinearity*

The influence of feed additives based on humic substances on animal productivity

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Abstract

In the Republic of Kazakhstan, the problem of increasing the productivity of agricultural animals of meat and dairy direction and the production of domestic organic products is acute. Recently, the use of humic acids as feed additives has become very relevant. The aim of the work is a comparative analysis of the quality of feed additives based on humic substances and their effect on animal productivity. The object of research was the feed additives "Gumka-KZ," "Qogyr-su," "Biogumid," made by various manufacturersa using the domestic potassium humate brand "Kazuglegumus." The physical and biological characteristics of feed products were analyzed according to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan. In the course of the studies, it was found that potassium humate feed additives have stable properties, are not toxic and safe for animals, differ in microbiological purity, have a biologically active effect in the form of an increase in the body weight of laboratory animals. The purity and shine of the coat in experienced mice, mobility, and increased appetite were noted. Analysis of the effectiveness of feed additives in laboratory animals showed that in the experimental groups there was a clear increase in animal body weight. The best effect on laboratory animals was shown by the food additive "Qogyr-su" with an average increase in the live weight of laboratory mice of 2.3-2.8 g compared to the control.

Key words: *feed additive; potassium humate; yeast; organic products; animals*

INTRODUCTION

In the Republic of Kazakhstan, there is an acute issue of increasing the productivity of farm beef and dairy animals and the production of domestic organic products, since farms experience losses in the productivity of farm animals, both adults and young animals, due to a decrease in the overall resistance of animals, a weak forage base, a lack of quality feed, and violations in feeding rations (Musaev F.A. et al., 2015). To obtain organic products, the Order of the Minister of Agriculture of the Republic of Kazakhstan dated May 23, 2016 No. 231 legislatively introduced a list of permitted means used in the production of organic products (Anon, 2016). This imposes certain restrictions on the use of feed antibiotics, hormones, and some other biologically active components, previously often included in the diet when feeding animals.

The feed additives market today is very diverse, represented by both domestic and imported preparations with different compositions and functional purposes. Particularly attractive are feed additives that include biologically active substances that affect the normalization of metabolism and replenish the diet of animals (Anon, 2022).

The list of permitted substances used in the production of organic products includes humic acids of natural origin in the form of aqueous or alkaline extracts as fertilizers and soil-improving substances (Chapter 1), vitamins of natural origin and identical to natural synthetic vitamins A, D, E; microelements: iron, iodine, cobalt, copper, manganese, zinc, molybdenum in the form of carbonates, sulfates, oxides and other compounds corresponding to physiological nutritional standards (Chapter 6, paragraph 1). An important note is the reference

allowing the use of feed additives registered in Kazakhstan and (or) member states of the Eurasian Economic Union (Anon, 2016).

Recently, the use of humic acids as feed additives has become very relevant (Valitova H.Z. et al., 2022). Analysis of the market for proposed feed additives has shown that several feed additives based on humic substances have been developed and are currently being offered: for cattle (Brel-Kiseleva I.M. et al., 2021), horses (Nosenko N.A. et al., 2007), breeding boars (Nechitailo K. et al., 2021), broiler chickens (Kosolapova A.I. et al., 2009), for farm domestic animals and birds (Isaev V.A. et al., 2007, Kukhar E.V. et al., 2023). There are proposals for feed additives made based on humic acids (Beldin M.E., 2021), fulvic acids (Imbaeva D.S. et al., 2020), and enriched humic substances with various bioactive substances (Saulebekova M.E. et al., 2020, Dauthan U., et al., 2020).

In addition to vitamin and mineral substances, yeast is used as a source of lipids, proteins, amino acids, and enzyme preparations (Wang A., et al., 2011). Lactobacilli or bacilli are used as a source of probiotic components, organic acids, and vitamins (Kukhar E.V., 2022), and algae are used as a source of fiber, polysaccharides, and lipids (Nurpeisov A., et al., 2019)

The work aims to compare the quality of domestic feed additives based on humic substances and their influence on the productivity of animals.

MATERIALS AND METHODS

The objects of the research were feed additives "Gumka-KZ" (manufactured by LLP NPO "Kaztekhnougol"), "Qonyr-su" (manufactured by NCJSC "S. Seifullin Kazakh Agro Technical Research University "), "Biogumid" (manufactured by IP "BioNanoPreparat"), manufactured by various manufacturers using domestic potassium humate of the brand "Kazugle-gumus" with a humic acid content of up to 56% by dry matter (Yermagambet B.T., 2021).

Experimental studies on the analysis of feed additives were carried out in the microbiology laboratory of the agricultural biotechnology research and production platform of NCJSC "S. Seifullin Kazakh Agro Technical Research University", IR spectrometry of the basic potassium humate and feed additives - in the Office of the Provost - the Office of Collective Use of JSC "Nazarbayev University".

IR spectrometry conditions: Nicolet iS10 FT-IR Spectrometer (Thermo Scientific), glass installed: KBr. Experimental conditions: results were recorded on an ATR (attenuated total internal reflection) attachment, number of scans: 32, resolution: 4.

The chemical composition of the feed was studied using a NIRSDS-2500 infrared analyzer manufactured by FOSS Analytical (Denmark) and a Polispec NIR spectrophotometer manufactured by ITPhotonics S.r.l. (Italy). Feed samples were collected following Standard GOST 27262-87, prepared for analysis following Standard GOST ISO 6498-2014, and dry matter was determined following Standard 31640-2012.

Studies using animals were conducted following the principles established by the International Animal Ethics Committee with the approval of the local ethics commission of the Seifullin Kazakh Agro Technical Research University.

The analysis of physical and biological characteristics of feed additives was carried out according to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan (St.Ph. of RK, 2008). To study the microbiological purity of humic feed additives, liquid and dense nutrient media of Sabouraud, Chapek, corn, potato-glycerol agar (PGA), meat-peptone agar, Giss media with sucrose, mannitol, lactose, maltose, glucose were used, which were prepared according to the manufacturer's instructions.

Laboratory tests of the effectiveness of the feed additive were carried out on white laboratory mongrel mice with appropriate feeding and maintenance conditions, at a temperature of 20-22 ° C, humidity in the range of 45-65%, kept in rooms isolated from noise and other stimulating effects. For the experiment, clinically healthy mice were selected, from which control and experimental groups were formed according to the principle of pairs-analogues of 5 individuals in each. The control group of mice received standard feed, the experimental groups additionally received feed additives for 30 days.

The obtained results were processed biometrically using statistical tools of the Microsoft Excel program.

RESULTS

The analysis of organoleptic and physical properties of feed additives intended to increase the productivity of farm animals and poultry of any age showed that they all have specific features: they had a color from light brown to dark brown shades, a specific smell and taste, were opaque when shaken, had a liquid consistency, two of them were in the form of a suspension that easily separated into liquid and solid fractions. When stored at rest, a uniform sediment of yeast ("Qonyr-su", "Biogumid") or finely dispersed suspension of coal ("Gumka-KZ") falls out, and has stable organoleptic properties.

The analysis of the sterility of the feed additive "Gumka-KZ" and the microbiological purity and sterility of the feed additives "Qonyr-su", "Biogumid" during observation for 10 days showed that there is no growth of foreign microflora. In the feed additives "Qonyr-su" and "Biogumid" homogeneous yeast saccharomyces cerevisiae grow, have a milky beige color, smooth surface, the shape of the colonies is round, the size is small, the surface is smooth, the profile is convex, the edge is even, the structure is fine-grained. Microscopy of smears confirmed the microbiological purity of the feed additives "Qonyr-su" and "Biogumid", and the complete sterility of the drug "Gumka-KZ".

Analysis of the biochemical activity of yeast on Giss media with sucrose, glucose, lactose, mannitol and maltose showed that yeast actively ferments all sugars except lactose.

Determination of the concentration of potassium humate introduced into the composition of feed additives by IR spectrometry and UV spectrometry showed that the preparation "Gumka-KZ" with a concentration of potassium humate of 10% has full compliance with the declared concentration of humic substances in dry matter and percentage concentration. Analysis of the feed additives "Biogumid" and "Qonyr-su" during IR spectrometry showed the presence of three main groups of spectra and several additional ones, which is due to a change in the composition of the preparation, which is additionally enriched with proteins and polysaccharides included in the composition of the milk replacer and yeast.

The study of acute and chronic toxicity of feed additives showed that all additives are harmless and non-toxic to animals. Analysis of the nutritional value of the feed additives "Biogumid" and "Qonyr-su" showed that both feed additives have a reduced dry matter content and an increased moisture content. In the "Biogumid" preparation, the protein content is increased by 0.8%, ash - by 0.22%, fat content is reduced by 0.4%, starch - by 2.72%. Crude fiber has the same indicators as the base values. In the feed additive "Qonyr-su" the protein content is 1.9% higher than in the base, ash - 0.57% higher, which is twice as high as in the feed additive "Biogumid". The fat content is reduced by 0.8%, which is two times less than the indicators of the drug "Biogumid", the starch content - by 0.42%, and crude fiber - by 0.23%. Data on the feed additive "Gumka-KZ" is not presented, since it contains only one component - potassium humate, containing organomineral substances.

Laboratory tests of the effectiveness of feed additives showed that the live weight of mice in the experimental groups has an average value of 22.2-28.9±0.5, which is 2.3-3.1 g higher than in the control group. Cleanliness and shine of the fur of the experimental mice, activity, mobility, and increased appetite were noted. The best effect was shown by the biopreparation "Qonyr-su". The data indicate that the biopreparation has a beneficial effect on the body of the experimental mice, stimulates growth, increases live weight, compared to the control (Table 1).

Table 1. Live weight gain of laboratory mice during feed additives efficacy testing (Average biomass growth, M±m = 5)

Biopreparation	Experimental group	Control group	Average for the group
«Gumka-KZ»	8,60	6,30	2,30
«Biogumid»	7,99	5,62	2,37
«Qonyr-su»	9,06	6,26	2,80

The results of determining the effectiveness of feed additives "Gumka-KZ", "Qonyr-su", "Biogumid" on farm animals showed that in the experimental groups there is a clear increase in the body weight of animals (Table 2).

Table 2. Increase in live weight of beef cattle during tests of the effectiveness of feed additives (Average daily weight gain, g/head / %)

Biopreparation	calves	feeding
«Gumka-KZ»	8,60	6,30
«Biogumid»	7,99	5,62
«Qonyr-su»	9,06	6,26

As can be seen from the results of determining the effectiveness of the feed additives "Gumka-KZ" and "Biogumid" on yearling calves of meat breeds, the best result is observed when feeding the feed additive "Biogumid". The results of determining the effectiveness of the feed additive "Qonyr-su" on dairy cows showed that in the experimental groups there is a clear increase in milk productivity (Figure 1).

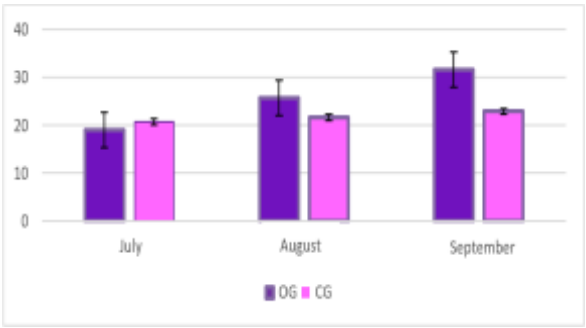

Figure 1. Average daily milk yield in the experimental and control groups when feeding the feed additive "Qonyr-su": OG – experimental, CG – control

An analysis of the quality and effectiveness of feed additives made it possible to establish the compliance of finished biological products with the requirements of "Regulation (EC) No. 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed" (Anon, 2009) and the requirements of the List of permitted means for the production of organic products (Anon, 2016).

DISCUSSION

According to recent research by scientists, humic acids, by improving digestion and assimilation of feed, optimize the condition of the gastrointestinal tract of animals. The replacement of antibiotics (added to feed as growth stimulants) with humic acids improves the productivity and condition of animals: daily weight gain and feed consumption (Thomassen B.P. et al., 2000).

In addition, we receive organic products that do not harm human health. This is since humic acids, as natural components of humus, constantly enter the animal body with pasture grasses, with feed or with natural feed additives (green, coal, peat, etc.). In the animal's body, humic substances are included in metabolic processes, completely metabolized in the cell, assimilated without a trace, bringing it benefits in the form of additional sources of biologically active substances (Kisileva N.V., et al., 2013). It has been proven that humic acids have a positive effect on the general condition of animals, normalizing the body's metabolic processes at the molecular level. In a complex combination with fulvic acid, humic acids form a bioavailable complex for the improvement of a living organism, which has the properties of antibiotics. Humic substances and other BAS cause a variety of positive effects of humic acids on living organisms (Platonov V.V., et al., 2016), therefore, the products obtained after the application of the drug can be used without any restrictions (Bezuglova O. et al., 2022).

In Russia, humic preparations, in the form of ballast-free sodium humate, have been widely used as feed additives in the diets of cattle and poultry since the beginning of the second half of the 20th century (Bezuglova O. et al., 2016). Humic preparations are being actively introduced into livestock production in Kazakhstan. This is evidenced by the appearance of feed additives containing humic substances in the list of the "State Register of Veterinary Drugs and Feed additives" of the Republic of Kazakhstan: AL KARAL, Biogumid, Gumka-KZ (Anon, 2023).

It has been proved that humic substances introduced into feed or drinking water for agricultural animals and birds can become a promising feed biologically active additive

to the basic diet, increasing the daily increase in animal body weight (by 100...300 g), feed conversion rate (by 0.03...0.06) and productivity (Lubimova N.A. et al., 2020). The complex of humic substances with BAS, including bacteria, yeast, and algae, enhances the biologically active effect. For example, a feed additive for farm birds and animals based on humic substances, Far Eastern kelp, and succinic acid helps to increase the productivity and resistance of farm birds and animals (Kukhar E.V., 2023).

An analysis of the nutritional value and effectiveness of domestic feed additives containing humic substances from various manufacturers containing a pure potassium humate preparation – «Gumka-KZ», a potassium humate preparation with brewer's yeast – «Biogumid» and a potassium humate preparation with baking yeast – «Qonyr-su» showed that they have a different effect and act with the newborn period. These results are understandable if we consider in detail the composition of the two feed additives. The presence of yeast leads to an increase in protein and amino acid levels, as the biomass of microorganisms is accumulated. The higher protein level in the «Qonyr-su» feed additive is higher due to the additional introduction of a whole milk substitute into the composition of this drug, the proteins of which are not a nutrient substrate for yeast. This leads to the fact that yeast does not compete for this component with animals, it is preserved in dissolved form and therefore has a good effect on young cattle (Kukhar E.V., 2023). A similar effect is provided by brewer's yeast of the feed additive "Biogumid", which are not able to decompose lactose and, therefore, do not compete with young farm animals for nutrients, which allows the use of this feed additive from the newborn period (Kukhar E.V., 2022).

The decrease in the concentration of starch and fat in both cases is due to the peculiarities of the metabolism of yeast, which produces the enzyme amylase, which cleaves starch, and lipolytic enzymes. The increase in ash content in the samples in both cases is associated with the accumulation of yeast biomass. Unequal indicators of ash content, protein, and other parameters are associated with the peculiarities of the biology of beer and baking yeast, which are usually cultivated in barley malt and wheat flour, respectively.

Quality control of feed additives made it possible to establish compliance of finished biological products with the requirements of Regulation (EU) No. 767/2009 of the European Parliament and Council of July 13, 2009 on the placement and use of feed on the market (Anon, 2009) and the requirements of the List of Permitted Means for the production of organic products (Musaev, F.A. et al., 2015)

CONCLUSIONS

1 Feed additives "Gumka-KZ", "Qonyr-su", "Biogumid", produced by various manufacturers using domestic liquid potassium humate of the "Kazuglegumus" brand, have stable properties, are non-toxic and safe for animals, and are distinguished by microbiological purity.

2 Comparative analysis of domestic feed additives containing humic substances in their composition showed that they have a biologically active effect in the form of an increase in the body weight of laboratory animals. Cleanliness and shine of wool in experimental mice, mobility, and increased appetite were noted.

3 The results of determining the effectiveness of feed additives on animals showed that a clear increase in body weight is observed in the experimental groups. The best effect on laboratory animals was revealed when using the feed additive "Qonyr-su". On fattening calves, the best effect was revealed when using the feed additive "Biogumid".

FUNDING INFORMATION

The study was conducted within the framework of the program BR21882327 “Development of new technologies for organic production and processing of agricultural products”.

ACKNOWLEDGEMENTS

The authors thank the staff of Seifullin University for their advice and support.

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Impact of small businesses on animal welfare using multi-criteria decision making

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Abstract

It is often difficult to explain cattle enterprises with statistical methods in order to interpret and evaluate them in terms of animal welfare. Thus, there is a need to scale animal welfare features, which is an abstract concept, with concrete criteria. For this purpose, animal welfare evaluation criteria have been applied to measure cattle enterprises according to animal welfare. In practice, a total of 7 criteria were evaluated: space per animal, social relationship, barn floor type, ventilation, lighting, care and nutrition. To evaluate the 7 criteria considered in the application, the TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) method, which is the most preferred among the Multi-Criteria Decision Making (MCDM) methods, was applied. The Multi-Criteria Decision Making method enables reaching the most appropriate compromise solution by evaluating different approaches and alternatives according to their decision-making values. The aim of this research is to examine the applicability of the TOPSIS method, one of the Multi-Criteria Decision Making methods, in order to examine the animal welfare of small-scale cattle enterprises registered to the Şırnak-İdil District Directorate of Agriculture and Forestry. In the study, 7 different criteria were evaluated to evaluate small-scale cattle enterprises according to animal welfare. These criteria were evaluated as area per animal, social relationship, barn floor type, ventilation, lighting, care and nutrition.

Key words: *Multi-Criteria, Animal Welfare, TOPSIS*

Experimental study of the route for the stimulating factors and ınfluence of trace metals content ın soil on the growth of the Land Snail Helix Aspersa

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Abstract

This study was devoted to evaluate the metal soil contamination of some biotope of North-eastern Algeria by using the Helix aspersa snail as a bioindicator.The objective was aimed to assess the ecotoxicological impact of anthropogenic activities on soil quality by using the land snail Helix aspersa as a bioindicator. Soil samples and snails were collected from several sites of northeast Algeria during spring and winter. All sites were chosen in this study for the reason of their proximity to industrial factories as a potential source of heavy metal soil contamination. The concentration of heavy metals in soil samples was analyzed using the X-ray Fluorescence (XRF) spectrometer (Thermo Scientific Model Niton FXL 950) since the three metals of the highest levels in soil samples were examined in Helix aspersa hepatopancreas and feet by means of the atomic absorption spectrophotometry. Also, the highest levels of heavy metals were noticed during spring in Helix aspersa of the closest sites to the potential sources of pollution. Overall, the results show that H. aspersa is an efficient bioindicator to evaluate the heavy metal atmospheric pollution, due to several industrial factories, and vehicle traffic.

Key words: *Soil Contamination, Heavy metals, Helix aspersa, North-East Algeria, Bioindicator*.

Comparison of nonlinear growth curves models in Awassi and Romanov x Awassi (F1) crossbred lambs

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Abstract

In the study, 4 nonlinear models were used, namely Logistic, Brody, von Bertalanffy, and Gompertz, to determine the changes in growth in live weight from birth to 32 weeks of age in Awassi and Romanov X Awassi (F1) (RxA) lambs. In order to decide on the best-fitting model, the mean square error (MSE) and adjusted coefficient of determination (R2) values were used. The Brody model was the best-fitting model with the highest R2 and lowest R2 values in Awassi and RxA male lambs. While R2 was 98.2 for Awassi in the Brody model, it was determined as 99.6 for RxA's. It was generally observed that the Logistic model estimates were more deviant than the other models. As a result, it was determined that the Brody model was the model that best-explained growth by showing the best fit to the data obtained from Awassi and RxA male lambs.

Key words: *Awassi, Romanov, growth curves*

INTRODUCTION

In animals, growth is the expression of the change in weight and volume that occurs at certain stages depending on age. Growth curves reflect the innate ability of animals to grow and develop and the interaction of this ability with the environment throughout their lives (Efe 1990). Since growth is under the pressure of many environmental factors based on genetic potential, it shows a complex structure and change (Yakupoğlu 1999). Changes in growth can be explained with growth curves. Curves that indicate the change in growth that occurs within a certain period of time are defined as growth curves or age-weight curves. Growth characteristics in different age groups are emphasized in growth curves. The target is to interpret the change in values obtained at different points depending on age and to summarize the data with fewer parameters that can be resolved biologically. Summarization is done with the growth curve parameters estimated as a result of the statistical fit of the model used (Akbaş 1995).

Growth curves provide the opportunity to estimate the growth of any living creature at an early age and to separate animals that can be considered to be good for breeding at an early age (Efe 1990; Tekel 1998). A growth model that has been validated and accepted (for body measurements and live weight) allows the prediction of growth in a certain period of time and can be used for early selection (Tekel 1998). It is stated that studies conducted using nonlinear models can guide future breeding studies and as a result, better phenotypic and genetic progress can be achieved by making selection in the desired direction in breeding (Efe 1990; Akbaş 1996; Yakupoğlu 1999; Lambe *et al.* 2006**;** Yıldız vd 2009).

MATERIALS AND METHODS

The animal material of this study consists of 41 male lambs, 19 Awassi and 22 Romanov x Awassi (F1) (RxA), raised in the Sheep Farm affiliated to the Ataturk University Food and Livestock Application and Research Center. The birth weight of each lamb was weighed within the first 24 hours using a scale sensitive to 20 g. Weaning was performed at an average age of 72 days in the farm and the lambs were sent to pasture. The same care and feeding conditions were applied to the lambs in the farm.

In the study, live weight data measured at 22-day intervals from birth to 32 weeks of age were used to calculate the growth curve

parameters. Parameter means were calculated using the parameters determined for each animal and growth curves were drawn. Non-linear Brody, Logistic, von Bertalanffy, Gompertz models that explain growth depending on time were used in the study. These models were compared with R^2 and MSE values and the most suitable growth model was determined for Awassi and RxA male lambs.

Table 1. Nonlinear growth models

Growth Model	
Brody	$\boldsymbol{Yt = A[1 - \beta * \exp(-k * t)]}$
Bertalanffy	$\boldsymbol{Yt = A\ [1 - \beta * \exp(\ -k * t)]^3}$
Gompertz	$\boldsymbol{Yt = A * \exp[-\beta * \exp(-k * t)]}$
Logistic	$\boldsymbol{Yt = A\ [1 + \beta * \exp(\ -k * t)]^{-1}}$

The terms belonging to mathematical models are as follows; Y= Live weight, t= Age; Yt= Live weight observed at t months of age (kg); A= The parameter A, which expresses the asymptotic limit of weight as age approaches infinity, is the average highest live weight of the individual or the adult live weight; β= The parameter showing the ratio of the live weight gained after birth to the adult live weight, estimated with the initial value of weight (Yt) and time (t). Exp= Natural logarithm base, k= This parameter, expressed as the maturation rate, is the ratio of the maximum growth rate to the adult live weight. This parameter shows the speed at which the live weight (Yt) approaches the asymptotic weight (A).

R^2 and MSE were used to compare the models in terms of goodness of fit. The Marquardt method was used for iteration in the estimation of the models. The Marquardt method prefers a compromise between the Gauss-Newton and Steepest descent methods and combines the best aspects of these methods (Draper and Smith, 1981). R^2 is a measure of how much of the total variation in the data set can be expressed by the generated growth curve model and varies between 0 and 1. Data analysis and A, B, and k parameters and comparison criteria for growth curves were estimated using the IBM SPSS 26.00 (2020) statistical package program for eight different nonlinear models for each animal.

RESULTS and DISCUSSION

The fact that growth in lambs reaches an asymptote after a certain age makes the use of nonlinear models widespread in the examination of the live weight-age relationship. In estimating the growth curves of live weight in Awassi and RxA (F1) male lambs, parameter estimates (A, B, and k) were made using nonlinear growth models such as Brody, Bertalanffy, Gompertz and Logistic models and descriptive statistics of these parameters are presented in Table 2.

Table 2. Estimated parameter values, standard errors, root mean square error (MSE), and coefficients of determination (R^2) of Awassi and RxA (F1) male lambs using nonlinear growth models.

Model		A	B	k	MSE	R^2
Brody	Awassi	36.40±8.01	0.99±0.05	0.127±0.05	1.20	98.2
	RxA	22.39±1.63	1.00±0.03	0.162±0.03	0.127	99.6
Bertalanffy	Awassi	29.94±4.12	0.57±0.06	0.25±0.07	1.60	97.6
	RxA	19.53±1.13	0.57±0.03	0.28±0.04	0.223	99.3
Gompertz	Awassi	28.58±3.52	2.29±0.30	0.30±0.08	1.83	97.2
	RxA	18.84±1.04	2.27±0.19	0.34±0.05	0.283	99.1
Logistic	Awassi	26.59±2.77	5.55±1.43	0.46±0.11	2.50	96.2
	RxA	17.73±0.92	5.51±0.99	0.52±0.07	0.482	98.4

When Table 2 is examined, the A parameter, which expresses the maximum average live weight value independent of the effects of environmental conditions, was determined

as the highest with the Brody model (36.40 and 22.39) in Awassi and RxA (F1) male lambs, and the lowest with the Logistic model (26.59 and 17.73). The B parameter, which is a coefficient indicating the weight at age zero, was determined as the highest with the Logistic model (5.55 and 5.51) in females and males, and the lowest with the Bertalanffy model (0.57 and 0.57). The k parameter, which is a function of growth rate or maturation rate, gives the constant growth rate per unit time. The k parameter was determined as the highest with the Logistic model (0.46 and 0.52) and the lowest with the Brody model (0.127 and 0.162) in Morkaraman female and male lambs.

R^2 and MSE values were used to compare Brody, Logistic, Bertalanffy, and Gompertz models for Awassi and RxA (F1) male lambs and the most suitable model was estimated (Table 2). When Table 2 is examined, the lowest MSE value (1.20) in Awassi male lambs was estimated with the Brody model, and the highest value (2.50) with the Logistic model. While the lowest MSE value (0.127) in RxA (F1) crossbred male lambs was obtained with the Brody model, the highest value (0.482) was determined with the Logistic model. The highest R^2 value in Awassi lambs was determined with the Brody model as 98.2% and the lowest value as 96.2% with the Logistic model. The highest R^2 value in RxA (F1) lambs was determined with the Brody model as 99.6% and the lowest value as 98.4% in the Logistic model. According to the results obtained, the Brody model, which had the highest R^2 and lowest MSE values, showed the best fit in Awassi male and female lambs, while the Logistic model showed the worst fit.

Similar to our study, Bilgin et al. (2004), Kopuzlu et al., (2014), Nimase et al., (2018), Gautam et al., (2018) used different nonlinear models and emphasized that the Brody model can be recommended due to having fewer parameters and ease of interpretation compared to other models.

Table 3. Observed data and estimated live weight values with nonlinear models for Awassi and RxA (F1) male lambs.

Age	Observed		Brody		Bertalanffy		Gompertz		Logistic	
(week)	Awassi	RxA	Awassi	RxA	Awassi	RxA	Awassi	RxA	Awassi	RxA
1	4.1	3.10	4.53	3.35	5.03	3.63	5.27	3.03	5.90	4.15
4	8.2	6.40	8.33	6.20	8.21	6.04	8.19	4.91	8.28	6.02
8	12.3	8.80	14.63	10.68	14.53	10.62	14.46	9.12	14.13	10.50
12	15.9	10.60	19.51	13.92	19.65	14.03	19.71	12.73	19.68	14.26
16	17.4	12.50	23.30	16.26	23.31	16.26	23.33	15.25	23.32	16.33
20	18.1	13.50	26.24	17.96	25.75	17.62	25.59	16.80	25.18	17.21
24	20.1	14.80	28.52	19.19	27.33	18.43	26.91	17.71	26.01	17.54
28	23.8	16.80	30.29	20.07	28.33	18.90	27.65	18.22	26.36	17.66
32	25.7	17.10	31.66	20.71	28.95	19.17	28.07	18.50	26.50	17.71

The observed live weight values for Awassi and RxA (F1) male lambs and the estimated live weight values with nonlinear models are shown in Table 3. In Figure 1 and Figure 2, the growth curves of the realized and estimated live weight averages of Awassi and RxA (F1) male lambs with different models are plotted over time.

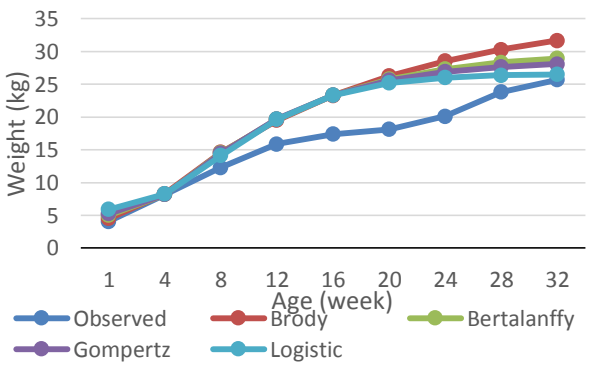

Figure 1. Growth curves of Awassi male lambs observed and estimated by different nonlinear models

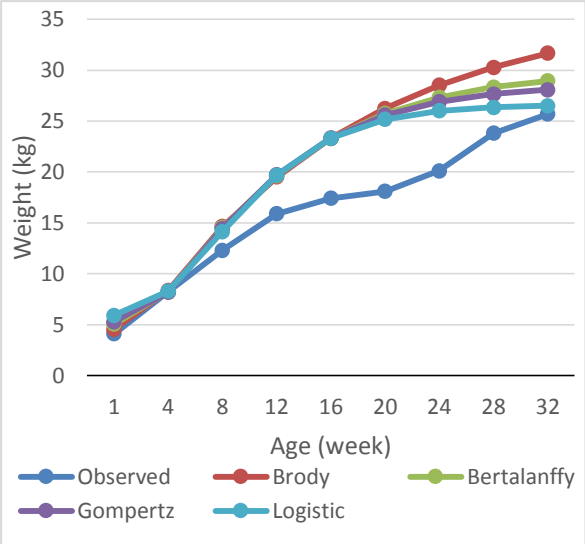

Figure 2. Growth curves of RxA (F1) male lambs observed and estimated by different nonlinear models

CONCLUSIONS

Following growth processes is extremely important for breeding. A slowdown, stoppage or decreasing trend in growth may indicate a problem related to care or feeding. Growth curve parameters are used as breeding criteria as well as defining growth, providing the opportunity to separate animals for breeding at an early age. It is possible to determine the most appropriate (optimum) slaughter age of the individual and evaluate it economically by estimating the later live weights from the obtained data. Four different models were used in this study and the Brody model was determined as the model that best predicted growth.

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Comparison of MARS and CART Data mining algorithms for prediction of body weight in male Ross 308 broiler chickens

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Abstract

Ross 308 broiler chickens are type of chicken raised specifically for meat production which are fast growing and can be sold at the age of 6 weeks of age. The aim of this study was to use multivariate adaptive regression splines and classification and regression tree data mining algorithm approaches to predict live body weight from morphological traits of Ross 308 male chickens and compare their predictive performance. A total of sixty birds were used in this study. The flock was reared under intensive system and kept in the same house. Body weight and morphological traits such as wing length, beak length, shank length, body girth, body length, and shank circumference were measured for every Ross 308 chicken. Two data mining algorithms MARS and CART were used for body weight prediction. The goodness of fit criteria were used to select the best model. The CART data mining algorithm was found to be the best decision model that gave the greater predictive accuracy with higher r, Rsq and ARSq test results when the criteria were taken into account as part of BW prediction. These result suggests that CART data mining algorithm might help farmers recognize morphological traits that are influential in body weight.

Key words: *Body girth, body length, correlation, goodness of fit, morphological traits*

Linkage disequilibrium in animal genetics – definition, measures and applications

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Abstract

The non-random connection of alleles at various loci is known as linkage disequilibrium (LD). Combinations of alleles inside haplotypes occur at frequencies that differ from those expected on the premise of independence when two alleles at two distinct loci are in LD. When genetic variation at a locus is linked to a trait, it means that either the genetic variation at that locus directly impacts the phenotype of interest or the locus is in LD with the causal mutation. The level of LD, which dictates how many markers should be typed in a genome scan to discover a quantitative trait locus (QTL) using LD, is critical to the practicality of association studies. This review explores the origin of LD in genetics and how it applies to animal breeding and genetics.

Key words*: Linkage disequilibrium, quantitative trait loci, alleles, haplotypes, genetic variation*

INTRODUCTION

Consider 2 hypothetical markers, A and B, that are on the same chromosomes. Alleles A1 and A2 are present in A, and alleles B1 and B2 are present in B. A1 B1, A1 B2, A2 B1, and A2 B2 are the four potential haplotypes of markers. If the population's frequencies of alleles A1, A2, B1, and B2 are all 0.5, we can anticipate the population's frequencies of the four haplotypes to be 0.25. Linkage disequilibrium (LD) occurs when the haplotype frequencies deviate from 0.25, indicating that the genes are not in random association. As an aside, the distinction between linkage and linkage disequilibrium mapping is somewhat arbitrary - in fact, linkage disequilibrium between a marker and a QTL is essential if the QTL is to be found in either type of analysis (Mueller 2004). The distinction is that linkage analysis only takes into account linkage disequilibrium within families, which can span tens of thousands of cM and is broken down by recombination after only a few generations. A marker must be in linkage disequilibrium (LD) with a QTL throughout the entire population for linkage disequilibrium mapping. The relationship must have persisted for a significant number of generations to be a property of the entire population; hence, the marker(s) and QTL must be closely related.

MEASURES OF LINKAGE DISEQUILIBRIUM

According to Hill (1981), one measure of LD is *D*, which can be calculated as:

$$D = Freq\ (A1_{B1}) \times Freq\ (A2_{B2}) - Freq\ (A1_{B2}) \times Freq\ (A2_{B1})$$

where *Freq (A1$_{B1}$)* is the population frequency of the A1$_{B1}$ haplotype, and similarly for the other haplotypes.

The *D* statistic is highly reliant on the frequencies of individual alleles, making it ineffective for assessing the degree of LD between numerous loci (for example, at different points along the genome). Hill and Robertson (1968) suggested the r^2 statistic is less dependent on the allele frequencies metric.

$$r^2 = \frac{D^2}{Freq\ (A1) \times Freq\ (A2) \times Freq\ (B1) \times Freq\ (B2)}$$

The frequency of the A1 allele in the population is *Freq (A1)*, and the same is true for the other alleles in the population. The value of r^2 ranges from 0 for a pair of loci with no linkage disequilibrium to 1 for a pair of loci in complete linkage disequilibrium.

For example, consider the following

hypothetical allelic frequencies.

$$Freq\ (A1) = Freq\ (A2) = Freq\ (B1) = Freq(B2) = 0.5$$

The haplotype frequencies are:

$$Freq\ (A1_{B1}) = 0.1$$
$$Freq\ (A1_{B2}) = 0.4$$
$$Freq\ (A2_{B1}) = 0.4$$
$$Freq\ (A2_{B2}) = 0.1$$

$$D = 0.1 \times 0.1 - 0.4 \times 0.4 = -0.15$$

$$D^2 = 0.0225$$

The value of r^2 is then

$$\frac{0.0225}{(0.5 \times 0.5 \times 0.5 \times 0.5)} = 0.36$$

This is a moderate level of r^2.

D' is another often used pair-wise LD measure. The value of D is standardized by the highest value it can achieve to determine D'.

$$D' = \frac{|D|}{D_{max}}$$

Where if D > 0,

$$D_{max} = \min[Freq(A1)\{1 - Freq(B2)\}, \{1 - Freq(A2)\}Freq(B1)]$$

If D < 0

$$D_{max} = \min[Freq(A1) \times Freq(B2), \{1 - Freq(A2)\}\{1 - Freq(B2)]$$

For two reasons, the statistic r^2 is recommended over D' as a measure of the amount of LD. Firstly, the r^2 between a marker and a (unobserved) QTL is the fraction of variation generated by alleles at a QTL that can be explained by markers. The decrease in r^2 with distance represents how many markers or phenotypes are needed to discover QTL in an initial genome scan using LD. When compared to the sample size for testing the QTL itself, the sample size for detecting an ungenotyped QTL must be raised by a factor of $1/r^2$. D', on the other hand, performs a terrible job of forecasting needed marker density for a genome scan using LD. The second rationale for using r^2 instead of D' to determine the level of LD is that D' is prone to be overstated when sample sizes are small or allele frequencies are low (McRae *et al.* 2002).

The LD measurements mentioned above are for bi-allelic markers. While they can be applied to multi-allelic markers like microsatellites, Zhao *et al.* (2005) suggested using the $\chi^{2'}$ measure of LD for multi-allelic markers.

$$\chi^{2\prime} = \frac{1}{l-1}\sum_{i=1}^{k}\sum_{j=1}^{m}\frac{D_{ij}^2}{Freq(A_i)Freq(B_j)}$$
$$D_{ij}^2 = Freq(A_i\_B_j) - Freq(A_i)Freq(B_j)$$

Freq (Ai) is the frequency of the i[th] allele at marker A, *Freq (Bj)* is the frequency of the j[th] allele at marker B, and *l* is the minimum of the number of alleles at marker A and marker B. Note that for bi-allelic markers, $\chi^{2'} = r^2$.

Zhao *et al.*'s (2005) study involved the use of simulation, which indicated a number of multi-allelic pair-wise measures of LD – and $\chi^{2'}$ was the most reliable predictor of useable marker-QTL LD; that is, the measure of QTL variance that can be explained by the marker.

We may want to quantify the extent of LD across a chromosome region that contains several markers, yet statistics like r^2 only consider two loci at a time. The chromosome segment homozygozity (CSH) is an alternative multi-locus definition of LD (Hayes *et al.* 2003). Consider an ancestral animal that lived many generations ago and has descendants now. The ancestor's chromosome is torn down with each generation, until only little portions of chromosome that may be traced back to the common ancestor remain. By descent, these chromosomal regions are identical (otherwise called identical by descent, IBD). The likelihood that two chromosomal segments of the same size and location picked at random from the population originate from a common ancestor (i.e., IBD) without intervening recombination is the

CSH. CSH refers to the length of a chromosomal segment, up to the entire length of the chromosome. The CSH cannot be determined directly from marker data, but must be inferred from marker haplotypes for chromosomal segments.

Consider a chromosomal segment with marker locus A on the left end and marker locus B on the opposite end. Alleles A and B define the haplotype. Two of these segments are randomly selected from the population. The haplotype homozygosity (HH) is the likelihood that two haplotypes are identical by state (IBS). The two haplotypes can be IBS in one of two ways: one, either they descended from a common ancestor without intervening recombination and are thus identical by descent (IBD); or two, they are identical by state but not IBD.CSH is the likelihood of one. Given that the segment is not IBD, the likelihood of two is a function of the marker homozygozities. The haplotype homozygosity (HH) is calculated by adding the probabilities of one and two.

$$HH = CSH + \frac{(Hom_A - CSH)(Hom_B - CSH)}{1 - CSH}$$

Where Hom_A and Hom_B are the homozygosities of marker A and marker B, respectively. When the haplotype homozygosities and individual marker homozygosities are observed from the data, this equation can be solved for CSH. The estimated haplotype homozygosity can be determined in a similar but more difficult manner for more than two markers.

Another advantage of employing multi-locus LD measurements over pair-wise measures is that they can be less variable. Two sampling mechanisms cause the variation in LD. The initial sampling process is based on finite population size and reflects the sampling of gametes to generate successive generations. The second sampling procedure is the selection of individuals from the population to be genotyped, which is determined by the sample size, n. The large variability of LD measurements is due to the first sampling step. Marker pairs located at different locations in the genome but separated by a comparable distance might have vastly varied r2 values, especially if the marker separation is small. This is because an ancestral recombination between one set of markers but not the other may have occurred by accident. Because they aggregate information across numerous loci in a time interval, multi-locus estimates of LD can minimize variability by averaging some of the impacts of accidental recombinations. Hayes *et al.* (2003) used simulation to evaluate the variability of r^2 and CSH. They used a mutation-drift model with a constant N of 1000 to generate a chromosomal region of 10 cM containing 11 markers. They discovered that when at least four loci were included in the CS computation, CSH was less variable than r^2.

ORIGINS OF LINKAGE DISEQUILIBRIUM IN LIVESTOCK POPULATIONS

Migration, mutation, selection, a tiny finite population size, or other genetic processes can cause LD in a population. In an F2 QTL mapping experiment, LD is established between marker and QTL alleles by crossing two inbred lines; in an F2 QTL mapping experiment, LD is created between marker and QTL alleles by crossing two inbred lines.

The fundamental source of LD in livestock populations is widely thought to be finite population size. This is due to the fact that

i. most livestock populations have tiny effective population sizes, resulting in huge quantities of LD;

ii. LD caused by crossbreeding (migration) is substantial when crossing inbred lines but minimal when crossing breeds with similar gene frequencies, and it fades within a few generations (Goddard 1991);

iii. mutations are likely to have occurred many generations ago; and

iv. while selection is most likely a major driver of LD, its impact is likely to be limited to specific genes, with little impact on the amount of LD 'averaged' across the genome.

LD EXTENT IN LIVESTOCK AND HUMAN POPULATIONS

If LD is primarily caused by finite population size, it should be less severe in humans than in cattle, because the effective population size in people is around 10,000 (Kruglyak 1999), whereas in livestock, effective population numbers might be as low as 100.

(Riquet *et al*. 1999). The image is a little muddied by the fact that animal numbers have been substantially bigger, although the effective population size of Caucasians has been much smaller (following the out of Africa hypothesis). As a result, we should anticipate to see that the r^2 values in livestock are significantly higher than in humans at long distances between markers, but the amount of LD is more equivalent
at short distances. This is exactly what has been observed. Moderate LD ($r^2 \geq 0.2$ in humans, for example) often spans less than 5kb (0.005cM) depending on the group investigated (Dunning *et al*. 2000, Reich *et al*. 2001, Tenesa *et al*. 2007). In humans and cattle, however, very high levels of LD (e.g., $r^2 \geq 0.8$) only reach a short distance.

The first whole-genome LD study in cattle, which used 284 microsatellite markers from 581 maternally inherited gametes in Dutch black and white dairy cows to quantify the extent and distribution of LD, was carried out, with high levels of LD extending over several tens of centimorgans (Farnir *et al*. 2000). LD in cattle has been confirmed in a number of following studies (Tenesa *et al*. 2003; Vallejo *et al*. 2003; Khatkar *et al*. 2006a; Odani *et al*. 2006). Only recently, a study in a large mildly selected cattle population from Western Africa conducted under an extensive breeding system revealed that LD extends over shorter distances than previous studies from developed countries, which was explained by increased selective pressure and/or an admixture process (Thevenon *et al*. 2007). All of these LD investigations used microsatellite loci that were highly informative but had a low locus density. With the conclusion of the bovine genome sequencing project, it is now possible to determine the extent of LD using dense single nucleotide polymorphism (SNP) marker maps, resulting in significantly higher resolution.

SNP markers have minimal genotyping costs, in addition to their abundance in the genome (Snelling *et al*. 2005). (Hinds *et al*. 2005). Khatkar *et al*. (2006b) used SNP loci to generate a first- generation LD map of bovine chromosome 6 in Australian Holstein–Friesian cattle, and D′ to estimate the extent of LD. The distance over which LD is expected to be beneficial for association mapping was discovered to be 13.3 Mb, indicating that the range of LD in Holstein–Friesian dairy cow is broad. McKay *et al*. (2007) used 2670 SNP markers to build LD maps for eight cow breeds from the Bos taurus and Bos indicus subspecies, and found that the amount of LD (calculated using r^2) available for association analysis does not surpass 500 kb. The disparities in the degree of LD between McKay *et al*. (2007) and prior investigations were related to differences in LD reporting measures, notably D' vs. r^2. Previous investigations have found that D′ overestimates the extent of LD (Ardlie *et al*. 2002; Ke *et al*. 2004), resulting in extensive LD at long intermarker distances (Farnir *et al*. 2000; Tenesa *et al*. 2003; Vallejo *et al*. 2003; Khatkar *et al*. 2006a; Odani *et al*. 2006).

Du *et al*. (2007) used 4500 SNP markers genotyped in six lines of commercial pigs to determine the degree of LD in pigs. Because paternal haplotypes were over-represented in the population, only maternal haplotypes of commercial pigs were utilized to calculate r^2 between SNPs. According to the findings of their investigation, pigs may have significantly greater LD than cattle. The average value of r^2 for SNPs separated by 1cM was roughly 0.2. In cattle, LD of this size barely extends 100kb. The average r^2 in pigs at 100kb was 0.371.

Heifetz *et al*. (2005) investigated the degree of LD in several breeding chicken populations. They employed microsatellite markers and applied the statistics to determine the degree of LD. They discovered considerable LD over large distances in their populations. For example, 57% of marker pairs separated by 5-10cM had an $\chi^{2`} \geq 0.2$ in one line of chickens and 28% in the other. Heifetz *et al*. (2005) pointed out that the lines they studied had small effective population sizes and were largely inbred, so the level of LD in other chicken populations with greater effective population sizes may differ significantly.

The extent of LD in domestic sheep was studied by McRae *et al*. (2002). Because they employed the D' parameter rather than the r^2 parameter, it's impossible to compare their findings to those of other species. They discovered that high levels of LD lasted for tens of centimorgans and then dropped as

marker distance increased. They also looked at D' bias under various conditions and discovered that *D'* can be skewed when uncommon alleles are present. To establish the true extent of LD, they suggested using the statistical significance of LD in conjunction with coefficients such as *D'*.

QTL MAPPING USING LINKAGE DISEQUILIBRIUM

The population level connections between markers and QTL are used in linkage disequilibrium (LD) mapping of QTL. Because there are little pieces of chromosome in the current population that are descended from the same common ancestor, these relationships occur. These chromosome segments with no intervening recombination will have identical marker alleles or haplotypes, and if there is a QTL inside the chromosome segment, they will have identical QTL alleles. The genome-wide association test with single marker regression is the simplest of the QTL mapping procedures that take advantage of LD.

Due to the availability of tens of thousands of single nucleotide polymorphism (SNP) markers in cattle, pigs, chickens, and sheep soon, doing trials to map QTL in genome-wide scans using LD has recently become practical.

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Farm assistant counts sheep

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Abstract

Small livestock farming in our country is mostly based on pasture. The most important advantage of this situation is that it reduces feed expenses and increases our profitability within the farm. However, the most important problem is in the counting of animals when they come from the pasture to the pen and when they go from the pen to the pasture. This situation depends on the shepherd's attention and follow-up. However, finding experienced shepherds in our country is becoming more and more difficult every day. It may be difficult or even impossible for a sheep giving birth in the pasture to follow the herd when the geographical conditions become difficult. Quick counting of sheep and lambs as the animals enter and exit the pen depends on the shepherd's practice and experience. In order for this situation to be more realistic and to prevent personal mistakes, different alternatives should be considered. For this reason, a system has been developed using deep learning techniques to automatically count the animals in the herd when entering the pen. This system will automatically count the animals at the entrance and exit of the farm, and in case of missing animals, the system users will automatically notify the system users via web and mobile applications. With the implementation of this system, it will be possible to determine the losses that will occur on the farm with an early warning system. In our study, animals will be detected with the deep learning-based YoloV8 pre-trained model on images taken from fixed cameras that will be placed at the entrance and exit of the pen. Counting results obtained from the developed system can be used on different devices by providing multi-platform support. By disseminating this practice, losses of sheep and lambs in the pasture can be prevented.

Key words: *Sheep, lamb, sheep counting, object detection, object counting,*

Heritabilities and genetic correlations between body conformation and milking efficiency of holstein-friesian cows milked by milking robots

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Abstract

Previous pilot studies conducted by scientists from the Bydgoszcz University of Science and Technology (Poland) show that it is possible to predict the suitability of Holstein-Friesian cows for milking with milking robots using decision trees based on information about the phenotype of detailed body conformation traits. However, constructing a selection index adapted to automatic milking requires estimating the genetic parameters of the traits being improved. For this reason, the current studies aimed to estimate the heritability of milking efficiency, detailed body conformation traits of primitive cows, and genetic and phenotypic correlations between them. The study examined 796 Polish Holstein-Friesian first-born cows from 7 herds that utilized the Lely Astronaut A4 automatic system in their barns. The study assessed the following linear features of udder conformation in the primiparous cows: udder depth (UD), teat length (TL), rear udder width (RUW), rear udder height (RUH), rear teat placement (RTP), front teat placement (FTP), fore attachment (FA), central ligament (CL), angularity (A), foot angle (FA), rear legs rear view (RLR), height sacrum (HS), body depth (BD), chest width (CW), rump angle (RA), rump width (RW), rear legs set (RL), and body condition score (BCS). Additionally, the studies recorded milking efficiency, i.e. (ME, kg/min) — milk yield per day divided by box time. In order to estimate the heritability of controlled traits as well as genetic and phenotypic correlations between the udder dimension traits and ME, the AIREMLF90 program (S. Tsuruta) and a linear two-trait animal model were used. Based on the estimates, it was determined that the heritability of ME was moderate and amounted to 0.376. The heritability of detailed conformation traits varied widely from 0.074 (RLR) to 0.584 (HS). The studies showed both positive (from 0.019 (BCS) to 0.617 (RW)) and negative (from -0.342 (RL) to -0.060 (RUH) genetic correlations between ME and conformation traits. A similar phenomenon was recorded in the case of phenotypic correlations: positive from 0.001 (UD) to 0.109 (A) and negative from -0.136 (TL)to -0.006 (RLR). The studies proved that it is possible to genetically improve milking efficiency based on conformation traits, particularly chest width, body depth, and rump width.

Key words: *automatic milking system, milking parameters, dairy cattle, European Union, United States*

Effectiveness of using extruded feed with symbiotic for fish

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Abstract

Currently, extruded fish feeds are very popular among fish farmers, since extrusion significantly increases the digestibility of nutrients. We hypothesize that, in addition to the extrusion of food, a symbiotic additive will positively affect the growth and development of tilapia. The article shows the effectiveness of using symbiotic extruded feed for fish. The main components of the developed feed are fish and soybean meal, as well as extruded wheat and peas. The symbiotic used in the recipe is our own development (a mixture of strains of lactobacilli Leuconostoc mesenteroides and Lactococcus lactis obtained as a result of cultivation on solid agar for the growth of lactobacilli). Experiments on evaluation of the efficiency of developed feed on productive qualities of tilapia were conducted in closed water supply plants based on international research center «Fish farm» (KATRU, Kazakhstan, Astana). Tilapia is divided into two experimental groups, each with 100: control (Con) and experimental (Exp). The differences in groups were only in feeding, with commercial feed received by Con and Exp developed. Chemical analysis of the feed showed no differences in nutritional value of the feed. The experiment lasted 45 days, of which 14 were adaptive. After the adaptation period, weekly 20 random fish were taken and weighed and measured in length (large, L and small, l), calculated Fulton's condition factor *(k_c). Evaluation of the effectiveness of the feed showed a significant difference between groups in favor of group Exp ($P \leq 0.05$): at the end of the first week for k_c 3.42±0.02 vs. 3.16±0.09; at the second week for l – 12.3±0.38 cm and 11.3±0.34; by the fourth week for k_c – 3.77±0.26 vs. 3.28±0.3. The results of the formulation and evaluation of the feed have been positive. This feed is recommended for use in feeding Tilapia in the supply of water basins.*

Key words: *tilapia, extrusion, symbiotic, Fulton's condition*

INTRODUCTION

The Tilapia meat market has been growing positively in recent years and the outlook is quite positive. According to FAO, in November 2023, high-cost producers struggled with low resources and market volatility. Trombeta T.D. (2017) reported that in the cost of tilapia production, feed costs can be 60%, Abdel-Latif H.M.R. (2022) also pointed out that feeding is included in more than half of the production costs in fish farming system. Researchers are conducting many studies on improving fish feeding technology beyond the cost of feeding. El-Dakar A.Y. (2023) in his review writes that in the world for production of 1 kg of protein fish products is spent 0.6 kg of protein suitable for human, to reduce this figure, he says, it is better to include in the fish feed is a soy meal and fish meal. For example, Hejdysz M. (2016) writes that the extruded pea has raw fiber, ADF and NDF, trypsin activity, fitinous and resistant starch, and the apparent ME is above. Willora F.P. (2021) reports on the use of fully extruded feed in feeding pinagoras, which are used to combat sea lice fish. Samuelsen T.A. (2021) uses extrusion as one of the physical factors in the production of feed for Atlantic salmon. On the use of eco-friendly feed additives such prebiotics and symbiotes writes in article M. Fazle R. (2022), he notes using them not only positively affects growth but also improves the immune response of fish.

Given the above and having analyzed the market of feed for fish in Kazakhstan (not presented feed for tilapia), we decided to include in the recipe about 70% of fish flour and soybean meal (Soybean meal was larger because tilapia prefer plant protein, FAO).
The filling was made with extruded wheat and peas in equal proportions, as well as starch and gelatin for water resistance, amino acids and mineral premix for fish. The distinctive feature of our feed is a symbiotic supplement, which consists of lactose bacteria of two strains of *Leuconostoc mesenteroides* and *Lactococcus lactis.*

MATERIALS AND METHODS

The aim of this study was to determine the effectiveness of the developed feed within project AP19576848. The following objectives were set for achieving the goal:
- development of symbiotic;
- determine the nutritional value of developed feed;
- study the growth and development of tilapia when using developed feed.

The chemical analysis of feed was carried out in the conditions of the accredited laboratory KATRU «Zootechnical analysis of feed and milk» using FOSS 2500 IR.
In 2023, the experience of evaluating the effectiveness of developed feed on productive qualities of tilapia in cultivation in closed water supply plants was carried out. The experiment was conducted on the KATRU platform. Two experimental groups were formed, each with 100: a control (Con) and an experimental (Exp). The age of the fish was the same (115 days), and the conditions of the fish were also similar. The differences between the groups were only in feeding, with commercial feed being crushed (Aller Aqua Production) and Exp feed developed by KATRU, 3% of the total fish mass per day for 1 month. The adaptation period was 2 weeks. Feed for the Exp group was created based on FAO recommendations for tilapia. After two adaptation weeks, randomly 20 fish were taken and weighed and measured (length small and large). In total during the experimental month was conducted 4 fish catching with same procedure of weighing and measuring.
Fish length was measured using the following commonly used method.
The Large length (L, cm) of the fish from the beginning of the head (with mouth closed) to the end of the tail fin (in folded position). For fish with different tail-fin extensions (e.g., a marker) the measurement is carried out to the end of the main part of the tail-fin. Additional processes are not considered.
The Small length (l, cm) of a fish from the beginning of the head (with closed mouth) to the beginning of the tail fin (more precisely - to the end of the hyparus bone). In most specialized articles (including articles with primary description of the species) is used standard length.
Using length determined the Fulton's condition factor (kc) or the coefficient of condition factor was estimated as:

$$k_c = 100*W/L^3,$$

where W is the total weight of the fish and L is its Large length (Fulton, 1911)
Statistical analysis is performed in the SPSS 25.0 (Pennsylvania State University).

RESULTS

Development of symbiotic. For develop the symbiotic, fish were gutted, cleaned and their intestines were washed. For better microflora release, the intestine was cut across. After preparation of fish intestines, work was done on accumulation of bacterial culture according to the accepted studies. For this intestinal was transferred to bottles with water and cultured in a thermostat at 28°C 14-16 hours. After the time of cultivation, the environment was cloudy, and white muddy sediment formed. To isolate pure lactose colonies and cultivate solid nutrient media, ten times the growth was carried out in a sterile physiological solution. Incubation was carried out in a thermostat at 37°C for 24 hours. From the petri dishes obtained, 20 colonies were selected that differ in their growth, consistency and size.
Microscopy was done by the method of Gram painting. DNA isolation was performed by a ready-made set of GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA). The PCR analysis yielded 25 samples of bacteria with a long fragment of 790 p.n. The PCR-assay results were then used to prepare the samples for sequencing. The results of the sequence analysis were as

follows: 2 sequences were not identified, 8 sequences were identified as pathogens of fish causing intestinal disorders. The three species are Kurthia gibsonii, Kurthia zopfii, Lactococcus garvieae. The remaining 10 strains are identified as lactobacilli belonging to two species: Leuconostoc mesenteroides, Lactococcus lactis.

To develop a symbiotic product based on lactose bacteria and yeast in liquid form, including the preparation of a nutrient medium with the addition of growth components, inoculation, biomass accumulation, cooling, dissimilar that the nutrient medium was prepared on an aqueous basis with the addition of up to 1.5% glucose, and as inoculum used activated lactobacteria strain Lactococcus lactis 03/1987 and Leuconostoc mesenteroides 08/1987 in a quantity of 0.05%. In distilled water gluconate - 1.5%, buffer salts - 0.1%, ascorbic acid - 0.1%, pepton - 5%, microbiological agar - 1.5%, yeast - 0.5% pH of the medium was set within (7.0.0.1). The finished medium was sterilized at t=121°C for 30 minutes, then cooled to a temperature of (37 1)°C and inoculum was injected - activated strain of Lactococcus lactis 03/1987 and Leuconostoc mesenteroides 08/1987 in quantity 0,05% and increased biomass under periodic cultivation conditions for (24 2) hours with single neutralization after 12 hours of sterile carbon sodium solution (Na_2CO_3). After the cultivation process was finished, the top layer of the culture fluid was separated, the bacterial suspension cooled to (4 2)°C, poured into aseptic bottles with a capacity of 10-12 ml, and sealed.

Nutritional value of developed feed. The main nutrients for tilapia according to FAO are: dry matter (DM), crude protein (CP), crude fat (CL), crude fiber (CF), ash, starch and Digestible energy (DE). Chemical analysis of commercial and developed feed was performed, which were then used in the experiment (Table. 1)

Table 1. Chemical analysis of feed used in the experiment

Nutrient	Con	Exp
DM, %	92,7±6,12	90,9±5,16
CP, %	33,4±0,17	30,1±0,15
CL, %	10,1±0,12	9,1±0,16
CF, %	5,7±0,06	4,9±0,03
Ash, %	6,4±0,07	6,9±0,05
Starch, %	-	16,2±0,19
DE, mg/kcal	123.3±4.8	122.1±3.4

According to the studies carried out, DM in the feed for the Con group was 92.7±0.12%, and for Exp 90.9±0.16%, according to the DM norms, there should be no less than 90% in the feed. The CP in the feed for Con contains more than 3% of Exp at a rate of 29.9-31% (33.4±0.17% versus 30.1±0.15%). CL, CF and ash in two feedlots was practical in equal quantities, within acceptable norms. But in our feed, we used potato starch as a binder, which gave it the ability to not break down for hours, and the starch level in our feed was 16.2±0.19%. The DE norm should be not lower than 120 mg/kcal, both feeds meet this standard, in feed Con contains 1.2 mg/kcal more than feed for group Exp. The nutrients requirements for tilapia are taken from FAO site.

Fish growth while using developed feed. Research on the effectiveness of newly developed feed is an important step in the development and introduction of new products in agriculture. They help create products that are as market relevant as possible, provide optimal results for animals and are economically and environmentally sustainable.

The following table shows the feed efficiency (Table. 2).

In the initial period of the experiment, the fish of the two groups did not differ significantly in the studied parameters. During the first week of the experiment, L of the Exp group increased by 0.8 cm and became 13.7±0.46 cm, l increased by 0.6 cm and became 11.6±0.4 cm. In the first week, a significant difference in kc was noted, the Exp group was 3.42±0.02, while the Con group was 3.16±0.09, while for the latter this indicator decreased by 0.24 from the beginning of the experiment ($P \leq 0.05$).

Table 2. Evaluation of feed efficiency

Period	Gr.	L, cm	l, cm	k_c
Start	Con	13.0±0,5	11.1±0,4	3.4±0,1
	Exp	12.9±0,5	10,9±0,4	3,39±0,1
1 wk.	Con	12.9±0,4	10.9±0,4	3,16±0,09[a]
	Exp	13.7±0,46	11.6±0,4	3.42±0,02[b]
2 wk.	Con	13,6±0,39	11,3±0,34[a]	3,09±0,07
	Exp	14,5±0,43	12,3±0,38[b]	3,32±0,07
3 wk.	Con	14,4±0,56	11,9±0,5	3,19±0,13
	Exp	15,7±0,97	13,3±0,87	3,55±0,81
4 wk.	Con	15,2±0,8	12,6±0,72	3,28±0,3 [a]
	Exp	17,8±1,27	15,3±1,13	3,77±0,26[b]

a, b - P≤0,05

In the 2nd week of the experiment, a significant difference was observed between the groups in the l indicator. If the Con group lengthened by only 0.2 cm relative to the initial indicators, then the Exp group by almost 1.5 cm (P≤0.05). kc in the 2nd week of the experiment decreased in both groups relative to the 1st week. By week 3, the difference between the groups increased even more. Although there is no reliability during this period of the experiment, L of the Exp group was 1.3 cm larger, l was larger by 2.2 cm. In the final week, Exp significantly increased kc in fish, and there was a significant difference between the groups, if in the Exp group it was equal to 3.77±0.26, then for the Con group it was equal to 3.28±0.3 (P≤0.05). It is worth noting that the fish of the Con group never returned to the initial values for the kc indicator, from the initial 3.4±0.1 at the end of the experiment it was below 0.12. The fish survival rate was 98% in the Con group and 97% in the Exp group, all losses were due to the fact that the pools were not covered and the fish jumped out of it in the first week. After the pools were covered, no other losses were observed during the experiment.

DISCUSSION

The use of components similar to ours and symbiotics in fish feeds has been used to conduct research on dietary indicators.

Use of extruded feed in feeding Atlantic salmon Jacobsen H.J. (2018) notes that the hardness of a given feed is negatively correlated with feed consumption and fish growth. At the same time, Aas T.S. (2020) in his study indicated opposite results and notes that the result was influenced by a combination of other factors.

Liao Q. (2023) studied the use of Metschnikowia sp. GXUS03 in feeding juvenile tilapia, the study examined growth performance, gut flora and liver health. 3 groups of fish were formed: the main diet, the main diet with the addition of 1 × 107 CFU/g GXUS03 and the main diet with the addition of 1 × 108 CFU/g GXUS03. The results of the study showed that fish with the third type of diet had higher indicators than the other two.

Ebrahimi G.H. (2012) investigated the use of the prebiotic Immunogen on feed intake, growth, immunity and meat composition of Carp. The results of his research showed that this additive in a volume of 1-1.5 g/kg increases the efficiency of feeding, positively affecting growth, and the fish are also more resistant to the infectious disease caused by A. Hydrophila.

From the above studies, it is obvious that the use of various components same as our in fish feeding had different effects on the studied indicators, but most of them were positive, which corresponds to our results..

CONCLUSIONS

The results obtained by other authors and ours make it possible to conclude that the use of extrusion as one of the methods of feed production and the addition of symbiotics has a positive perspective. The experiment used a commercial feed that advised on the nutritional value of the developed feed. Lactobacilli with a probiotic effect were isolated using generally accepted microbiological methods. Feed efficiency was assessed using indicators such as weight, Large and Small length, and the Fulton condition factor was calculated. During all periods of the experiment, the Exp group had advantages. There was a significant difference in the first and final weeks for the k_c indicator and in the second week for the l indicator (P≤0.05). Over the entire experiment, fish from the Con group increased by 2.2 cm, while Exp by almost 5 cm, which is 17% more.

ACKNOWLEDGEMENTS

Research carried out within the framework of the project AP19576848, with funding from the Ministry of Science and Higher Education of Kazakhstan.

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Expression of Lipoprotein Lipase, Peroxisome Proliferator Activated Receptor Gamma, Apo Lipoprotein Lipase A-1, and Apo Lipoprotein B Precursor Genes in Fulani Ecotype, Noiler and Arbor Acre Chickens

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Abstract

The study evaluated the expression patterns of the Lipoprotein Lipase (LPL), Peroxisome Proliferator-Activated Receptor Gamma (PPARG), Apo lipoprotein lipase A-1 (APOA-1), and Apo lipoprotein B precursor (APOB) genes in Fulani ecotype, Noiler, and Arbor acre chickens. These genes have been linked to key roles influencing partitioning between adipose and muscle tissues that increase fat storage or the delivery of energy in the form of fatty acids for muscular growth and, therefore, may influence meat sensory attributes. Indigenous chicken breeds have been the consumer's favourite or choice in Africa compared to the exotic breeds, principally because of their lower meat fat content and deliciousness. The way these genes are expressed in chickens may depict how fatty they are, which may affect their meat´s palatability. It is to test this hypothesis that 300 day-old chickens (100 of each) were used for the study, which lasted 21 weeks (Fulani ecotype, Noiler), and 8 weeks (Arbor acre), respectively. The birds were managed on deep litter and fed the same single-phase diet, supplying 21.1% CP and energy of 2908.52 kcal/kg ME. The study was fashioned in such a way that all the birds were harvested at the same time (the Arbor acre birds were stocked in the last 8 weeks of the trial). Five birds selected from each chicken group were slaughtered, liver tissue samples harvested, fixed in an RNA-later solution, transported to the laboratory, and used to study the expression pattern of the genes. The results showed no significant (P > 0.05) differences in the expression of the APOA-1 gene, while the expression of the LPL, PPARG, and APOB genes were all significantly (P < 0.001) influenced. The PPARG gene was most upregulated in the Fulani ecotype chicken (0.23 fold change), while it was downregulated in the Noiler and Arbor acre chickens (-0.002 and -2.23 fold changes, respectively). The LPL and APOB genes were most upregulated in the Fulani ecotype chicken (2.61; 2.19 fold changes, respectively), while being downregulated in the Noiler (-0.92, -1.03 fold changes), and Arbor acre (-1.64, -1.16 fold changes), respectively. Conclusively, the results showed that the expression of the four genes might be breed-dependent, and is most common in the Fulani ecotype chicken.

Key words: *Genes, chicken breeds, expression pattern, fold change, liver tissues*.

INTRODUCTION

Consumer preferences and concerns are legitimate influencers of productive activities by livestock farmers. Farmers, therefore, tailor their production in such a way that what they produce will meet the expectations of the final consumers or customers. When it comes to poultry production in Nigeria, this has played a critical role with most people having a preference for the hardier indigenous chickens. A consequence of this is that about 90% of chickens reared in Nigeria are of the indigenous type (Nwanta *et al.,* 2006) representing approximately 83 million chickens (Lasagna *et al.,* 2020) which are raised extensively. Their production has helped in no small measure in alleviating poverty through being sources of income and animal protein supply to not only the

resource-poor folks but also the generality of people. The massive importation of the quick-growing exotic broiler chicken breeds has not dampened indigenous chicken production. This stems largely from the ease of keeping them, their better adaptability to the climate, and most importantly, the perceived palatability of their meat. This perceived palatability of the meat of indigenous Nigerian chickens is the main reason it is sought after by most people compared to broiler chickens. Equally, the tender nature of broiler chicken meat is also not so appealing to many consumers who prefer the tougher meat of the indigenous chicken. The question then arises, as to what is/are the main reason(s) for the perceived palatability of the meat from indigenous chickens.

Meat palatability is somewhat a function of its marbling score and fatty acid composition (Oh *et al.,* 2013). The fatty acid composition of the adipose tissue in livestock has been recognized as an important carcass trait that affects meat quality because a higher Monounsaturated Fatty Acid (MUFA) level leads to a lower fat melting point, which affects the softness of fat and improves meat flavour (Melton *et al.,* 1982; Yang *et al.,* 1999). The composition of fatty acid also determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour (Wood *et al.,* 2008). Reports suggest that the fatty acid composition of meat might be controlled by genetic factors, such as lipid synthesis and fatty acid metabolism-related genes (Narukami *et al.,* 2011).

To be specific, the Lipoprotein Lipase (LPL) gene regulates the plasma levels of triglycerides and High-Density Lipoprotein (HDL) via the action of lipoprotein lipase, an enzyme mediating the lipolysis of triglycerides derived from triglyceride-rich lipoproteins, and bringing about the release of fatty acids. The free fatty acids are then transported to other tissues through the bloodstream. Muscle also stores triglycerides for its energy requirements and the presence of triglycerides manifests as the ''marbling'' in meat.

Peroxisome Proliferator Activated Receptor Gamma (PPARG) gene regulates or controls adipocyte differentiation, fat deposition in mammals (Grindflek *et al.,* 2004), and lipid and energy metabolism while at the same time inhibiting inflammation (Hashizume *et al.,* 2011). It also exerts lipid-lowering and antioxidant effects. It is one of the members of the peroxisome proliferator-activated receptors which are expressed in many metabolically active tissues but are especially high in the liver (Li *et al.,* 2018).

Apo lipoprotein lipase A-1 (APOA-1) gene provides the instructions necessary for the synthesis of a protein called apoliprotein A-1 which is a component of HDL. HDL is a molecule that transports cholesterol and certain fats called phospholipids through the bloodstream from the body's tissues back to the liver (a process called reverse cholesterol transport) and is involved in cholesterol homeostasis (Mangaraj *et al.,* 2016). Once in the liver, the cholesterol and phospholipids are redistributed to other tissues or removed from the body. The reverse cholesterol transport pathway is, therefore, a way through which APOA-1 acts as an essential co-factor for several key components including lecithin: cholesterol acyltransferase (Fielding *et al.,* 1972), ATP binding cassette A1 (Wang *et al.,* 2008), and scavenger receptor B1 (Rigotti *et al.,* 1997). HDL protects against atherosclerosis primarily via reverse cholesterol transport.

Apo lipoprotein B precursor (APOB) gene provides information for the synthesis of apolipoprotein B (Olofsson and Boren, 2005). APOB which exists in two forms, are constituents of lipoproteins, which are particles that carry fats and fat-like substances like cholesterol in the blood. These proteins which are building blocks of Very Low-Density Lipoproteins (VLDLs), Intermediate-Density Lipoproteins (IDLs), and Low-Density Lipoproteins (LDLs), are all involved in the transport of fats, and cholesterol in the bloodstream. APOB attaches to specific receptors on the surface of cells, predominantly in the liver, and transports LDLs into the cell, where they are broken down to release cholesterol; the cholesterol is then either stored, used by the cell, or removed from the body. When not taken out of the body, they contribute to the development of atherosclerosis (Sadovsky, 2003).

All the genes enumerated above are lipogenic and are involved in fatty acid metabolism. Their interplay no doubt will have a massive impact on meat marbling and hence, palatability. It is possible that how palatable meat from the three chickens is will depend on the interplay of the four genes and other closely related ones.

There is scarce if not no literature about the expression of the genes above in the birds studied. It is expected that results emanating from the study will also reveal reasons for the preference shown by Nigerians for the indigenous chicken breeds over the exotic breeds. The study is therefore, aimed at understanding the level of expression of lipoprotein lipase, peroxisome proliferator activated receptor gamma, apo lipoprotein lipase A-1, and apo lipoprotein B precursor genes and how this may influence the palatability of meat obtained from the Fulani ecotype, Noiler, and Arbor acre chickens.

MATERIALS AND METHODS

The poultry research farm of the Department of Animal Production, Federal University of Technology, Minna, Niger State, Nigeria was used in conducting the research. Minna lies between latitude 9° 28' and 9° 37' N, and longitude 6° 23' and 6° 33' E with a mean annual rainfall of between 1102.6 to 1361.7mm. The vegetation is Southern Guinea Savannah and at an altitude of 147m above sea level.

Three chicken breeds were used in the study. The Fulani ecotype chicken is native to the harsh drier parts of Nigeria. Its purity has been largely preserved by the isolated family group lifestyle of the Fulani nomadic tribe who keep them. This isolation makes it hinders interbreeding with other indigenous chickens in the country (Fayeye *et al.,* 2005). It is one of the most commonly found chicken breeds in the country and shows a great propensity for quick growth reaching a market weight of 0.9-2.5kg (Jesuyon and Salako, 2013). Its ancestry is said to contain some exotic bloodline; Ogundipe (1990), and Tiamiyu (1999) opined that the Fulani ecotype chicken could have been developed from crosses between exotic cockerels (Rhodes Island Red) used in previous chicken improvement programmes, and indigenous hens.

The Noiler chicken is a hybrid breed. They were produced from a cross between the White Plymouth Rock renowned for its meat and quick growth, and the Nigerian indigenous chicken renowned for hardiness, resilience, and ability to thrive in resource-challenged environments; Noiler is dual purpose, multi-coloured and can reach a market weight of 2.2-2.6kg by 13 weeks of age (Afrimash.com).

The Arbor acre Plus is probably the most popular commercial breed of broiler chickens in Nigeria. A product of Aviagen, it was bred to produce chicken efficiently through consistent parental performance and excellent performance with good processing yield (Aviagen.com). They are fast-growing, with excellent conformation and tender breast meat. They can reach a market weight of 2793g (6 weeks), and 4374g at 8 weeks.

300-day-old unsexed birds (100 each) were allotted into 3 experimental treatments, replicated 5 times with 20 birds/replicate in a completely randomized design arrangement. The Fulani ecotype were grouped as treatment 1 (T_1), Noiler birds as treatment 2 (T_2), Arbor acre Plus birds as treatment 3 (T_3). The birds were all fed the same single-phase diet formulated to provide 21% CP and 2900 kcal/kg ME (Table 1). Feed and water were served *ad libitum* for the experimental duration.

Table 1. Composition of experimental diet fed

Ingredient	Percentage
Maize	55.32
Maize offal	5.00
Protein concentrate (30%)	39.68
Proximate analysis	
Crude Protein (%)	21.00
ME (kcal/kg)	2,900.00
Crude fibre (%)	5.50
Ether extract (%)	7.24
Ash (%)	9.00
Nitrogen free extract	53.05

The birds were managed on deep litter. Adequate temperature was maintained and routine cleaning, vaccinations, and drug administration were observed until the birds attained maturity. A total of 12 birds (4 each

from the Fulani ecotype, Noiler, and Arbor acre Plus) were selected and slaughtered at the end of the trial. After processing, fresh liver samples were collected from the birds. The samples collected (5g) were placed in properly labelled sterile Eppendorf tubes, and fixed in RNA later solution (an aqueous non-toxic tissue and cell storage reagent that stabilizes and protects cellular RNA). The samples were placed on an ice pack and transported to the laboratory for the gene expression study.

The RT-qPCR protocols of the Geneaid kit were used for gene extraction and quantification. The forward and reverse primers used are given in Table 2. The synthesized cDNA was amplified using the My IQ single colour real-time cycler. The qPCR mix used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25µl reactions consisting of 4µl of the 5x HOT Firepol qPCR Mix, 0.4µl each of the forward and reverse primers, and a specific probe which had a concentration of 250nM, 18.2µl of nuclease-free water and 2µl cDNA template (100ng). The cycling conditions were an initial activation at 95°C for 12 minutes, followed by denaturation at 95°C for 15 seconds, then annealing at 53 and 55°C for 20 seconds (for the genes and GAPDH, respectively), and a final elongation at 72°C for 20 seconds.

Each sample was run in triplicate and averaged triplicates were used to assign cycle threshold (CT) values. GAPDH was used as the reference gene. The ΔCT values were generated by subtracting experimental CT values from the CT values for β-actin targets co-amplified with each sample. The group with the highest means ΔCT value (lowest gene expression) per amplified gene target was set to zero and the mean ΔCT values of the other groups were set relative to this calibrator (ΔΔCT). The ΔΔCT values were calculated as powers of 2 (-2ΔΔCT), to account for the exponential doubling of the PCR.

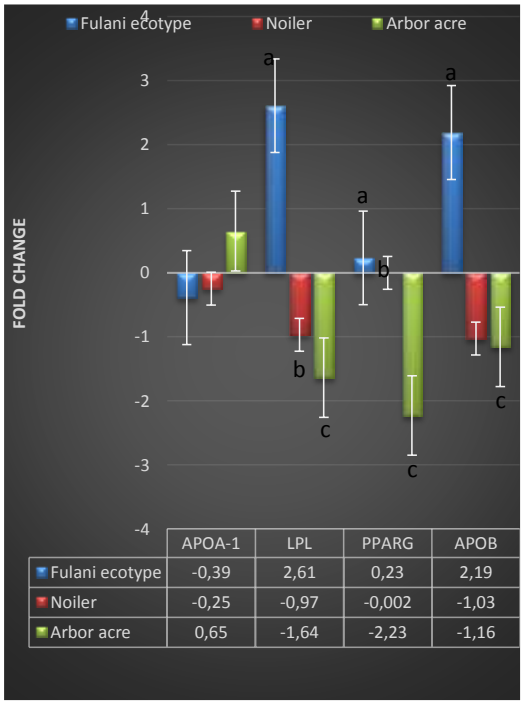
Data generated were analyzed using the One-way ANOVA procedure of SPSS software version 16.00 (IBM, USA). The probability level was set at $P<0.05$.

Table 2: Primer sequence used for the study

Gene name	Accession number	Primer sequence	Primer length	Product length	Exon-exon junction	Species
Lipoprotein lipase (LPL), mRNA	NM_205282.2	5' GCGACTCAGTTCTACTTCGTG 3' TTCATCTCAGCTTCGGGATCG	21 21	250	Yes	*Gallus gallus*
Apolipoprotein A-I (APOA-1), mRNA	NM_205525.5	5' TGGGCAAACAGCTTGACCTGA 3' CCGTCCACTTGGCAGAGAAC	21 20	216	Yes	*Gallus gallus*
Apolipoprotein B (APOB), mRNA	NM_001044633.2	5' CTTTAGAGGCCTCCGCCAG 3' TGCCTCTCCAGAACCTTTCA	19 20	170	Yes	*Gallus gallus*
Peroxisome proliferator activated receptor alpha (PPARA), mRNA	NM_001001464.1	5' TAGTAAGCTCTCAGAAACTTTGTTG 3' GAAACAGAAGCCGCTTTCCA	25 20	157	Yes	*Gallus gallus*

RESULTS

The expression patterns of APOA-1, LPL, PPARG, and APOB genes are presented in Figure 1. The results from the study showed no significant ($P > 0.05$) differences in the expression of the APOA-1 gene, while the expression of the LPL, PPARG, and APOB genes were all significantly ($P < 0.001$) influenced, respectively. The PPARG gene was most upregulated in the Fulani ecotype chicken (0.23 fold change) while being downregulated in the Noiler and Arbor acre chickens (-0.002 and -2.23 fold changes, respectively). The LPL and APOB genes were most upregulated in the Fulani ecotype chicken (2.61; 2.19 fold changes, respectively), while being downregulated in the Noiler (-0.92, -1.03 fold changes), and Arbor acre (-1.64, -1.16 fold changes), respectively.



	APOA-1	LPL	PPARG	APOB
Fulani ecotype	-0,39	2,61	0,23	2,19
Noiler	-0,25	-0,97	-0,002	-1,03
Arbor acre	0,65	-1,64	-2,23	-1,16

Figure 1: Expression patterns of APOA-1, LPL, PPARG, and APOB genes in Fulani ecotype, Noiler, and Arbor acre chickens

DISCUSSION

Meat sensory properties mainly comprise meat colour, tenderness, juiciness, and flavour, which greatly influence consumer choice at the point of visual and in-mouth and or nose perception (Font-i-Furnols and Guerrero, 2014). The upward regulation of the LPL gene in the Fulani ecotype chicken is an indication of a better regulation of fatty acid released from lipoproteins, and increased mediation of the uptake and storage of triglycerides in adipocytes. This will translate to better marbling of the meat influencing its palatability. According to Smith and Carpenter (1974), fat could affect meat juiciness by augmenting the water-holding capacity, through lubrication of the muscle fibres during cooking, via increasing the tenderness of the meat, and thus, the seeming sensation of juiciness, or by stimulating salivary flow during mastication. Since better-marbled meat tends to be juicier, and more tender, these could be the reasons for consumer's appreciation for meat from the Fulani ecotype chicken. One thing that's not too clear though is, how to reconcile this with the actual toughness of meat obtained from the Fulani ecotype chicken particularly when compared to meat from Arbor acre chicken which is more tender and fattier. Further study will be needed to elucidate this discrepancy.

PPARG gene was also upregulated in the Fulani ecotype chicken. This gene has a synergistic relationship with the LPL gene and hence, is expected to also play a role in marbling. Well-marbled meat has a good network of intermuscular fats. Studies using mutton indicate that a certain degree of marbling is indispensable for optimum palatability, as there's a positive relationship between intermuscular fat and meat tenderness, juiciness, and taste (Pannier *et al.,* 2018). The expectation, therefore, is for the meat from the Fulani ecotype chicken to be juicier, and to taste better; this has been alluded to by many Nigerians when asked about their preference for its meat.

The APOB gene regulates the two types of apolipoprotein B, which are building blocks of Very Low-Density Lipoproteins (VLDLs), Intermediate-Density Lipoproteins (IDLs), and Low-Density Lipoproteins (LDLs), involved in the transport of fats, and cholesterol in the bloodstream. APOB attaches itself to specific receptors on the surface of cells (predominantly found in the liver), and helps in the transportation of LDLs into the cell, where they are metabolized to release cholesterol; the cholesterol is then either stored, used by the cells, or removed from the body. Sadovsky (2003) had opined that when not taken out of the body, cholesterol contributes to the development of atherosclerosis. This is because, LDLs are known as 'bad cholesterol' which are capable of increasing heart disease, and increasing the risk of stroke. In terms of meat fattiness, tenderness, palatability, and storage stability, the APOB gene may not have a direct impact. It should be noted however, that, these characteristics of meat are influenced by many other factors such as the animal's breed, its diet, age, and the method used in processing it. However, it is possible that the APOB gene could indirectly affect meat quality through its role in lipid metabolism.

The low levels of APOA-1 gene expression in the Fulani ecotype and Noiler chickens in the face of a correspondingly higher expression of APOB gene in the same chickens, however, calls for caution in the

consumption of their meat. This is because a low APOA-1 level points to an amplified possibility of cardiovascular disease, more so in the presence of a raised APOB (Frondelius *et al.,* 2017). Other factors that could be connected with low APOA-1 levels are chronic liver and kidney diseases. These conditions were however, not the subject of the study and hence, were not evaluated.

CONCLUSIONS

The study has revealed that the expression of LPL, APOA-1, PPARG, and APOB genes in the chickens studied is breed-specific. The surprising thing, however, is that fewer of the genes were expressed in the fattier Arbor acre chicken compared to the leaner Fulani ecotype and Noiler chickens. This may be due to the diet fed. Parts of the reasons given by consumers for their preference for the Fulani ecotype and Noiler chickens is their lean meat nature and the higher expression of LPL, PPARG, and APOB genes may be contributing factors. These genes tend to regulate fatty acid metabolism leading to their storage in the muscle. The presence of intermuscular fat renders meat to be well marbled and such meat tends to be juicier and tastier. Too much consumption of meat with high contents of intermuscular fat should, however, be cautioned against as there's a high chance of exposure to cardiovascular diseases and other associated maladies.

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Challenges and opportunities in sustainable beef production: a review ririn siti

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Abstract

This narrative review delves into the multifaceted landscape of sustainable beef production, offering a comprehensive analysis of the challenges and opportunities encountered within the industry. Recognizing the growing global demand for beef and the imperative to balance production with environmental, economic, and social sustainability, this review synthesizes current research findings, emerging trends, and innovative practices. Key areas of exploration include genetic advancements, nutritional strategies, management practices, and the integration of technology to enhance overall sustainability. The critical evaluation of existing frameworks and the identification of gaps in knowledge aim to guide future research and policy initiatives toward fostering a more sustainable and resilient beef production system. Achieving sustainability in beef production requires a multifaceted and holistic approach that addresses the growing global demand, environmental impact, economic viability, and societal considerations.

Key words: *Challenges, Environment, Opportunities, Sustainability.*

A sulfonylurea herbicide, Sekator, induced hepatic histological damages in male rabbits

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Abstract

The present study was conducted to investigate the effect of a sulfonylurea herbicide "SEKATOR" recently introduced in Algeria on liver histology of male rabbits (Oryctolagus Cuniculus). Herein, 24 male rabbits were equally divided into the control group (Control) received distilled water and a standard diet, and three groups administered orally with three different doses of Senator (0.213 (G1); 0.426 (G2), and 1.066mg/kg body weight (G3)) respectively for 21 consecutive days. At the end of the treatment period, the rabbits were sacrificed by decapitation, and the liver of each animal was removed, weighed, and then fixed in 10% formalin for the histopathological evaluation. Moreover, sekator induced alterations in the biochemical markers of liver function were obviously supported by the histopathological observations, showing dilation of veins, congestion, inflammation, dilation of sinusoids, inflammatory infiltrates and congestion of portal veins.

Key words: *Toxicity, SEKATOR, histopathological, Oryctolagus cuniculus.*

Determination of the relationship between fattening performance and cellular characteristics of skeletal muscles in Saanen kids

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Abstract

Protein, DNA and RNA content of skeletal muscle tissue and protein/DNA, protein/RNA and RNA/DNA ratios show the transcriptional and translational capacity of muscle tissue and can directly affect postnatal growth and development. Therefore, the aim of this study is to determine the relationship between cellular characteristics such as protein, DNA and RNA content and protein/DNA, protein/RNA and RNA/DNA ratios in the skeletal muscles of Saanen kids at different slaughter weights, and the relationship between body weight and body weight at the end of fattening. In the study, 10 male Saanen kids were fattened for 60 days (up to 5 months of age) after 3 months of weaning. From the end of fattening, the kids were divided into two groups as low and high at 1 standard deviation according to their live weight. Samples of Longissimus dorsi (LD) and Semitendinosus (ST) skeletal muscles taken from the kids after slaughter were used as study material. In the isolation of genomic DNA from muscle samples, Miller et al. (1988) and RNA isolation were performed using the Favor Prep Tissue Total RNA Purification Mini commercial kit of the brand FAVORGEN, as recommended by the company that produced the kit. DNA amount was found to be 134.30 ± 7.49 µg/g in LD muscle in low cut weight kids and 187.74 ± 24.16 µg/g in high cut weight kids, 171.83 ± 31.42 µg/g in ST muscle in low cut weight kids. g was found as 133.99 ± 22.12 µg/g in high cut weight kids (p>0.05). The amount of RNA was found to be 69.65 ± 4.75 µg/g in the LD muscle in low cut weight kids and 77.51 ± 8.99 µg/g in high cut weight kids, 60.54 ± 4.11 µg/g in ST muscle in low cut weight kids. It was found 60.00 ± 2.99 µg/g in kids with g high cut weight (p>0.05). Total protein amount was determined by Bradford (Coomasie brillant blue) method. Total DNA, RNA and protein amounts were determined by spectrophotometric method. Protein content is 4.48 ± 0,69 mg/g in LD muscle in low cut weight kids and 6.81 ± 0,64 mg/g in high cut weight kids, 6.65 ± 0,68 mg/g on ST muscle low cut weight kids it was measured as 6.03 ± 0,42 mg/g in high cut weight kids (p>0.05). The data obtained at the end of the study were subjected to variance analysis in the SPSS 20.0 package program. The relationship between end-feeding body weight and cellular properties of muscle mass was evaluated with the Pearson coefficient of correlation. In the light of the obtained data, the relationship between cellular properties of muscle mass and body weight at the end of fattening was determined in male Saanen kids.

Key words: *Saanen kids, Skeletal muscle, Fattening performance, DNA, RNA, Protein*

INTRODUCTION

The goat is a farm animal that stands out for its milk yield but is highly productive in various aspects, with nearly all of its products being utilized. Saanen is the most well-known goat breed. It originates from the Saanen region of Switzerland and is widely bred in the western and northwestern parts of Switzerland. (Kaymakçı, 1996). Goat meat accounts for approximately 5% of global meat consumption (USDA, 2023). In our country, goat meat makes up 5.3% of red meat consumption. Goat farming in Turkey is almost entirely conducted extensively under pasture conditions.

In the developing and changing world, one of the most important and persistent problems facing humanity is adequate and balanced nutrition. In this regard, animal-based foods are indispensable and cannot be replaced by other food products due to their biological properties. Due to the limited availability of natural resources, it is important to utilize these resources in the

most efficient and high-quality way while meeting people's protein needs.

Small ruminant livestock, including sheep and goat farming, forms an important sector of animal husbandry in our country. Compared to sheep, goats can better utilize low-quality pastures. Goats differ from other ruminants in terms of their ability to utilize roughage and pastures, the retention time of feed in the digestive system, the ratio of daily feed intake to body weight, the activity of their metabolism and energy, and their daily movement and activity levels.

The building block of animal tissues is protein. The growth and regeneration of body tissues occur through proteins. Since goats are ruminants, the quantity of the protein they consume is more important than the source. In animal husbandry, body weight and body condition are key factors affecting the productivity of animals (Robinson, 1990). Animals try to maintain their body weight by using the fat and protein reserves in their bodies while being exposed to environmental conditions and physiological effects such as mating, pregnancy, birth, and lactation during the production season (Butler-Hogg, 1984; Fattet et al., 1984).

In goats, birth weight is influenced more by maternal factors (about 70% genetic, 30% environmental) than environmental factors, so birth weight and growth rate should be considered as selection parameters. Muscle tissue is formed by the aggregation of muscle cells called muscle fibers. The number and diameter of muscle fibers determine muscle mass. The number of muscle fibers is closely related to the quantity, quality, and flavor of the meat, as well as the postnatal growth rate and development potential of the animal. The formation of muscle fibers begins early in the embryonic period. Factors affecting the embryo during the prenatal period also affect myogenesis (Maltin et al., 2001; Rehfeldt et al., 2000; Stickland and Handel 1986).

Some studies on sheep have shown that the development of muscle fibers in skeletal muscle begins around the 30th day of pregnancy (primary muscle fibers on the 32nd day and secondary muscle fibers on the 38th day) and is completed by around the 80th day of pregnancy. This period is considered critical for fetal muscle fiber development (Wilson et al., 1992). In the postnatal period, exercise, severe food restriction, mechanical load, and other factors affect the diameter and type of muscle fibers but do not affect the number of muscle fibers (Lefaucher and Gerrad, 2000; Timson et al., 1985; Parsons et al., 1982; Goldspink and Ward, 1979; Stickland et al., 1975). The formation of secondary myofibrils partly coincides with the formation of intramuscular adipocytes and fibroblasts, and together, myocytes, adipocytes, and fibroblasts form the basic structure of skeletal muscle. A large proportion of the myocytes, adipocytes, and fibroblasts in fetal muscles originate from the same pool of mesenchymal stem cells (Uezumi et al., 2011). Therefore, identifying the mechanisms that regulate the differentiation of fetal muscle mesenchymal stem cells is crucial for improving productivity in animal husbandry. The increase in muscle mass during the postnatal period is due to the growth of muscle fibers through hypertrophy.

Many studies have found that animals with a higher total number of muscle fibers tend to have higher birth weights compared to those with fewer muscle fibers. This birth weight difference increases the lamb's survival rate. It is also known that animals with higher birth weights tend to gain more weight during fattening than those with lower birth weights (Gondret et al., 2006).

Cellular development characteristics such as protein, DNA, and RNA content in skeletal muscles, and the ratios of protein/DNA, protein/RNA, and RNA/DNA are directly related to growth and development.

Therefore, quantitative analysis of protein, DNA, and RNA in muscle tissue provides a good method for estimating the growth and development potential of farm animals.

In this study, the cellular development characteristics of male Saanen kids were investigated to determine the relationship between final live weight and live weight at the end of the fattening period. It also aimed to shed light on which of the low and high birth weight kids have better growth and fattening potential. Improving the development of skeletal muscle is very

important for livestock farming. Skeletal muscle is the source of protein-rich meat for human consumption. Myogenesis during the prenatal period is crucial for the postnatal growth rate, development potential, meat quality, and flavor of the animal. Maternal nutrition and other factors, as well as permanent changes in the environment or program, affect prenatal growth and body composition, impacting the fetus (Barker, 1998; Martorell et al., 2001). These permanently determine the phenotype. An adverse intrauterine environment is associated with the programming of postnatal growth and composition, as well as an increased risk of adult diseases (Barker, 1998). Different maternal feeding practices during the critical period of pregnancy, which is vital for fetal muscle and fat tissue development, can affect the muscle fiber composition of the offspring in adulthood, increase marbling, and the number of muscle fibers in the offspring (Bozbay et al., 2014).

Live weight is significantly influenced by muscle mass. Muscle mass consists of muscle fibers, and the formation of muscle fibers begins early in the embryonic period. Cellular development characteristics such as protein, DNA, and RNA content in skeletal muscles, and the ratios of protein/DNA, protein/RNA, and RNA/DNA are directly related to growth and development. Therefore, quantitative analysis of protein, DNA, and RNA in muscle tissue provides a good method for estimating the growth and development potential of farm animals.

This study aims to investigate the cellular development characteristics in male Saanen kids, determine the relationship between final live weight and live weight at the end of the fattening period, and shed light on which of the low and high birth weight kids have better growth and fattening potential.

MATERIALS AND METHODS

The study was conducted in the small ruminant barn of the Livestock Unit within the Faculty of Agriculture at Ondokuz Mayıs University, Samsun. In the study, 10 male kids born from Turkish Saanen goats were used as animal material. After birth, all the kids were kept with their mothers for 15 days to ensure they received adequate colostrum. Following this period, the kids were treated with internal and external parasite control medications and standard health protection measures. To ensure adequate rumen development, the kids were initially provided with high-quality dry hay (dried alfalfa) for the second week after birth within the barn, and then allowed to graze with their mothers in the pasture. Up until weaning age, the kids were allowed to freely nurse from their mothers, and during this period, they were provided with a growth feed (at least 90% dry matter, 16% crude protein, and 2500 kcal/kg metabolic energy) and high-quality dry alfalfa ad libitum.

Evaluation of Live Weight and Fattening of Kids

The birth weights and 90-day weaning weights of the male kids included in the study were determined. After weaning age (day 90), all the kids were subjected to a 60-day fattening period (until 150 days of age). Prior to the fattening period, all the kids were gradually adapted to the commercial kid fattening feed (containing at least 90% dry matter, 12% crude protein, and 2700 kcal/kg metabolic energy) and high-quality dry alfalfa over a period of 4 to 5 days. Before starting the fattening period, all the kids were fasted for 1 day to determine their pre-fattening live weights. During the fattening period, the kids were provided with a mixture of commercial kid fattening feed and roughage ad libitum. The live weights of the fattened kids were recorded at 10-day intervals throughout the fattening period. After the 60-day fattening period, the kids were fasted for 1 day to determine their live weights, and then they were divided into two groups low and high based on their final live weights. The groups were formed based on one standard deviation from the average slaughter weight of all the kids.

Slaughter and Muscle Sampling

At the end of the fattening period, the goats included in the study were slaughtered in a commercial abattoir authorized by the Ministry of Agriculture and Forestry, following regulations. Immediately after slaughter, approximately 50 g samples were taken from the Longissimus dorsi (LD) and Semitendinosus (ST) muscles on the left side of the carcass. The fat and connective tissues

were removed from the muscle samples, and pieces measuring 2×5×2 cm were cut, frozen in liquid nitrogen, and stored at -80 ºC until analysis.

DNA Isolation and Quantification

Muscle samples designated for DNA isolation were crushed with a mortar and pestle after being supplemented with liquid nitrogen. The genomic DNA isolation from LD and ST muscle samples was carried out using the DNA isolation protocol reported by Miller et al. (1988) in a laboratory setting. Mechanically processed muscle samples were removed from -80°C and allowed to thaw at room temperature (24-25°C) until fully dissolved. DNA isolation was performed by weighing 100 mg of each sample with a precision balance. The quantity and purity of the genomic DNA isolation were assessed using a NanoDrop Spectrophotometer. The isolated genomic DNA molecules from the samples were checked using a 1% agarose gel.

Total Protein Isolation and Quantification

From the muscle samples, which were previously mechanically fragmented with liquid nitrogen, 100 mg was weighed, and 1500 µl of buffer solution containing 1 mM EDTA, 0.1 M KCl, and 0.1 M KH2PO4 (pH:7.6) was added. The mixture was vortexed for approximately 1 minute and then centrifuged at 15,000 g for 45 minutes. After centrifugation, the supernatant was carefully collected, and the total protein content was determined by measuring the absorbance at 595 nm using the Bradford (Coomassie Brilliant Blue) method with a spectrophotometer. The samples with determined protein content were subjected to SDS-PAGE electrophoresis for 1.5 hours at 40 A (amperes) in running buffer. Bio-RAD Kaleidoscope marker was used as the standard marker.

RNA Isolation and Quantification

In the study, RNA isolation was performed from 100 mg of mechanically fragmented muscle samples, following the Favorgen protocol using the Favor Prep Tissue Total RNA Purification Mini Kit. The quantity and quality of the total RNA preparations were spectrophotometrically determined at a wavelength of 260 nm using the NanoDrop™ 2000/2000c Spectrophotometers (ThermoFisherScientific). The purity of the total RNA was determined by the A260/280 and A260/230 ratios. The samples were loaded onto a 1% agarose gel. Then, the images were obtained using a gel electrophoresis imaging system.

Statistical Analyses

The data obtained at the end of the study were analyzed using the Shapiro-Wilk test, Levene's test, and one-way analysis of variance (ANOVA) with SPSS version 20.0 under the OMÜ license. According to the results of the Shapiro-Wilk test, it was determined that the data were normally distributed ($P>0.05$), and the Levene's test results indicated that the variances were homogeneous ($P>0.05$). One-way analysis of variance was used to compare the experimental groups.

RESULTS

The total amounts of DNA, RNA, and protein in the Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Saanen goat kids with low and high slaughter weights are presented in Table1. In the study, it was determined that the total genomic DNA amount in the LD muscle of Saanen goat kids with high slaughter weight was higher than that of kids with low slaughter weight ($p>0.05$). The total genomic DNA amount in the ST muscle showed similarity between goat kids with different slaughter weights. Similarly, the total RNA amount in the LD and ST muscles showed similarity between goat kids with different slaughter weights. However, a higher amount of protein was detected in the LD muscle of goat kids with high slaughter weight compared to those with low slaughter weight ($p>0.05$). The total protein amount in the ST muscle showed similarity between Saanen goat kids with different slaughter weights.

Table 1. Total DNA, RNA, and protein amounts in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Saanen goat kids with low and high slaughter weights

Traits	Muscle	Groups	
		Low	High
DNA (µg/g)	LD	134,30±7,49[b]	187,74±24,16[a]
	ST	171,83±31,42	133,99±22,12
RNA (µg/g)	LD	69,65±4,75	77,51±8,99
	ST	60,54±4,11	60,00±2,99
Protein (mg/g)	LD	4,48±0,69[b]	6,81±0,64[a]
	ST	6,65±0,68	6,03±0,42

[a,b,c] Means with different letters in the same row are significantly different (p<0.05).

The RNA/DNA, Protein/DNA, and Protein/RNA ratios in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Saanen goat kids with low and high slaughter weights are presented in Table 2. The study found that the RNA/DNA ratio in the LD muscle of Saanen goat kids with high slaughter weight was higher compared to those with low slaughter weight (p>0.05). The RNA/DNA ratio in the ST muscle was similar across different slaughter weights. The study also found that the Protein/DNA ratio in the LD muscle of Saanen goat kids with high slaughter weight was higher than that of those with low slaughter weight (p>0.05). The Protein/DNA ratio in the ST muscle showed similarity across different slaughter weights. No significant differences were found in the Protein/RNA ratio between Saanen goat kids with different slaughter weights in both LD and ST muscles.

Table 2. RNA/DNA, Protein/DNA, and Protein/RNA (µg/gg) Ratios in Longissimus-dorsi (LD) and Semitendinosus (ST) Muscles of Saanen Kids with Low and High Slaughter Weights

Traits	Muscle	Group	
		Low	High
RNA/DNA	LD	0,48±0,11[b]	0,63±0,09[a]
	ST	0,45±0,03	0,39±0,05
Protein/DNA	LD	29,94±6,07[b]	56,50±11,30[a]
	ST	51,25±8,08	34,44±4,96
Protein/RNA	LD	67,20±14,70	91,40±11,70
	ST	113,20±15,80	88,52±8,60

[a,b,c] Means with different letters in the same row indicate significant differences (p<0.05).

The live weight averages and carcass characteristics of Saanen kids with low and high slaughter weights are presented in Table 3. The study found no statistically significant differences between Saanen kids with low and high slaughter weights in terms of birth weight, weaning weight at 90 days, and live weight at 110, 120, and 130 days. However, it was observed that the live weights at 140 and 150 days were higher in Saanen kids with high slaughter weights compared to those with low slaughter weights (p>0.05). Similarly, Saanen kids with high slaughter weights had heavier hot and cold carcasses compared to those with low slaughter weights (p>0.05). The study did not find any statistically significant differences in cold and hot carcass yield between live weight groups.

Table 3. Live weight averages and carcass characteristics of Saanen kids with low and high slaughter weights

Traits	Group	
	Low	High
Birth Weigh (g)	2396,0±43,2	2580,0±84,6
Weaning Weight at 90 Days (g)	18280,0±414,9	19820,0±949,0
Live Weight at 110 Days (g)	24520,0±676,3	25850,0±859,1
Live Weight at 120 Days (g)	26000,0±687,7	27210,0±629,4
Live Weight 130 Days (g)	26530,0±599,5	28070,0±511,7
Live Weight 140 Days (g)	27360,0±609,6[b]	30130,0±473,9[a]
Live Weight 150 Days (g)	27730,0±593,6[b]	31175,0±373,3[a]
Pre-slaughter Live Weight (g)	27940,0±607,33[b]	31440,0±390,3[a]
Hot Carcass Weight (g)	14065,0±296,3[b]	15558,0±348,5[a]
Cold Carcass Weight (g)	13677,0±316,1[b]	15164,0±348,0[a]
Loss (g)	387,60±50,63	393,20±32,42
Hot Carcass Yield (%)	50,35±0,29	49,49±0,79
Cold Carcass Yield (%)	48,96±0,47	48,23±0,75
Loss (%)	1,39±0,19	1,25±0,11

[a,b,c] Differences between means with different superscripts in the same row are significant (p<0.05).

DISCUSSION

In this study, the relationship between skeletal muscle development, molecular

characteristics, and fattening performance of Saanen kids with low and high slaughter weights was examined. Skeletal muscle mass is an important parameter associated with the growth process in vertebrate animals.

The study found that Saanen kids with high slaughter weight had higher total genomic DNA levels in the Longissimus dorsi (LD) muscle compared to those with low slaughter weight. Factors affecting the embryo during the prenatal period also impact myogenesis (Maltin et al., 2001; Rehfeldt et al., 2000; Stickland and Handel, 1986). This finding suggests that kids with higher slaughter weight have larger and more developed skeletal muscles, and that the muscle development in high slaughter weight animals is more effective compared to low slaughter weight animals during the prenatal period. Additionally, the protein content in the LD muscle was also found to be higher in kids with high slaughter weight, indicating that protein synthesis, which is crucial for muscle mass growth and development, is greater in these animals.

Moreover, the RNA/DNA ratio in the LD muscle was higher in kids with high slaughter weight compared to those with low slaughter weight. The RNA/DNA ratio is considered an indicator of cellular activity, with higher ratios possibly indicating faster growth and cell division. This finding suggests that skeletal muscles in high slaughter weight kids grow and develop more rapidly.

However, no statistically significant differences were found between kids with different slaughter weights regarding DNA, RNA, and protein levels or RNA/DNA ratios in the Semitendinosus (ST) muscle. This may suggest that the ST muscle has less impact on skeletal muscle development compared to the LD muscle. For assessing skeletal muscle development in high and low slaughter weight kids, examining the LD muscle may provide clearer results.

In terms of fattening performance, no statistically significant differences were found between low and high slaughter weight kids regarding birth weight, 90-day weaning weight, and fattening live weights at 110-130 days of age. However, at 140 and 150 days of age, kids with high slaughter weight had higher values for fattening live weights and carcass characteristics (hot and cold carcass weights) compared to those with low slaughter weight. This indicates that high slaughter weight kids have better feeding and growth potential. As the prenatal period extends, the animals' potential for feed utilization and survival increases. Among kids with different birth weights, those with higher birth weights benefit more from fattening performance compared to those with lower birth weights.

CONCLUSIONS

In conclusion, this study has shown that Saanen kids with high slaughter weights exhibit better skeletal muscle development and feeding performance. The higher DNA, protein levels, and RNA/DNA ratios in the LD muscle indicate that high slaughter weight kids have a larger and more developed muscle mass. Consequently, high slaughter weight kids have demonstrated better growth compared to low slaughter weight kids under the same feeding conditions. These findings highlight the significant role of genetic and nutritional factors in the relationship between slaughter weight, muscle development, and feeding performance.

ACKNOWLEDGEMENTS

This project was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) under the 2209-A University Students Research Projects Support Program with the application number 1919B012202084.

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Phenotypic and morphometric analysis of Zambian Indigenous Chickens in Kabwe District, Zambia

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Abstract

This study aimed to characterize indigenous chickens in Kabwe district on which basis breeding programs can be instituted. For this study, 434 chickens comprising 206 females and 228 males were sampled. Each chicken was observed for shank color, comb type, ear lobe color, and eye color. Linear body measurements were also taken individually using a tailor's tape in centimetres, and comprised corpus length (CL), chest circumference (CC), thigh length (TL), thigh circumference (TC), shank circumference (SC), shank length (SL), keel length (KL), and body length (BL). Results revealed that the most common phenotypes were white shanks (33.70%), single comb type (98.91%), red-white earlobe color (50%) and orange eyes (80.44%). The average body weight was 1698.24g which correlated positively and significantly ($p<0.05$) with all linear body measurements in both males and females except which had an insignificantly ($P>0.05$) negative correlation with body weight in the males where. A stepwise regression analysis revealed that keel length, chest circumference, shank circumference, thigh circumference, body length, and corpus length CL can be used to significantly predict body weight with an $R2=76.90\%$ ($P<0.01$). CHAID data mining algorithm was also used to develop a model that identified keel length as a key predictor for body weight with corpus length and thigh length being other predictors with an $R2=61.20\%$. The findings of this study can be used by breeders and farmers to make selection decisions aimed at the improvements of their chickens.

Key words: *Body weight, CHAID data mining algorithm, Characterization of chickens, Correlation, Linear body measurements, stepwise regression analysis*

Comparison of expression levels of Myogenic factor 5 and 6 genes in Peking ducks of different ages

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Abstract

Myogenic factor 5 (Myf5) and 6 (Myf6) genes, which are members of the myogenic regulatory factors family, are among the genes that regulate the development and differentiation of muscle cells and the main regulators of skeletal muscle tissue formation. Determining the expression levels of Myf5 and Myf6 genes in the Pekin duck with different ages could help reveal growth potential at the molecular level. Therefore, this study aimed to determine the expression levels of Myf5 and Myf6 genes in the pectoral major (PM) skeletal muscles of the Pekin ducks of different ages. Total RNA was isolated from muscle samples using a commercial RNA extraction kit as recommended by the manufacturer. The isolated RNA was converted into cDNA using a commercial cDNA kit in a Thermal Cycler. Myf5 and Myf6 gene expression levels in PM muscle were determined by real-time quantitative polymerase chain reaction. In male Pekin ducks, Myf5 gene expression fold change in PM skeletal muscle was higher at 8 weeks than at 4 weeks (P<0.05). No statistically significant difference was found regarding Myf5 gene expression fold change in female Pekin ducks of different ages. Again, no statistically significant difference was found in terms of Myf5 gene expression fold change between 8-week-old female and male Pekin ducks, while Myf5 gene expression fold change in 4-week-old female ducks was found to be lower than in male ducks (P<0.05). Myf6 gene expression fold change was higher at 8 weeks of age than in 4-week-old ducks in both male and female Pekin ducks (P<0.05). Similarly, it was found that male Pekin ducks at 4 and 8 weeks of age had higher Myf6 gene expression fold change in PM skeletal muscle than female ducks (P<0.05). Study results showed that age affected the expression level of Myf5 and Myf6 genes and that the expression levels of these genes changed with growth.

Key words: *Pekin ducks, Pectoralis major muscle, Growth, Myf5, Myf6*

INTRODUCTION

The rapid increase in the world population, disruptions in meat production, and, more importantly, raising farm animals with quality meat are some of the necessities of our day. By changing the skeletal muscle development profiles (muscle fiber type, diameter, intramuscular fat ratio) of the animals to be fattened with gene expression organizations to be made in farm animals, the feed utilization rate can be affected for more meat production or more meat production can be achieved with lower amounts of feed from the animals. In addition, determining the cellular or molecular properties in the skeletal muscle tissue of the animal species and breeds to be used in meat production is of great importance in terms of pre-determining the meat production potential of the animals to be fattened, increasing meat production, reducing production costs and reducing meat prices.

Skeletal muscle development is controlled by myogenic regulatory factors (MRFs), a family of muscle-specific basic helix-loop-helix (bHLH) transcription factors. Skeletal muscle differentiation occurs when MRF genes of the MyoD family are activated in muscle progenitors, and this genetic program operates in both the trunk and head regions myogenic factor 5 (Myf5), myogenic differentiation (MyoD), myogenic regulatory factor 6 (Mrf4), and myogenin are essential components of the myogenic pathway (Zhong et al., 2013). The main

functions of myogenic regulatory factors are as follows.

The MyoD gene is responsible for the growth and proliferation phase of the early formation process of muscle fibers (Braun and Arnold, 1995). The MyoG gene regulates postnatal muscle maturation and differentiation (Braun and Arnold, 1995). The Mfy5 gene is considered the first expressed MRFs and is controlled by a 140 kb enhancer complex in its regulatory region (Carvajal et al. 2008). The Myf5 gene is mainly involved in the physiological process of muscle fiber elongation and development. The Myf6 gene is responsible for postnatal muscle maturation and differentiation. It has been shown that Mfy6 can also be effective in the growth and proliferation phase of muscle fibers during their early formation (Kassar-Duchossoy et al. 2004).

By changing the skeletal muscle development profiles (muscle fiber type, diameter, intramuscular fat ratio) of the animals to be fattened by gene expression organizations in farm animals, the feed conversion ratio can be affected for higher amounts of meat production (Kassar-Duchossoy et al., 2004). In addition, determining the cellular or molecular characteristics in the skeletal muscle tissue of farm animals to be used in meat production is of great importance in predetermining the meat production potential of fattening animals, increasing meat production, reducing production costs, and reducing the price.

Peking ducks exhibit rapid growth and high meat yield, and only phenotypic values were taken into account in studies on this breed's development, meat yield, and fattening performance. The underlying mechanism of the differences observed in the studies has not been fully revealed and has been attributed to environmental factors.

One of the aims of this study is to prove that the reliability of the results of studies conducted at the molecular level and understanding skeletal muscle development at the cellular level can be an indispensable basic building block in studies aimed at increasing animals' fattening performance. However, limited studies on Myf5 and Myf6 genes related to Peking ducks are in the literature. Due to all these facts, the present study aims to determine the expression levels of Myf5 and Myf6 genes, which act as the primary regulators of skeletal muscle myogenesis (formation of muscle tissue) in the pectoralis major (PM) skeletal muscles of Pekin ducks of different slaughtering ages, and to determine the relationship between gene expression level and growth. Thus, the real fattening potential of Pekin ducks can be revealed at the molecular level.

MATERIALS AND METHODS

The study was conducted at the Ondokuz Mayıs University Agricultural Faculty's Farm. The study used Pekin ducks, bought from a commercial hatchery, as animal material. All ducklings were transferred to a production house at daily age. Each animal was sexed from the cloaca, and the wing numbers were attached to each individual. Ducks were reared with a feeding program to standard commercial practices. Ducks were fed a diet containing 22% crude protein (CP) and 2950 kcal/kg ME for the first four weeks and 16% CP and 3100 kcal/kg ME from the 5th to 8th weeks. Sawdust + straw were used as bedding material. The lighting schedule was 24 h lights (L): 0 h dark (D) during the first week, 22L: 2D for 2-4 weeks, and 18L: 6D for 5-8 weeks. The temperature was between 28-35 °C during the first week and maintained between 20-32 °C from the 2nd week onwards (Thinh et al., 2021). Ducks were reared at a stocking density of 5 birds/m^2.

After the first 4 weeks, 20 randomly selected ducks (10 males and 10 females) were subjected to standard slaughtering in the slaughterhouse within the enterprise. Immediately after slaughter, the Pectoralis major (PM; breast muscle) muscle on the right side of the carcass was isolated and cleaned of fat and connective tissues. Then, 5 (length) × 2 (width) × 2 (height) cm pieces were taken from the PM muscle, frozen in liquid nitrogen, and stored at -80 °C until analysis. The remaining ducks were fed until 8 weeks of age. Similarly, at the end of 8 weeks, 20 randomly selected ducks (10 males and 10 females) were subjected to standard slaughtering in the slaughterhouse within the enterprise. Immediately after slaughter, the Pectoralis major (PM; breast muscle) muscle on the right side of the

carcass was isolated and cleaned of fat and connective tissues. Then, 5(length) × 2(width) × 2(height) cm pieces were taken from the PM muscle, frozen in liquid nitrogen, and stored at -80 ºC until analysis.
Total RNA of PM muscle samples were isolated by a commercial RNA (PureLink ™, RNA Mini Kit, Invitrogen™, 12183018A) purification kit using the TRIzol Reagent (Thermo Fisher Scientific, US) as suggested by the manufacturer. Genomic DNA was eliminated by digestion with DNase I (Thermo Fisher Scientific Inc., Waltham, MA, USA). The purity and concentration of isolated RNA were evaluated by the A260/A280 ratio using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), and all RNA samples showed A260/A280 values within the range of 2.01 to 2.08 and A260/ A230 values above 2. The integrity of collected RNA was checked with 1 % w/v agarose gel electrophoresis. Total RNA was converted to cDNA using a commercial cDNA kit (BIORAD iScript cDNA, 1708890) following the manufacturer's instructions in the Thermal Cycler (BIORAD) device. For GADPH the prepared cDNA samples were further purified, quantified, diluted to the same initial concentration, and stored at −20 °C until subsequent quantitative real-time PCR analysis.

qRT-PCR Analyses

Primers used for the amplification of genes were designed using online tools (https://www.ncbi.nlm.nih.gov/tools/primerblast/) (accessed on 12 April 2022) based on the related gene sequences of duck (Table 1).

Table 1. Primer Sequences for the mRNA expression analysis of genes

Genes	Primer sequence (5′-3′)		PS (bp)
	Forward	Reverse	
MyoD	AGGAGGAGGCTGAAGAAAGTGA	CTGTCTCGGCAGGTGATA	131
MyoG	GGAGCGCCATCAGCTACATC	CGAGGAAGTCCGAGCCATT	87
GAPDH	AGGAGGAGGCTGAAGAAAGTGA	CTGTCTCGGCAGGTGATA	118

PS= product size

GADPH was selected as housekeeping gene to normalize the expression of target genes. All primers were synthesized by Sentebiolab (Ankara, Türkiye). The specificity of each of the designed primers was checked via online Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) and melt curve analysis was carried out during qRT-PCR. Relative quantification of all transcripts was performed by qRT-PCR using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Real-time quantitative PCR were run with EvaGreen mastermix (5× HOT FIREPol EvaGreen qPCR Mix Plus, Solis BioDyne, Tartu, Estonya). The reaction mix was in a total volume of 10 µL comprising 5 µL of 5X HOT FIREPol mix, 0.5 µL of forward primer (10 µmol/L), 0.5 µL of reverse primer (10 µmol/L), Dye, 2 µL of DEPC treated water, and 2 µL of template cDNA. PCR amplification was carried out as follows: denaturation of 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, specific annealing temperature of 60 °C for 30 s. The relative mRNA expression levels of the genes were calculated by the $2^{-\Delta\Delta Ct}$ method.
The data obtained at the end of the study were conducted using the Shapiro-Wilk test, Levene test, and one-way analysis of variance using the SPSS 20.0 version OMU license. According to the Shapiro-Wilk test results, it was determined that the data were suitable for normal distribution ($P<0.05$) and according to the Levene test results, the variances were homogeneous ($P<0.05$). One-way analysis of variance was used to compare the trial groups.

RESULTS

The fold changes of expression of myogenic factor 5 (Myf5) and myogenic factor 6 (Myf6) genes in the Pectoralis major (PM) skeletal muscle of female and male Pekin ducks of different ages are presented in Table 2. In the study, significant differences were observed between age groups (except Myf5 gene expression fold change of 4 and 8

weeks old female Pekin ducks) and genders (except Myf5 gene expression fold change of 8 weeks old male and female Pekin ducks) in terms of expression fold changes of Myf5 and Myf6 genes (P<0.05). While Myf5 gene expression fold change of PM skeletal muscle in male Pekin ducks was found to be higher at 8 weeks of age than at 4 weeks of age (P<0.05), no statistical difference was found in terms of Myf5 gene expression fold change in female Pekin ducks of different ages. Again, no statistical difference was detected between 8-week-old female and male Pekin ducks regarding Myf5 gene expression fold change, while 4-week-old female ducks had lower Myf5 gene expression fold change than male ducks (P<0.05). Myf6 gene expression fold change was higher in male and female Pekin ducks at 8 weeks of age than in 4-week-old ducks (P<0.05). Similarly, 4-week-old and 8-week-old male Pekin ducks had higher Myf6 gene expression fold change in PM skeletal muscle than female ducks (P<0.05).

Table 2. The expression fold changes of myogenic differentiation factor (MyoD) and myogenin (MyoG) genes in the Pectoralis major (PM) skeletal muscle of male and female Peking ducks with low and high slaughter weight

Traits	Sex	Groups	
		Low	Low
Myf5	M	24.51 ± 1.05^{Ab}	34.22 ± 3.93^{a}
	F	11.28 ± 5.04^{B}	28.53 ± 9.59
Myf6	M	74.69 ± 8.04^{Ab}	131.78 ± 17.59^{Aa}
	F	27.34 ± 2.05^{Bb}	46.06 ± 3.93^{Ba}

^{a,b} Mean values with different superscripts in the same row indicate a significant difference (P<0.05)
^{A,B} Mean values with different superscripts in the same column indicate a significant difference, (P<0.05). M= male, F= female.

The expression levels of the myogenic factor 5 (Myf5) gene in the Pectoralis major (PM) skeletal muscle of female Pekin ducks at different ages are presented in Figure 1. The study found that 8-week-old female Pekin ducks had higher Myf5 gene expression fold change than 4-week-old ducks (P<0.05).

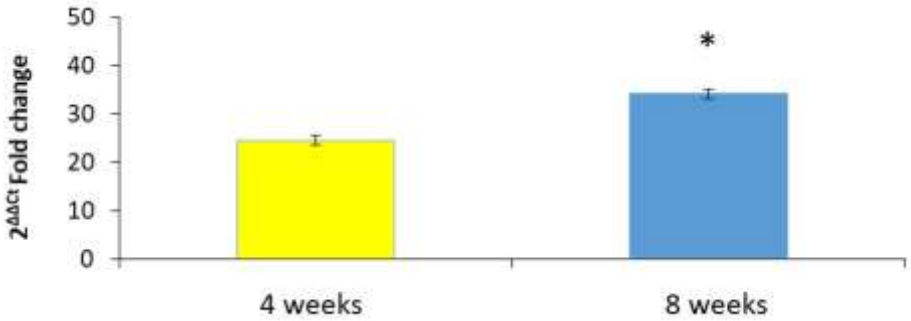

Figure 1. The expression levels of the myogenic factor 5 (Myf5) gene in the Pectoralis major (PM) skeletal muscle of female Pekin ducks at different ages. *P<0.05.

The expression levels of the myogenic factor 5 (Myf5) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages are presented in Figure 2. The study detected no statistically significant difference regarding Myf5 gene expression fold change among male ducks of different ages.

The expression levels of the myogenic factor 6 (Myf6) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages are presented in Figure 3. The study determined that female Pekin ducks at 8 weeks of age had higher Myf6 gene expression fold change than those at 4 weeks of age (P<0.05).

The expression levels of the myogenic factor 6 (Myf6) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages are presented in Figure 4. The study found that 8-week-old male Pekin ducks had higher Myf6 gene expression fold change than 4-week-old male ducks ($P<0.05$).

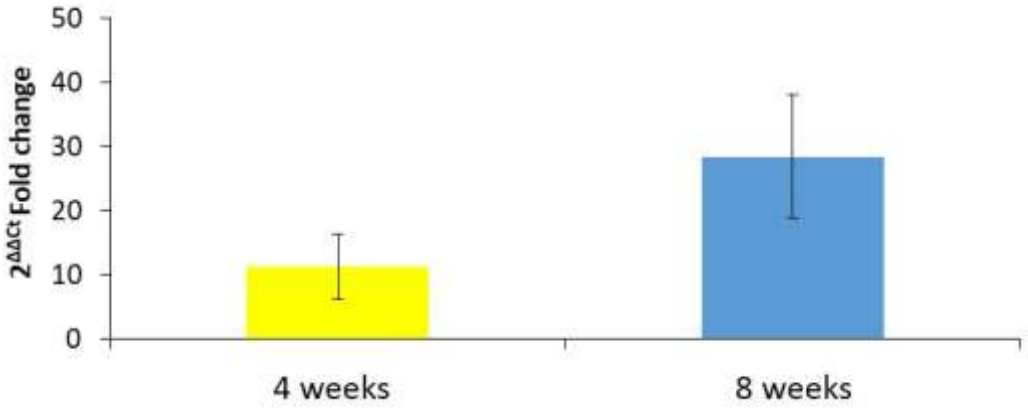

Figure 2. The expression levels of the myogenic factor 5 (Myf5) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages.

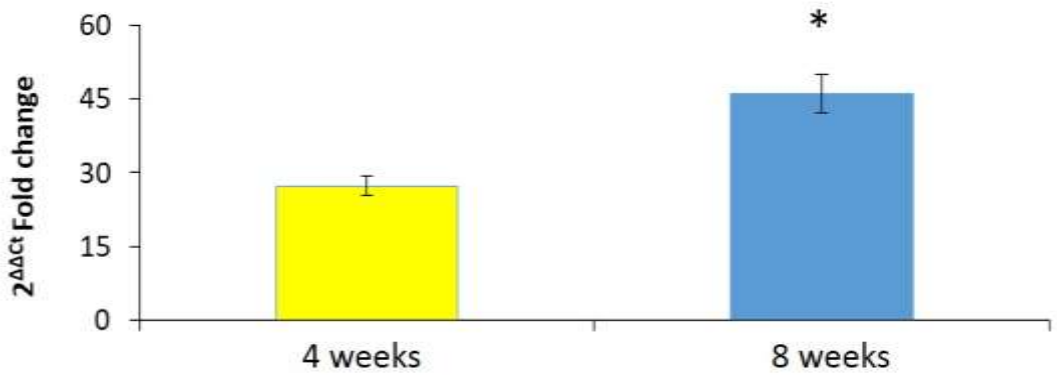

Figure 3. The expression levels of the myogenic factor 6 (Myf6) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages. *$P<0.05$.

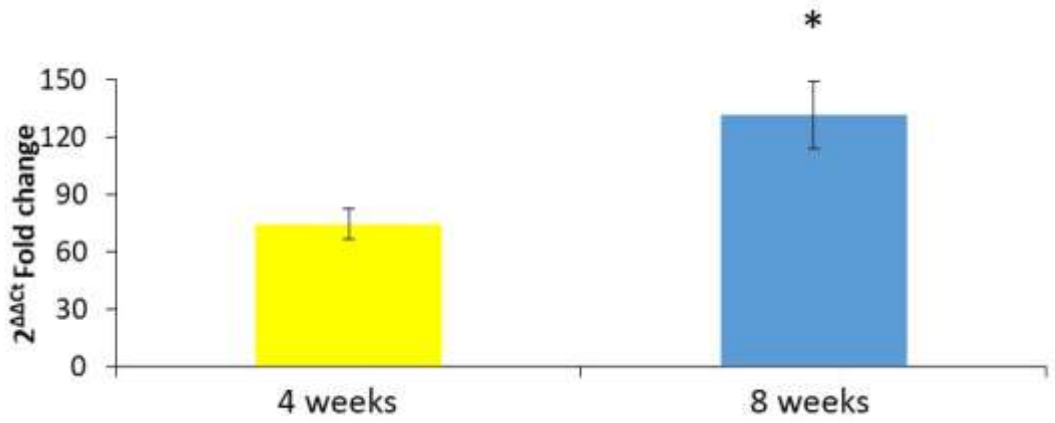

Figure 4. The expression levels of the myogenic factor 6 (Myf6) gene in the Pectoralis major (PM) skeletal muscle of male Pekin ducks of different ages. *$P<0.05$.

DISCUSSION

Many genetic and environmental factors affect meat yield in farm animals. Muscle fiber growth in organisms is regulated by the MRFs gene family, which consists of the myogenin gene (MyoG), Myf3 (MyoD), Myf5, and Myf6. These four genes contain 3 exons, and their expression shows a specific regulation during myogenesis. The MRFs gene family has an essential role in myogenesis and, thus, in muscle tissue characteristics (Bhuiyan et al., 2009). MRFs constitutes a selected family of transcription factors whose function and activity represent a paradigm in which a series of molecular switches determine the fate of the entire cell lineage (Te Pas, 2004). In addition to these biochemical properties, their temporal and spatial expression dynamics allow the definition of a clear and hierarchical relationship between the four MRFs. There are many studies on Myf5 and Myf6 genes in farm animals. Genxi et al. (2014) investigated the relationship between the Myf5 gene and growth traits in Jinghai yellow chickens using PCR-SSCP, RT-PCR, and sequencing methods. In the study, 3 different genotypes (CC, CD, and DD) of the Myf5 gene were obtained. It was determined that individuals with the CD genotype of the Myf5 gene had higher birth weights than individuals with the CC genotype.

Meat yield traits such as live weight or carcass weight are related to growth, increased muscle cell development, and tissues. In addition, live gain and carcass weight are related to the growth traits of farm animals. These traits are closely related to the increase in the number or diameter of muscle fibers of the animal and the development of muscle mass.

Growth related to carcass weight, one of the critical indicators of meat yield, is classified into two categories: prenatal and postnatal growth. Muscle development is a multidimensional chain of events that includes cellular increase and specializations in the embryonic period during growth, maturation, and development of functions (Ujan et al., 2011). The MRFs gene family mainly controls this chain of events. It is known that MRFs regulate myogenesis from the stages of muscle fiber formation, development, and proliferation to postnatal muscle maturation, differentiation, and functions (Zhong et al., 2013; Patel et al., 2014; Siqin et al., 2017).

Myf5 and Myf6 genes from the MRFs gene family play essential roles in the growth and development of muscle cells in farm animals. Evolutionary analyses of the amino acid sequences of this transcriptional activator family have reported that the vertebrate genes MyoD, Myf5, MyoG, and Myf6 are derived from a single ancestral gene by gene copies (Haghes and Schiaffino, 1999). These genes shape the formation of muscle cells in the embryonic period and control the maturation and functions of muscle fibers (Hughes and Salinas, 1999).

Comprehensive studies are needed to determine the relationships between the MRFS gene family and meat quality parameters more precisely. Muscle expression of specific genes, such as myogenic transcription factors, can significantly affect carcasses' meat content and meat quality. Therefore, transcription analysis of genes related to muscle development will provide valuable genetic information for meat production. In the present study, the Myf5 gene expression fold change in PM skeletal muscle in male Pekin ducks was higher at 8 weeks than at 4 weeks. Furthermore, Myf5 gene expression fold change in 4-week-old female ducks was lower than in males. Myf6 gene expression fold change was higher at 8 weeks of age than at 4 weeks in both male and female Pekin ducks.

Similarly, Myf6 gene expression fold change was higher in PM skeletal muscle in male Pekin ducks at 4 weeks and 8 weeks of age than in female ducks. The genetic information obtained from this study can be used to develop ways to accelerate genetic improvement in breeding and possible future research directions. In other words, determining the expression levels of the MRFs gene family, especially Myf5 and Myf6, can provide important information to more descriptively and clearly define the meat production of female and male Pekin ducks and to increase meat production.

CONCLUSIONS

This study presents a significantly different expression of Myf5 and Myf6 genes in duck

skeletal muscles at various ages. This study has shown that age affected the expression level of Myf5 and Myf6 genes and that the expression levels of these genes changed with growth.

ACKNOWLEDGEMENTS

This study was supported within the scope of TUBITAK 2209/A University Students Research Projects Support Program.

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Use of multivariate adaptive regression splines in animal science

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Abstract

In this study, it is aimed to examine the possibilities of using Multivariate Adaptive Regression Splines algorithms in the field of animal science for more than one result. For this purpose, attachment time (min/day) and milk yield (lt) were used as multiple outcome variables, and cabin residence time (min/day), milking speed (lt/min) and lactation day (days) were used as explanatory variables in a data set taken from the Polish Holstein population. The methodology and goodness-of-fit criteria of multi-outcome MARS were examined in detail. According to the results obtained, it was determined that the explanatory variables of duration of stay in the cabin (min/day), milking speed (lt/min) and lactation day (days) were capable of explaining the attachment time variable by 0.72% and milk yield by 79.6%. It was determined that while the success of predicting milk yield was high, the success of predicting the attachment time variable was quite low. It has been evaluated that the success of multi-outcome prediction may also depend on the relationship between outcome variables. When the findings and similar literature were evaluated, it was understood that multi-outcome multivariate adaptive regression curves (MARS) can be used successfully in the field of dairy cattle studies.

Key words: *Multiple outcome prediction, dairy cattle farming, Holstein, Multivariate statistics*

INTRODUCTION

Dairy cattle play a critical role in meeting the global demand for milk and dairy products, which are essential nutritional sources for many people. Some of the primary reasons for the importance of dairy cattle include the following:

1- Milk Production: Dairy cattle are bred primarily for milk production, which holds a significant place in human nutrition. Milk is rich in proteins, vitamins, and minerals, and serves as the fundamental raw material for various dairy products such as cheese, butter, and yogurt (Diler et al., 2022; Górska-Warsewicz et al., 2019).

2- Economic Value: Dairy farming significantly contributes to the economy by providing employment and income. Additionally, dairy products are valuable export commodities in international trade, generating billions of dollars in revenue for countries (Quénon et al., 2021; Dagtekin et al., 2023).

3- Nutritional Value: Milk is rich in calcium, vitamin D, vitamin B12, and potassium, all of which are essential for healthy bones, teeth, and overall health (Pereira, 2014; Bezie, 2019).

4- Cultural Importance: Dairy products are a staple of traditional diets in many cultures and hold a significant place in cultural events and practices (Santoze & Gicheha, 2019; Dagtekin et al., 2023).

In conclusion, dairy cattle are an indispensable part of the global food system, contributing economically, nutritionally, and culturally.

The nutritional value of cow's milk is high in protein, calcium, phosphorus, and vitamins B12 and D. These nutrients are essential for healthy bone growth, muscle development, and overall health (Massey, 2001; Guetouache et al., 2014). Cow's milk can be

used to produce various dairy products such as cheese, butter, yogurt, and ice cream, and it is widely available in many parts of the world. Cow's milk has played a significant role in many cultures for centuries, often being used in traditional dishes and celebrations (Górska-Warsewicz et al., 2019). Dairy farming, which relies heavily on the production of milk from cattle, is a vital industry with substantial economic value, providing employment opportunities and contributing to local economies in many countries. Cow's milk is a critical source of nutrition and a versatile ingredient appreciated by people worldwide (Massey, 2001; Guetouache et al., 2014; Górska-Warsewicz et al., 2019).

Milk fat and protein are essential components of milk and dairy products and provide a range of nutritional and functional benefits. The nutritional value of milk fat and protein is in essential nutrients such as amino acids, vitamins and minerals, as well as important sources of energy. For example, milk fat contains vitamins A, D, E and K, as well as healthy fatty acids such as conjugated linoleic acid (KLA), which has been linked to a variety of health benefits (Jenkin and McGuire, 2006; Bezie, 2019). Milk fat contributes to the rich and creamy flavour of dairy products such as butter, cheese and ice cream, while protein plays an important role in determining the texture and consistency of these products (Mehta, 2015). Research has shown that consuming foods high in protein and healthy fats can help increase feelings of satiety and reduce cravings, which may be beneficial for weight management (Priyadarshini et al., 2018). Dairy fat and protein have been associated with a range of health benefits, including improved heart health, better brain function and reduced risk of type 2 diabetes (Jenkin and McGuire, 2006; Ejtahed et al., 2015; Bezie, 2019).

Dairy farming is crucial for maintaining and enhancing the efficiency, profitability, and sustainability of the dairy industry. By selecting breeding candidates with desirable traits, the industry can continue to provide high-quality dairy products to consumers while also promoting animal welfare and environmental conservation (Quénon & Magne, 2021; Diler et al., 2022).

Robotic milking in dairy cattle, as an application of modern agricultural technology, is used to optimize milk production processes and enhance efficiency. Robotic milking systems automate the milking process by minimizing human intervention. These systems utilize sensitive sensors and robotic arms to attach milking machines to the cow's teats, monitor milk flow, and stop the milking process when necessary. By adjusting the frequency and timing of milking according to the cows' natural cycles, robotic milking reduces stress levels and increases milk yield. Additionally, these systems continuously collect data on the cows' health and milk quality, enabling farmers to manage their herds more effectively. Consequently, robotic milking is an innovative technology that enhances both efficiency and animal welfare in dairy cattle farming.

In the field of animal husbandry, identifying the environmental factors that influence production traits is of critical importance. The accuracy and reliability of the collected data, the inclusion of relevant independent variables in the model, and the fulfillment of certain fundamental assumptions related to the statistical methods used are crucial for ensuring that the results obtained are consistent and dependable. If the assumptions of statistical methods (such as linearity, homoscedasticity, and normal distribution) are not met, it becomes essential to employ more robust data mining and artificial neural network algorithms (such as CART, CHAID, Exhaustive CHAID, MARS, ANNs) instead of traditional methods. These approaches are vital for accurately and reliably identifying the independent variables that affect the dependent variable under investigation (Eyduran et al., 2019).

One of the primary objectives in animal breeding studies is to identify independent variables that influence the dependent variable under investigation, using these variables as indirect selection criteria to achieve desired levels of the dependent variable. In this context, the use of the aforementioned effective statistical methods can facilitate the accurate identification of other traits that serve as indirect selection criteria.

In the field of animal husbandry, the use of data mining algorithms for the characterization of animal species and breeds in terms of general traits, the characterization of sex, the characterization of mastitis, and the characterization of candidate genes related to meat and milk traits will contribute to the development of accurate and effective strategies.

In recent years, the use of CART, CHAID, MARS, and ANN algorithms, which enable the analysis of classification and regression problems, has gained significance in identifying the specific breed characteristics of the studied breeds (Grzesiak & Zaborski, 2012). The application of these algorithms, which are relatively easy to analyze and interpret, provides a foundation for breeding programs by identifying the independent variables and their combinations that contribute to the emergence of desirable or undesirable traits in animal husbandry.

This study aims to examine the potential applications of MARS algorithms for multiple outcomes in the field of animal science. To achieve this, a dataset from the Polish Holstein population was used, where attachment duration (minutes/day) and milk yield (liters) served as the multiple outcome variables, and time spent in the stall (minutes/day), milking speed (liters/minute), and days in lactation were employed as explanatory variables.

MATERIALS AND METHODS

As data material for the study, in a data set obtained from a Polish Holstein population, bonding time (min/day), milk yield (lt) were used as multiple outcome variables and duration of stay in the cabin (min/day), milking speed (lt/min) and lactation day (days) were used as explanatory variables.

Milk recording data were collected according to the A4 method accredited by the International Committee for Animal Recording (ICAR), which involves two consecutive recordings of two daily milkings with an interval of 28 to 33 days, during the period from 2016 to 2019. All data were provided by the SYMLEK IT system at the Milk Analysis Laboratory of the Polish Federation of Cattle Breeders and Dairy Farmers (PFCB&DF), which is ISO 17025 certified and ICAR accredited (Kolenda et al., 2021).

In this study, the application of the multi-response MARS algorithm, particularly in the MARS model, which is one of the alternative methods that can be used to model complex and non-linear data structures, has been carried out in the field of Animal Science.

One of the topics that should be evaluated in terms of relationship statistics is regression analysis, which allows for the examination of the functional cause-and-effect relationship between two or more variables (Arı & Önder, 2013). In regression analysis, the primary consideration is not only that the relationships are within a cause-and-effect framework but also the number of explanatory and outcome variables involved in these relationships. In this context, regression analysis involving one outcome (Y) and one explanatory (X) variable is referred to as simple linear regression. Additionally, when there is a single outcome variable and multiple explanatory variables, the appropriate method to apply is multiple linear regression analysis. However, in some cases, the number of outcome variables may also be more than one, similar to the number of explanatory variables. In such cases, multivariate multiple regression analysis is employed to examine the linear relationships between explanatory and outcome variables (Günaşdı, 2014). If the dependent and independent variables are continuous, multivariate multiple linear regression is used to investigate the linear relationship between sets of dependent and independent variables (Dattalo, 2013; Günaşdı, 2014).

The validity of the model obtained from multivariate multiple linear regression analysis and the estimation of coefficients are determined in a manner similar to the process in univariate multiple linear regression analysis (Özdamar, 2004; Günaşdı, 2014). In this context, certain assumptions that apply to classical simple and multiple linear regression analysis are also applicable to multivariate multiple linear regression.

1- The distribution of dependent variables should be in accordance with the multivariate normal distribution.

2- Sampling should be done by chance.

3- There should be no multicollinearity between and within dependent and independent variables.

4- The variance-covariance matrix must be homogeneous.

The MARS algorithm, developed by the famous physicist Jerome Friedman (Friedman, 1991), is a non-parametric regression method that defines and models the relationship between dependent and independent variables in the most efficient way. MARS algorithm does not require assumptions in terms of functional relationships between dependent and independent variables as in classical regression approaches. In addition, there is no need for any assumption about the distribution of all variables for the MARS algorithm. Methods such as cross-validation, repeated cross-validation and resampling can be used during the separation into training and test sets. The training sets are divided into piecewise linear segments with different slopes at the analysis stage. These pieces are called splines. Splines are interconnected and allow the definition of basis functions, also referred to as piecewise curves. This allows the linear and non-linear effects in the model to be defined more effectively. The connection points between splines are called knots. Candidate knots are located within the limits of variation of each independent variable. The MARS algorithm generates the basis functions by taking into account all possible knots and interaction effects within the stepwise procedure. In order to identify pairs of basis functions, a forward pass procedure is used to identify candidate nodes at a random location within the limits of variation of each independent variable. At each stage, the model generated by the MARS algorithm customises the nodes and the pairs of basis functions to achieve the lowest error variance. When the complex model is reached, the inclusion of the basis functions continues. In the model created with MARS, unnecessary functions that reduce the prediction performance are eliminated by backward pass procedure (Sevgenler, 2019).

The prediction model for the MARS algorithm is expressed as in equation 2.1.

$$\hat{y} = \beta_0 + \sum_{m=1}^{M} \beta_m \prod_{k=1}^{K_m} h_{km}(X_{v(k,m)}) \qquad \textbf{(2.1)}$$

Here, $\hat{y}$ is the predicted value of the dependent variable, β_0 is the regression constant, $h_{km}(X_{v(km)})$ is the basis function.

The pruning algorithm is performed by the technique expressed in equation 2.2 with generalised cross validation (Kornacki and Ćwik, 2005).

$$GCV(\lambda) = \frac{\sum_{i=1}^{n}(y_i - \hat{y_i})^2}{\left(1 - \frac{M(\lambda)}{n}\right)^2} \qquad \textbf{(2.2)}$$

Here, n is the number of individuals in the training set, y_i is the value of the dependent variable belonging to the i. individual, y_{ip} is the prediction value of the dependent variable belonging to the i. individual. In addition, M(λ) is the correction function for the complexity of the model including λ terms.

2.1. Multi-result MARS Analysis

In this study, the use of multivariate adaptive regression curves methodology with multiple outcomes in a situation where more than one dependent variable is analysed simultaneously is considered. MARS is a regression technique for modelling complex and non-linear data structures. This method has the capacity to flexibly identify interactions and non-linear relationships between variables.

2.2. Basic Principles of Multi-result MARS

MARS estimates the dependent variables using a set of basis functions defined as functions of the independent variables. Multi-result MARS constructs a separate MARS model for each dependent variable and integrates the results of these models when more than one dependent variable is modelled.

2.3. Model Formulation

The mathematical expression of the multi-outcome MARS model is as in equation 2.3:

$$Y_i = \beta_0 + \sum_{m=1}^{M} \beta_m B_m(X) + \varepsilon_i \quad \textbf{(2.3)}$$

Basis functions $B_m(X)$ are piecewise polynomial functions, usually generated using specific cut points (knots) in the data set. These functions are used to model non-linear relationships and interactions in the data set.

2.4. Model Selection and Optimisation

Model setup is a two-stage process:

1. Model Expansion (Forward Pass): A large number of basis functions are added to create a large model. Initially, the model starts with only one constant term. Then, in an iterative process, one basis function is added one by one to minimise the error of the data. At each step, the most appropriate basis function is selected and included in the model to improve the prediction accuracy of the model. These basis functions are usually piecewise linear functions around the splitting points and help to capture the complex characteristics of the model. As a result, a flexible and powerful regression model is created by adding a large number of basis functions. This process is highly effective because of its ability to model possible complex relationships in the data.
2. Model Pruning (Backward Pass): With the backward elimination method, some terms are removed from the model to reduce the complexity of the model and to identify the basis functions that will provide the best performance. In MARS analysis, this process aims to create a more parsimonious and generalisable model by eliminating the redundancy of basis functions added during the model expansion phase. First, the model starts at the highest level of complexity; this is the point at which all potential basis functions are included. Then, progressively, the basis functions that contribute least to the performance of the model are removed one by one. This removal is based on a set of evaluation criteria, usually done without increasing the error of the model or with as little error as possible. The result is a simpler and more efficient model that prevents the model from overfitting and provides better predictive performance on general data sets. This method also makes the model more understandable and helps to identify important variables.

To evaluate the performance of the model and to avoid the problem of overfitting, the k-fold cross-validation method is used.

The results of the model have been evaluated from various perspectives, such as prediction accuracy, sensitivity analysis of the model, and the importance of variables. Additionally, graphical analyses and tables were used to interpret the model's predictive performance and the effects of independent variables on the dependent variables.

The goodness-of-fit criteria that will be used to compare the performance of the algorithms under investigation are provided in Equations 4-10 (Eyduran et al., 2017).

The Pearson correlation coefficient (r) between the actual values and the predicted values with respect to the dependent variables.

1. The Akaike information criterion (AIC) is provided in Equation 2.4.

$$AIC = n.ln\left[\frac{1}{n}\sum_{i=1}^{n}\left(y_i - y_{ip}\right)^2\right] + 2k, eğer\ \frac{n}{k} > 40$$
$$\mathrm{AIC}_c = \mathrm{AIC} + \frac{2k(k+1)}{n-k-1}, \text{ aksi halde} \quad \textbf{(2.4)}$$

The Akaike Information Criterion (AIC) is a criterion used to assess the quality of statistical models. AIC aims to select the most appropriate model by balancing model complexity and goodness of fit to the data. In MARS analysis, AIC plays a crucial role in model selection and optimization. The use of AIC during the expansion and pruning stages of the MARS model enhances the overall performance of the model and leads to more generalizable results.

2. The root-mean-square error (RMSE) is provided in Equation 2.5.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(y_i - y_{ip}\right)^2} \quad \textbf{(2.5)}$$

RMSE is a statistical measure used to quantify the difference between predicted values and actual values. By measuring the magnitude of errors, RMSE helps us understand how well the model performs.

3. The mean error (ME) is provided in Equation 2.6.

$$ME = \frac{1}{n}\sum_{i=1}^{n}(y_i - y_{ip}) \quad \textbf{(2.6)}$$

The mean error (ME) is a statistical measure that quantifies the average difference between the predicted values and the actual values of a model. ME is used to evaluate whether the model is biased and to determine if the predictions contain any systematic errors.

4. The mean absolute deviation (MAD) is provided in Equation 2.7.

$$MAD = \frac{1}{n}\sum_{i=1}^{n}|y_i - y_{ip}| \quad \textbf{(2.7)}$$

It is a statistical indicator that measures the average deviations from the central tendency of a data set. MAD is used to determine how much the data deviate from the mean (or median).

5. The standard deviation ratio (SDratio) is provided in Equation 2.8.

$$SD_{ratio} = \frac{S_m}{S_d} \quad \textbf{(2.8)}$$

It is a measure that compares the standard deviations of two data sets or variables. This ratio is used to compare the variations or dispersions of the two data sets. SDratio is particularly useful for comparing or scaling variability between two different groups.

6. The global relative approximation error (RAE) is provided in Equation 2.9.

$$RAE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - y_{ip})^2}{\sum_{i=1}^{n} y_i^2}} \quad \textbf{(2.9)}$$

It is a measure that evaluates the total amount of error in a model or prediction system. RAE is commonly used to determine how closely the predictions align with the actual data. It can be useful for assessing model performance across various fields, particularly in regression analyses and modeling studies.

7. The mean absolute percentage error (MAPE) is provided in Equation 2.10.

$$RAE = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{y_i - y_{ip}}{y_i}\right| * 100 \quad \textbf{(2.10)}$$

It is a measure used to assess the accuracy of predictions. MAPE expresses how close the predictions are to the actual values as a percentage. This measure is used to evaluate the magnitude of forecasting errors and the overall performance of the model. MAPE works accurately when the actual values are greater than zero. However, when actual values are zero or very small, MAPE can be misleading, requiring positive actual values for accurate results. Large deviations can lead to high MAPE values, so relying solely on MAPE can sometimes be misleading. Therefore, it is recommended to use MAPE in conjunction with additional metrics.

Here, *n* represents the number of individuals in the data set, *k* denotes the number of parameters in the model, s_m refers to the standard deviation of the model's error terms, and s_d indicates the standard deviation of the dependent variable.

RESULTS

Table 3.1 presents the descriptive statistics for the variables used in the study.

Table 3.1. Descriptive statistics (n=22,613)

	Mean	Standard Deviation	min	max	Standard error
Attache time (min/day)	10,16	7,75	2,33	116,33	0,05
Box Time (min/day)	20,28	7,21	6,67	45	0,05
Milking Speed (lt/min)	2,56	0,88	0,58	6,99	0,01
Milk Yield (lt)	29,97	9,28	5,1	73,1	0,06
DIM (day)	148,42	82,57	5	305	0,55

Attache Time: The mean bonding time is given with approximately 10.16 minutes and the standard deviation is 7.75 minutes. This indicates a significant variability in bonding times. The minimum bonding time was 2.33 minutes, while the maximum was 116.33 minutes. The standard error is 0.05, which shows the precision of the measurement.

Box Time: The mean cabin stay time was 20.28 minutes with a standard deviation of 7.21. The minimum time was recorded as 6.67 minutes and the maximum time as 45 minutes. The standard error was again determined as 0.05.

Milking speed: The average milking speed was expressed as 2.56 litres per second (lt). The standard deviation is 0.88, which shows that there are differences in milking speed between individuals. The lowest milking speed was 0.58 and the highest milking speed was 6.99. The standard error was determined as 0.01.

Milk yield: The average milk yield was calculated as 29.97 litres (lt). The standard deviation is 9.28 litres, which indicates that there is a considerable variation in milk yield. The minimum milk yield was 5.1 litres and the maximum was 73.1 litres. The standard error was determined as 0,06.

Days In Milk: The mean lactation day was 148.42 and the standard deviation was calculated as 82.57 days. This shows that the lactation period varies greatly among individuals. The minimum lactation period was 5 days and the maximum was 305 days. The standard error was determined as 0.55.

These statistics are important for understanding the distribution of variables of the population under study and provide the basis for further statistical analyses. However, high values of standard deviation indicate the degree of variability and potentially high variance in the data set, which may indicate large differences between individuals. Standard error values are the variability of a given mean corrected for sample size and can be used for comparisons.

The results of Pearson correlation analysis between the variables are given in Figure 1. The prediction model obtained as a result of multi-result MARS analysis is given in Table .

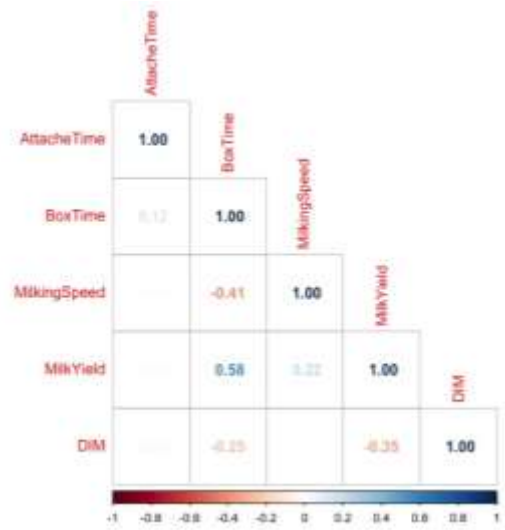

Figure 3.1. Correlation analysis results

Table 3.2. Forecasting model obtained as a result of Multi-result MARS analysis

	AttacheTime	MilkYield
(Intercept)	18,9976466	41,196967
MilkingFrequancy2	-4,097361	-1,95122
MilkingFrequancy3	-7,9163342	-4,920519
MilkingFrequancy4	-11,0749	-7,355715
MilkingFrequancy5	-12,665283	-8,412165
Lactation2	-2,8834757	7,99346
h(21.9667-BoxTime)	-0,6457132	-1,981818
h(BoxTime-21.9667)	0,8008123	2,055634
h(BoxTime-29.3)	-0,7047581	-1,462495
h(2.36841-MilkingSpeed)	-1,4280929	-13,99652
h(MilkingSpeed-2.36841)	3,3304832	6,629761
h(51-DIM)	0,0038909	-0,025796
h(DIM-51)	0,0075791	-0,008185
MilkingFrequancy2 * h(MilkingSpeed-2.9746)	0,0807494	-2,506713
MilkingFrequancy2 * h(2.9746-MilkingSpeed)	-0,7343794	-0,318473
h(40.8833-BoxTime) * Lactation2	0,0589587	-0,174818
h(BoxTime-40.8833) * Lactation2	1,5241545	-1,41575
h(3.47663-MilkingSpeed) * Lactation2	-0,1430273	-1,699032
h(MilkingSpeed-3.47663) * Lactation2	0,3586303	-1,372031
Lactation2 * h(DIM-21)	0,0061917	-0,008099
Lactation2 * h(21-DIM)	0,1707473	-0,173802
h(19.8167-BoxTime) * h(2.36841-MilkingSpeed)	0,3835378	0,73316
h(BoxTime-19.8167) * h(2.36841-MilkingSpeed)	-0,3948961	-0,785232
h(BoxTime-21.9667) * h(MilkingSpeed-1.77681)	0,1700641	-0,952909
h(22.6667-BoxTime) * h(MilkingSpeed-2.36841)	-0,211755	0,012998
h(BoxTime-22.6667) * h(MilkingSpeed-2.36841)	-0,7348788	0,112404
h(BoxTime-29.3) * h(MilkingSpeed-2.53648)	0,4277946	0,983717
h(BoxTime-29.3) * h(2.53648-MilkingSpeed)	0,6202812	0,957656
h(12.3667-BoxTime) * h(DIM-51)	-0,002951	0,000012
h(BoxTime-12.3667) * h(DIM-51)	-0,0002924	-0,000966
h(3.26825-MilkingSpeed) * h(DIM-51)	-0,0027111	0,008122
h(MilkingSpeed-3.26825) * h(DIM-51)	-0,0178811	-0,033673
MilkingFrequancy2 * h(BoxTime-21.9667) * h(MilkingSpeed-1.77681)	-1,6519291	-3,189227
MilkingFrequancy2 * h(BoxTime-22.6667) * h(MilkingSpeed-2.36841)	1,5860518	3,713305
MilkingFrequancy3 * h(BoxTime-21.9667) * h(MilkingSpeed-1.77681)	-0,7615773	-0,907606
MilkingFrequancy3 * h(BoxTime-22.6667) * h(MilkingSpeed-2.36841)	0,8407352	0,777969
h(22.3667-BoxTime) * h(MilkingSpeed-3.26825) * h(DIM-51)	0,0019066	0,002498
h(BoxTime-22.3667) * h(MilkingSpeed-3.26825) * h(DIM-51)	0,0015215	0,002444

Each basis function and the coefficients of these basis functions in the table show the effects of various factors related to milking on attachment time and milk yield. The MARS model uses piecewise-linear functions (basis functions) to capture non-linear interactions and complex relationships. The 'h()' functions in the model are known as hinge functions and can model the effects of variables below and above a certain threshold in different ways.

For example, the negative coefficients for milking frequency indicate that both attachment time and milk yield decrease as frequency increases. More frequent milking may lead to shorter attachment times and lower milk yields, perhaps because the teats have less resting time.

The second lactation period increases milk yield while decreasing attachment time, which may indicate that cows are more productive later in the lactation period.

As for the hinge functions, expressions such as 'h(21,9667-Cabin residence time)' and 'h(21,9667-Cabin residence time-21,9667)' represent the asymmetric effects of changes around a certain threshold value of cabin residence time on attachment time and milk yield. Changes below and above the threshold value may have different

coefficients, suggesting that there may be an optimum range of the time the cow spends in the milking parlour. Likewise, the terms related to milking speed represent the different effects on milk yield and attachment time around certain values of milking speed.

Interaction terms, for example 'Milking frequency2 * h(Milking Speed-2,9746)', show how the combination of two factors can have a different effect on milk yield and attachment time. This implies that the effects of the variables on each other are not independent and that the level of one factor can change how the effect of the other factor is felt.

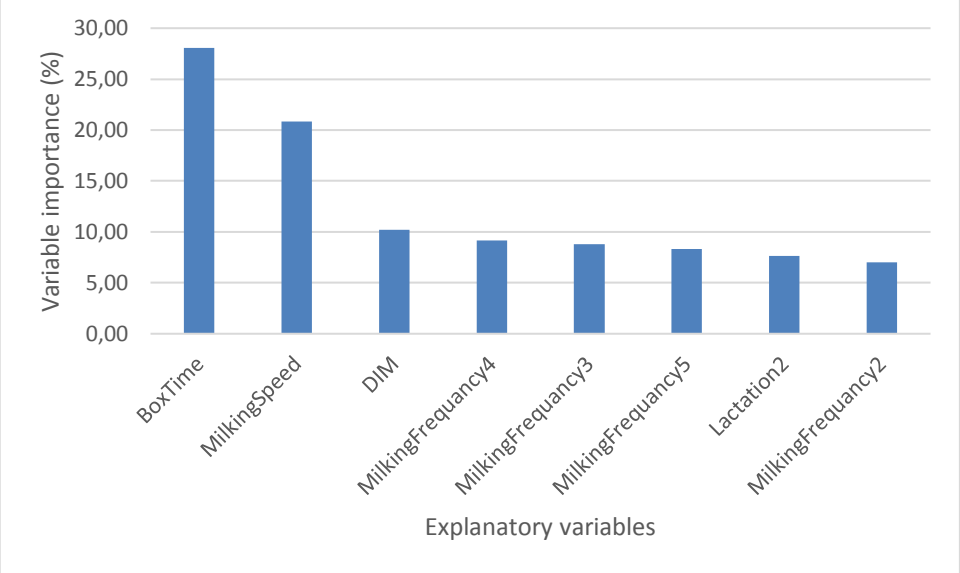

Figure 3.2. Importance levels of variables

The graph shows the relationship between the percentage of 'Variable Importance' and some explanatory variables.

Box Time (Approximately 27%): This variable stands out as the most important variable in the model. The length of stay in the cabin has the highest percentage compared to the other variables and makes the greatest contribution to the explanation of the model.

Milking Speed (Approximately 20%): The second most important variable, milking speed, plays an important role in the model, although it has a slightly lower percentage compared to the duration of stay in the cabin.

Lactation Day (Approximately 10%): Lactation day ranks third and has a moderate importance in the model.

Milking Frequency 4 (Approximately 8%): This variable has a moderate importance in the model and has a higher percentage compared to other milking frequencies.

Milking Frequency 3 (Approximately 7%): Milking frequency 3 makes a certain contribution among the explanatory variables, ranking fifth in importance in the model.

Milking Frequency 5 (Approximately 6%): This variable also has a moderate importance in the model, but it has a lower percentage compared to other milking frequencies.

2nd Lactation Period (Approximately 5%): The second lactation period is one of the less important variables in the model.

Milking Frequency 2 (Approximately 4%): Milking frequency 2, which is the least important variable, makes the lowest contribution to the explanation of the model.

As a result, this graphical analysis shows that the duration of stay in the cabin and milking speed have a higher importance than the other variables in the model. These findings provide important clues about which factors should be considered more in animal

husbandry and milk production. In particular, optimising the length of stay in the cubicle and milking speed may be critical to increase productivity and improve animal health. Other variables, such as lactation day and milking frequency, should also be taken into account in specific conditions and over specific time periods. Such analyses help to make science-based decisions for more efficient and healthier animal husbandry.

This visualisation points to potential areas for optimising decision-making processes and management practices, which can have important implications for interactions between variables and farm management strategies.

Table 3.3. Goodness of fit criteria

Criteria	Attache Time	Milk Yield
Root mean square error (RMSE)	7,465	4,19
Relative root mean square error (RRMSE)	73,45	13,98
Standard deviation ratio (SDR)	0,963	0,452
Coefficient of variation (CV)	73,45	13,98
Pearson's correlation coefficients (PC)	0,268	0,892
Performance index (PI)	57,927	7,388
Mean error (ME)	0	0
Relative approximation error (RAE)	0,341	0,018
Mean relative approximation error (MRAE)	0,004	0,001
Mean absolute percentage error (MAPE)	68,761	10,573
Mean absolute deviation (MAD)	5,17	2,916
Coefficient of determination (Rsq)	0,072	0,796
Adjusted coefficient of determination (ARsq)	0,07	0,796
Akaike's information cCriterion (AIC)	90991,356	64873,26
Corrected Akaike's information criterion (CAIC)	90991,487	64873,391

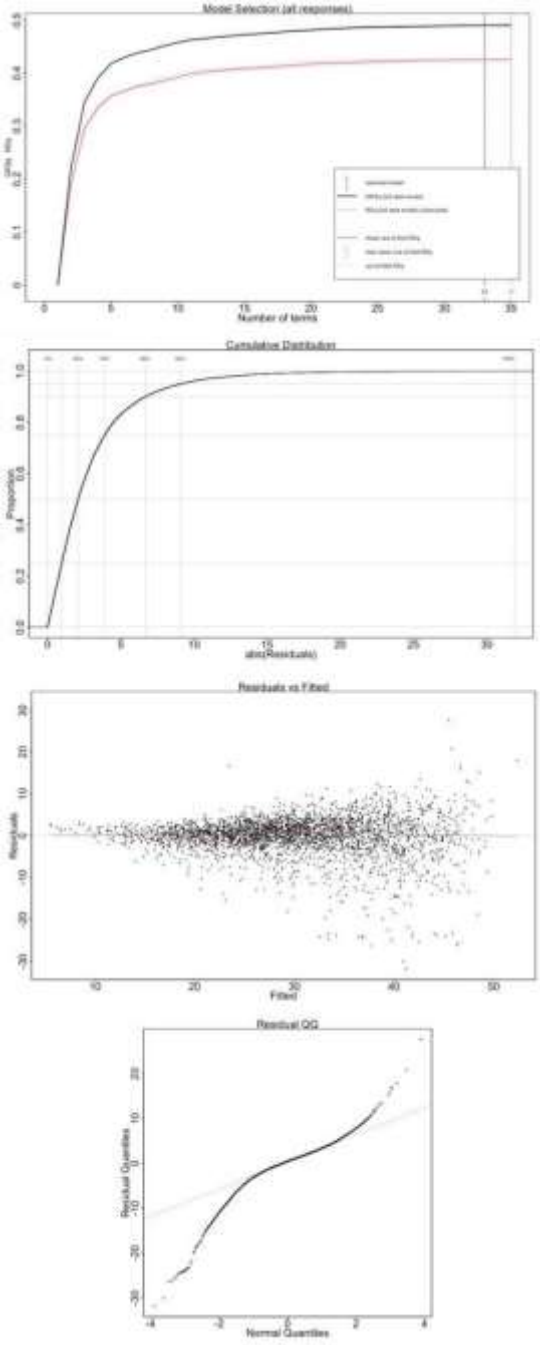

Figure 3.3. a) Milk Yield Model Selection, b) Cumulative Distribution, c) Residuals vs Fitted, d) Residual QQ

Model Selection (All responses): To evaluate the performance of the Multiresponse Adaptive Regression Splines (MARS) model used, the model selection plot shows the RSq and out-of-fold RSq values according to the number of terms. The RSq value of the model obtained on the full dataset is shown in the black line, while the average out-of-fold RSq value is shown in the pink line. The graph shows that the performance of the model increases as the number of terms increases. However, when the number of terms reaches a certain point, it shows that the added terms make only a limited contribution to the performance of the model. The selected model has the highest out-of-fold RSq value, indicating that the generalisation ability of the model is optimised.

Cumulative Distribution: The cumulative distribution of the absolute values of the residuals is shown in this graph. The graph shows that most of the residuals are concentrated at low error values and only a small amount at high error values. This indicates that the model generally makes predictions with high accuracy and that significant errors are rare. High values of a low percentage of errors may indicate that the model is underperforming at certain data points.

Residuals vs Fitted: The residuals and prediction plot compares the values predicted by the model and the errors corresponding to these predictions. The residuals are randomly distributed around the predicted values as shown in the graph. This shows that the model fits the data well and does not make systematic errors. The horizontal distribution of the residuals indicates that the model's prediction accuracy is typically high and does not show any signs of heteroskedasticity (where the variance of the error terms is not constant).

Residual QQ: The QQ plot for Residuals is used to determine how well the errors of the model conform to a normal distribution. The graph shows that the errors are largely close to a 45-degree line. This is comparable to the theoretically expected normal distribution. This shows that the errors are fairly well fitted to the normal distribution and that the model errors typically have a normal distribution. The model can be said to work well overall, although the presence of a few outliers indicates that the model may make significant errors at some data points.

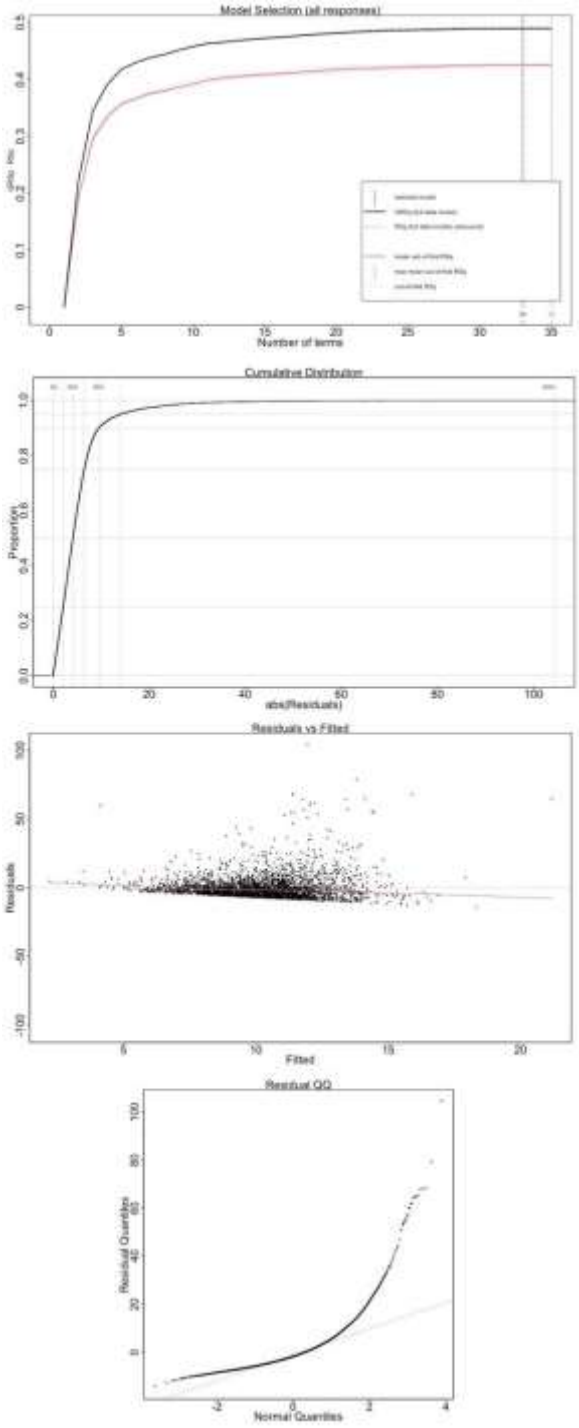

Figure 3.4. a) Model Selection for Attache Time, b) Cumulative Distribution, c) Residuals vs Fitted, d) Residual QQ

Model Selection (All responses): This model selection plot evaluates the performance of

the second MARS model depending on the number of terms. This evaluation is performed using RSq and out-of-fold RSq values. The RSq value of the model obtained on the full dataset is shown with black lines, while the average out-of-fold RSq value is shown with pink lines. The graph shows that the performance of the model increases as the number of terms increases. However, when the number of terms reaches a certain point, it shows that the added terms make only a limited contribution to the performance of the model. In this model, although the model with the highest out-of-fold RSq value was selected, but it was observed that the performance was lower than the first model.

Cumulative Distribution: This graph shows the cumulative distribution of the absolute values of the errors. This shows that the error values are spread over a wider area. The graph shows that the majority of residuals are concentrated at low error values, while some residuals have high error values. This suggests that the model is making larger errors and underperforming at certain data points. As a result of the widespread nature of the residuals, it suggests that the generalisation ability of the model may be limited.

Residuals vs Fitted: The residuals vs. prediction plot compares the values predicted by the model and the errors corresponding to these predictions. The graph shows that the residuals are randomly distributed around the predicted values. However, the model failed to predict some data points correctly. The fact that the points are located over a large area and that there are some outliers suggests that the model may not perform adequately at some data points and may make systematic errors.

Residual QQ: It uses the QQ plot for Residuals to assess the fit of the residuals of the model to the normal distribution. The graph shows that the residuals have some extreme values and are largely not close to the 45 degree line compared to the theoretical normal distribution. This indicates that the residuals do not fit the normal distribution and the model errors deviate from the normal distribution. These deviations in the residuals suggest that the model may make significant errors at some data points and may underperform overall.

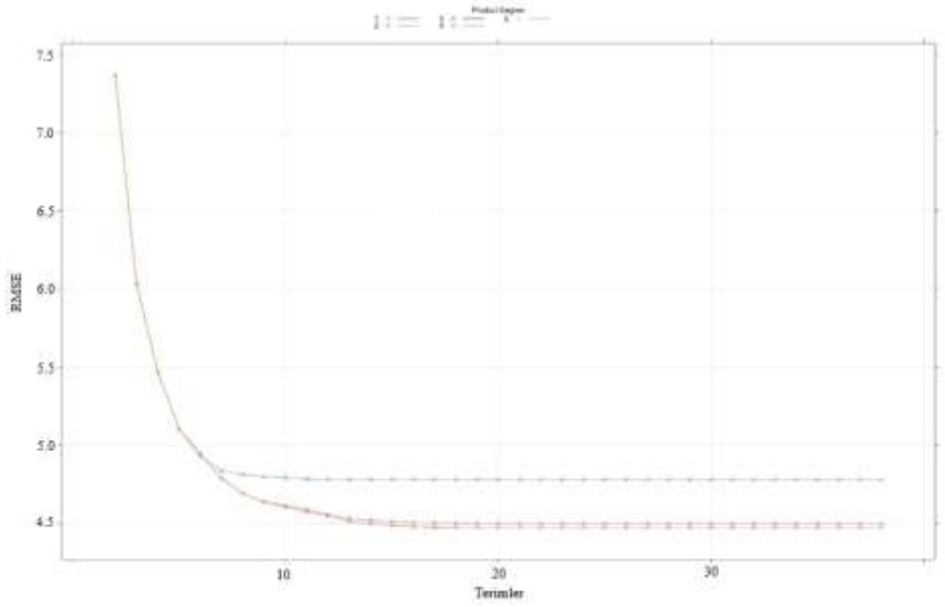

Figure 3.5. Milk Yield plot (Degree of multiplication, Root Mean Square Error (RMSE), Terms)

The graph shows the error rates (RMSE - Root Mean Square Error) of polynomials of different degrees for the milk yield prediction model. On the horizontal axis is the number of terms used (#Terms) and on the vertical axis is the root mean square error (RMSE). The lines represent polynomials of different degrees. Blue line:

Degree 1, Red line: Degree 3, Green line: Degree 4, Yellow line: For all degrees, we observe a decrease in the RMSE (root mean square error) value as the number of terms used increases. This indicates that the model performs better with more terms and its predictions are more accurate. Higher degree polynomials (e.g., degree 5) initially have higher RMSE values, but the error rates decrease rapidly as the number of terms increases. The degree 5 polynomial reached the lowest RMSE value after about 10 terms, leaving the other degrees behind. In the degree 1 polynomial (blue line), the RMSE value stabilises at a certain level as the number of terms increases and does not decrease further. This shows that low degree polynomials are inadequate to capture more complex relationships. As a result, it can be said that higher degree polynomials achieve lower error rates with more terms and perform better in milk yield estimation. However, it should be noted that as the complexity of the model increases, the risk of overfitting should be considered.

Table 3.4. Milk Yield prediction model obtained as a result of Multiple Results MARS analysis

	Coefficients
(Intercept)	36.494169
MilkingFrequancy2	1.586054
Lactation2	8.513750
h(21.9667-BoxTime)	-1.629705
h(BoxTime-21.9667)	0.801923
h(2.36841-MilkingSpeed)	-13.595395
h(MilkingSpeed-2.36841)	4.170487
h(51-DIM)	-0.040693
h(DIM-51)	-0.016145
h(40.5667-BoxTime) * Lactation2	-0.231384
h(BoxTime-40.5667) * Lactation2	-2.573986
h(3.44414-MilkingSpeed) * Lactation2	-1.985292
h(MilkingSpeed-3.44414) * Lactation2	-0.879978
h(21.15-BoxTime) * h(2.36841-MilkingSpeed)	0.795389
h(BoxTime-21.15) * h(2.36841-MilkingSpeed)	0.368668
h(BoxTime-21.9667) * h(MilkingSpeed-1.76434)	-0.440024
h(BoxTime-21.9667) * h(1.76434-MilkingSpeed)	-0.822253
MilkingFrequancy2 * h(BoxTime-21.9667) * h(MilkingSpeed-1.76434)	-0.702135

This table shows the coefficients of the model constructed to predict milk yield as a result of MARS analysis. Each row indicates a term in the model and the coefficient of that term.
(Intercept): 36.494169 : This is the initial value of milk yield predicted by the model when all other variables are zero. Milking Frequency 2: 1.586054 : Second order effect of milking frequency. When this term is positive, it indicates that milk yield increases as milking frequency increases. 2nd Lactation Period: 8.513750 : Second order effect of lactation period. The positive sign of this term indicates that milk yield increases as the lactation period increases. h(21.9667-Lactation period): -1.629705 : This term refers to the situation where the length of stay in the cabin is less than a certain threshold value (21.9667). The negative coefficient indicates that milk yield decreases when the length of stay in the cabin is less than this value. h(Length of stay in the cabin-21.9667): 0.801923 : This term refers to the situation where the length of stay in the cabin is greater than a certain threshold value (21.9667). A positive coefficient indicates that milk yield increases when the length of stay in the cabin increases from this value. h(2.36841-Milking Speed): -13.595395 : refers to the situation where the milking speed is less than a certain threshold value (2.36841). A negative coefficient indicates that milk yield is severely reduced when the milking speed is less than this value. h(Milking Speed-2.36841): 4.170487 : It expresses the situation where the milking speed is greater than a certain threshold value (2.36841). A positive coefficient indicates that milk yield

increases when the milking rate increases from this value. h(51-Lactation days): -0.040693 : It expresses the situation where the lactation day is less than a certain threshold value (51). Negative coefficient indicates that milk yield decreases when lactation day is less than this value. h(Lactation day-51): -0.016145 : It expresses the situation where the lactation day is greater than a certain threshold value (51). A negative coefficient indicates that milk yield decreases when the lactation day increases from this value, but the effect is smaller. h(40.5667-duration of survival) * 2nd lactation: -0.231384 : When the length of stay in the cabin is less than a certain value and Lactation is quadratic, this term has a negative effect. h(Length of stay-40.5667) * 2nd Lactation: -2.573986 : When the length of stay in the cabin is greater than a certain value and Lactation is quadratic, this term has a stronger negative effect. h(3.44414-Milking speed) * 2nd Lactation: -1.985292 : When milking speed is less than a certain value and Lactation is quadratic, this term has a negative effect. h(Milking Speed-3.44414) * 2nd Lactation: -0.879978 : This term has a smaller negative effect when the milking speed is greater than a certain value and Lactation is quadratic. h(21.15-Time in cabin) * h(2.36841-Milking Speed): 0.795389 : When the length of stay in the cabin is less than a certain value and the Milking speed is less than a certain value, this term has a positive effect. h(Length of stay-21.15) * h(2.36841-Milking Speed): 0.368668 : When the length of stay in the cabin is greater than a certain value and the milking speed is less than a certain value, this term has a smaller positive effect. h(Length of stay-21.9667) * h(Milking Speed-1.76434): -0.440024 : This term has a negative effect when the length of stay in the cabin is greater than a certain value and the Milking Speed is greater than a certain value. h(Length of stay in the cabin-21.9667) * h(1.76434-Milking Speed): -0.822253 : When the length of stay in the parlour is greater than a certain value and the milking speed is less than a certain value, this term has a larger negative effect. Milking frequency 2 * h(Length of stay in the parlour-21.9667) * h(Milking Speed-1.76434): -0.702135 : Milking Frequency 2, When the length of stay in the parlour is greater than a certain value and the milking speed is less than a certain value, this term has a negative effect.

Table 3.5. Goodness of fit values used for the Milk Yield model

	criterion	value
1	Root mean square error (RMSE)	4.464
2	Relative root mean square error (RRMSE)	14.894
3	Standard deviation ratio (SDR)	0.481
4	Coefficient of variation (CV)	14.890
5	Pearson's correlation coefficients (PC)	0.877
6	Performance index (PI)	7.937
7	Mean error (ME)	0.000
8	Relative approximation error (RAE)	0.020
9	Mean relative approximation error (MRAE)	0.001
10	Mean absolute percentage error (MAPE)	11.529
11	Mean absolute deviation (MAD)	3.170
12	Coefficient of determination (Rsq)	0.768
13	Adjusted coefficient of determination (ARsq)	0.768
14	Akaike's information cCriterion (AIC)	67697.604
15	Corrected Akaike's information criterion (CAIC)	67697.634

The goodness of fit values used for the model were determined as nprune = 18 and degree = 3.

Root mean square error (RMSE) - 4.464: This value indicates the average magnitude of error in the model's predictions. A lower RMSE indicates better performance of the model. Relative root mean square error (RRMSE) - 14.894: It is calculated as the ratio

of RMSE to the mean value. Expressed as a percentage, this value indicates how large the error size is compared to the size of the data. A low RRMSE indicates that the model performs well. Standard deviation ratio (SDR) - 0.481: The ratio of the standard deviation of the model's predictions to the standard deviation of the actual data. A value close to 1 indicates that the model is good, while a value considerably lower than 1 indicates that the model is not good enough. Coefficient of variation (CV) - 14.890: The ratio of the error rate to the mean value, usually expressed as a percentage. A low CV value indicates that the model's predictions are consistent. Pearson correlation coefficient (PC) - 0.877: This value indicates the linear relationship between the model's predictions and actual values. Values close to 1 indicate a strong linear relationship. 0.877 indicates that the model has a fairly good linear relationship. Performance index (PI) - 7.937: An index that evaluates the overall performance of the model. Higher values indicate better performance. Mean error (ME) - 0.000: It shows the average error between the predicted values and the actual values. A value close to 0 indicates that the model is error-free. Relative error of approximation (RAE) - 0.020: The ratio between the average error and the true values. A low RAE indicates that the model performs well. Mean relative approximation error (MRAE) - 0.001: Calculated as the average value of the RAE. Close to 0 indicates that the model performs quite well. Mean absolute percentage error (MAPE) - 11.529: Indicates the mean absolute percentage error between predicted and actual values. A lower MAPE indicates a better model performance. Mean absolute deviation (MAD) - 3.170: Indicates the mean absolute deviation between predicted and actual values. A lower MAD indicates a better model performance. Coefficient of determination (Rsq) - 0.768: Indicates the explanatory power of the model. Values close to 1 indicate that the model explains most of the data. 0.768 indicates that the model has good explanatory power. Adjusted coefficient of determination (ARsq) - 0.768: It is the corrected version of the Rsq value and takes into account the complexity of the model. 0.768 indicates that the model performs well in general. Akaike information criterion (AIC) - 67697.604: Measures the complexity and appropriateness of the model. Lower AIC values indicate better model performance. Corrected Akaike information criterion (CAIC) - 67697.634: It is the corrected version of AIC and takes into account the complexity of the model. Lower CAIC values indicate better model performance.

In general, most of these criteria indicate that the model performs quite well. Especially when we look at values such as PC, Rsq, ARsq, we can say that the model establishes the linear relationship well and explains most of the data. However, high values in some criteria (for example, RRMSE and CV) indicate that the model still needs to be improved.

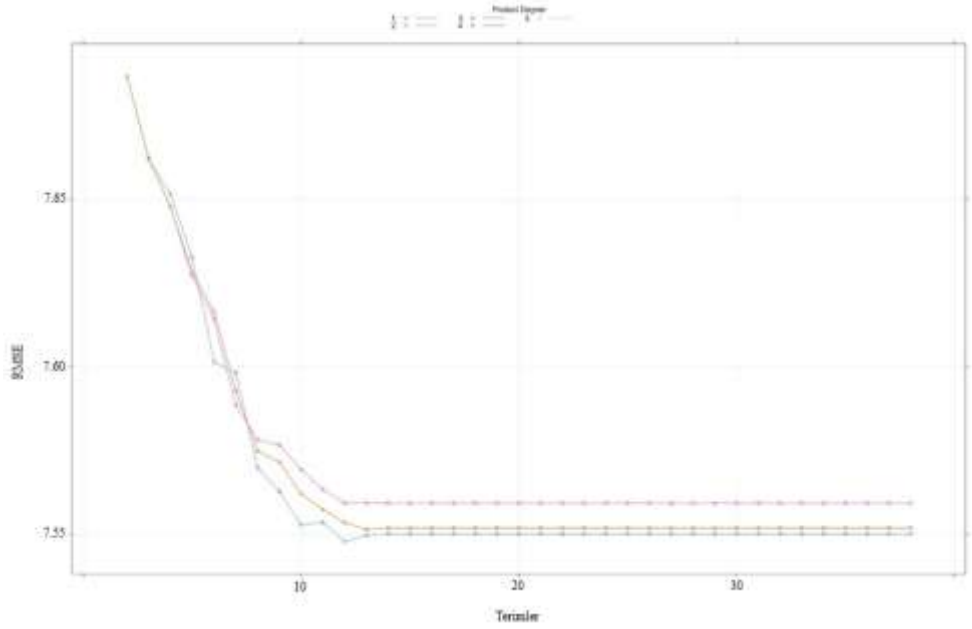

Figure 3.6. Time-to-Connect graph (Degree of multiplication, Root Mean Square Error (RMSE), Terms)

This graph shows the variation of the root mean square error (RMSE) of the model for various numbers of terms and product degrees. In the graph, we can observe how the RMSE values change as the number of terms increases.

Number of Terms on X Axis: On the X axis, it shows the number of terms used in the model. As the number of terms increases, the model becomes more complex and contains more parameters.

RMSE on the Y-Axis (Cross-Validation): The Y-axis shows the root mean square error (RMSE) of the model obtained during cross-validation. RMSE shows how close the predicted values of the model are to the actual values. Lower RMSE means better model performance.

Effect of Number of Terms: RMSE values generally decrease as the number of terms increases. This implies that the model shows better prediction performance when it contains more terms.

Downward Trend: A significant decrease in RMSE is observed until the first 10 terms. After this point, RMSE values stabilise and it is seen that the added terms do not make a significant contribution to the model performance.

Effect of Product Grade: When the effects of different product grades on RMSE are analysed, it is observed that product grade 1 has higher RMSE values, especially at low term counts, but as the number of terms increases, the effect of product grades on RMSE decreases. It is observed that the RMSE values between product grades 2, 3, 4 and 5 are similar and the performance becomes closer to each other as the number of terms increases.

Optimum Number of Terms and Product Degree: The graph shows that the RMSE values stabilise after about 15 terms and the lowest RMSE values are obtained in this range. This means that the performance of the model does not improve with the addition of more terms and even the risk of overfitting may increase.

The graph highlights the importance of choosing the appropriate number of terms and product grade to optimise the performance of the model. A term count of around 15 seems to be sufficient to achieve the lowest RMSE values and models with a product degree of 2 or higher show similar performance. These findings are used to achieve optimal model performance by balancing model complexity and the risk of overfitting.

Table 3.6. Attachment Duration prediction model obtained as a result of Multiple Results MARS analysis

	coefficients
(Intercept)	50.836480
MilkingFrequancy2	-5.563425
MilkingFrequancy3	-8.487326
MilkingFrequancy4	-10.259124
MilkingFrequancy5	-10.902340
Lactation2	-0.715459
h(BoxTime-12.3833)	-0.566128
h(BoxTime-20.4333)	-0.253338
h(43.0333-BoxTime)	-1.080865
h(4.59556-MilkingSpeed)	-1.482112
h(12-DIM)	0.435907
h(DIM-12)	0.003206

This table shows the regression coefficients of the attachment duration prediction model obtained as a result of the MARS analysis. Let us interpret these coefficients to understand the effect and importance of each variable on the prediction model.

(Constant Term) - 50.836480: This represents the baseline value predicted by the model when all independent variables are set to zero.

Milking Frequency 2) -5.563425: This is the coefficient for the second level of milking frequency. A negative coefficient indicates that milking frequency at this level reduces the bonding time.

Milking Frequency 3) -8.487326: This is the coefficient for the third level of milking frequency. At this level, milking frequency further reduces the bonding time.

Milking Frequency 4) -10.259124: This is the coefficient for the fourth level of milking frequency. At this level, milking frequency reduces bonding time even more.

Milking Frequency 5) -10.902340: This is the coefficient for the fifth level of milking frequency. At this level, milking frequency has the greatest reduction in bonding time.

Lactation 2) -0.715459: This is the coefficient for the second level of lactation. A negative coefficient suggests that lactation at this level slightly reduces bonding time.

h(Time spent in the stall - 12.3833): -0.566128: This is the coefficient for the time spent in the stall variable, below a specific threshold (12.3833). In this case, time spent in the stall reduces bonding time.

h(Time spent in the stall - 20.4333): -0.253338: This is the coefficient for the time spent in the stall variable, below a specific threshold (20.4333). Here, time spent in the stall reduces bonding time by a smaller amount.

h(43.0333 - Time spent in the stall): -1.080865: This is the coefficient for the time spent in the stall variable, above a specific threshold (43.0333). In this case, time spent in the stall reduces bonding time to a greater extent.

h(4.59556 - Milking speed): -1.482112: This is the coefficient for the milking speed variable, below a specific threshold (4.59556). In this case, milking speed reduces bonding time.

h(12 - Days in lactation) - 0.435907: This is the coefficient for the days in lactation variable, below a specific threshold (12). In this case, days in lactation increase bonding time.

h(Days in lactation - 12) - 0.003206: This is the coefficient for the days in lactation variable, above a specific threshold (12). In this case, days in lactation increase bonding time by a very small amount.

In general, an increase in milking frequency (especially at the 5th level) significantly reduces bonding time. This could indicate that more frequent milking is more efficient. The time spent in the stall variable has different effects on bonding time, depending on whether it is above or below certain threshold values. In particular, high stall time values (above 43.0333) reduce bonding time to a greater extent. A milking speed below a certain threshold reduces bonding time. As for the number of days in lactation, being below a certain threshold

increases bonding time, while being above it has a very small effect.

Table 3.7. Goodness-of-Fit Values for the Attache Time Model

	criterion	value
1	Root mean square error (RMSE)	21.644
2	Relative root mean square error (RRMSE)	72.210
3	Standard deviation ratio (SDR)	0.940
4	Coefficient of variation (CV)	29.090
5	Pearson's correlation coefficients (PC)	0.399
6	Performance index (PI)	51.598
7	Mean error (ME)	19.810
8	Relative approximation error (RAE)	0.476
9	Mean relative approximation error (MRAE)	0.005
10	Mean absolute percentage error (MAPE)	63.186
11	Mean absolute deviation (MAD)	19.825
12	Coefficient of determination (Rsq)	0.0528
13	Adjusted coefficient of determination (ARsq)	0.0528
14	Akaike's information cCriterion (AIC)	139080.994
15	Corrected Akaike's information criterion (CAIC)	139081.008

The goodness-of-fit values used for the model were determined with nprune = 12 and degree = 1.

Root Mean Square Error (RMSE) - 21.644: RMSE represents the average error in the model's predictions. A lower RMSE value indicates that the model is making more accurate predictions. The value 21.644 reflects the average magnitude of the model's error.

Relative Root Mean Square Error (RRMSE) - 72.210: RRMSE is the ratio of RMSE to the mean value, typically expressed as a percentage. This high value indicates that the model's error rate is quite large relative to the magnitude of the data.

Standard Deviation Ratio (SDR) - 0.940: SDR is the ratio of the standard deviation of the model's predictions to the standard deviation of the actual data. A value close to 1 indicates that the model captures the variance in the data well. The value 0.940 suggests that the model's predictions are reasonably consistent with the actual data.

Coefficient of Variation (CV) - 29.090: CV is the ratio of the error rate to the mean value, expressed as a percentage. A high CV value indicates that the model's predictions are inconsistent and its performance is poor.

Pearson Correlation Coefficient (PC) - 0.399: PC represents the linear relationship between the model's predictions and the actual values. Values closer to 1 indicate a strong linear relationship. The value 0.399 suggests a weak linear correlation between the model's predictions and the actual data.

Performance Index (PI) - 51.598: PI is an index that assesses the overall performance of the model. A high PI value indicates good model performance. The value 51.598 suggests that the model's performance is moderate.

Mean Error (ME) - 19.810: ME shows the average error between the model's predictions and the actual values. A positive value indicates that the model's predictions are systematically higher.

Relative Approximation Error (RAE) - 0.476: RAE is the ratio of the average error to the actual values. A low RAE indicates good model performance.

Mean Relative Approximation Error (MRAE) - 0.005: MRAE is the average value of RAE. A low MRAE value indicates that the model performs well.

Mean Absolute Percentage Error (MAPE) - 63.186: MAPE represents the average absolute percentage error between the predicted and actual values. Lower MAPE indicates better model performance. The value 63.186 suggests that the model has relatively high prediction errors.

Mean Absolute Deviation (MAD) - 19.825: MAD represents the average absolute deviation between the model's predictions and the actual values. A lower MAD indicates

better model performance. The value 19.825 reflects the average magnitude of the model's error.

Coefficient of Determination (R^2) - 0.0528: R^2 indicates how much of the variance in the data is explained by the model. Values closer to 1 suggest that the model explains most of the variance in the data. The value 0.0528 suggests that the model explains very little of the variance.

Adjusted Coefficient of Determination (AR^2) - 0.0528: AR^2 is the adjusted form of R^2, accounting for the complexity of the model. The value 0.0528 indicates that the model's performance is low.

Akaike Information Criterion (AIC) - 139080.994: AIC measures the trade-off between model complexity and goodness-of-fit. Lower AIC values indicate better model performance. A high AIC value suggests that the model is overly complex and performs poorly.

Corrected Akaike Information Criterion (CAIC) - 139081.008: CAIC is the corrected form of AIC, taking model complexity into account. A high CAIC value indicates that the model's performance is poor.

This table indicates that the overall performance of the bonding time model is poor. High RMSE, RRMSE, CV, and MAPE values suggest that there are significant errors in the model's predictions. Additionally, low PC and R^2 values show a weak relationship between the model's predictions and the actual data, as well as a limited explanatory power. To improve the model's performance, additional data and/or more appropriate modeling techniques need to be employed.

DISCUSSION

According to the results, it was determined that the explanatory variables of time spent in the stall (min/day), milking speed (L/min), and days in lactation (days) have the ability to explain 0.72% of the variation in bonding time and 79.6% of the variation in milk yield. While the model shows a high predictive accuracy for milk yield, its predictive performance for the bonding time variable is found to be significantly low.

The higher R^2 value in the Multivariate MARS model compared to the single model indicates that the multivariate model explains the data better and predicts with greater accuracy. The MARS model is more complex and flexible compared to single models like linear regression or simple regression. This flexibility allows the model to better capture the complex and non-linear relationships present in the dataset. Consequently, the explanatory power of the model is enhanced, resulting in a higher R^2 value. The MARS model can automatically model interactions and non-linear relationships between variables, which single models typically struggle to capture. The ability to account for these complex interactions between variables improves the model's performance and increases the R^2 value. Additionally, the MARS model employs specific techniques to control overfitting, enabling it to capture general trends more effectively rather than fitting the data too closely. This balance allows the model to perform well on test data, further contributing to a higher R^2 value. The fact that the MARS model achieves a higher R^2 value compared to the single model demonstrates that it better captures the complexities and relationships within the dataset and has superior predictive performance. This supports the preference for the MARS model, particularly in datasets with complex and non-linear structures.

Çanga Boğa et al. aimed to predict seasonal (autumn, winter, spring, and summer) milk yields as multiple outcomes and achieved prediction success rates of 95%, 97%, 92%, and 94%, respectively. It is thought that the low explanatory power in our study may be due to the structure of the data. Munoz-Osorio et al. aimed to estimate carcass tissue composition in Black Belly lambs using real-time ultrasound measurements (USM) to predict fat thickness and longissimus thoracis (LT) traits through multivariate adaptive regression splines (MARS) algorithms. They successfully predicted total bone weight, total muscle weight, and total muscle fat weight with accuracy rates of 76.3%, 95.3%, and 65%, respectively. The higher success rate compared to our study may be attributed to the high correlation of the predicted traits. Aguilar-Quiñonez et al. (Aguilar-Quiñonez et al., 2023) aimed to predict carcass tissue composition in Hair Sheep lambs using multivariate adaptive

regression splines algorithms and reported prediction success rates between 90% and 96% for the outcome variables. The higher success rate compared to our study may also be due to the strong correlation of the traits predicted in their work.

CONCLUSIONS

As a result of this study, which aimed to investigate the potential use of MARS algorithms for multiple outcomes in the field of animal science, it was determined that the explanatory variables of time spent in the stall (min/day), milking speed (L/min), and days in lactation (days) have the ability to explain 0.72% of the variation in bonding time and 76.8% of the variation in milk yield. Based on the findings and similar literature, it is understood that multivariate adaptive regression splines (MARS) can be successfully used in the field of dairy cattle farming. It is also apparent that this method could be applied in other studies within the field of animal science. It may be suggested that future research would benefit from evaluating and comparing MARS with other methods.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Prof. Dr. Dariusz PIWCZYŃSKI, faculty member at Bydgoszcz University of Science and Technology, for providing data support in this study, and to Assoc. Prof. Dr. Cem TIRINK, faculty member at Iğdır University, for his assistance.

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Prediction of body weight from body measurements with LightGBM algorithm

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Abstract

This study aims to use an advanced machine learning algorithm to estimate the body weight (BW) in Black Belly sheep raised in Mexico. For this aim, we used the LightGBM algorithm to get a prediction model. Body measurements were used as explanatory variables. The performances of LightGBM model was evaluated using R2, RMSE and MAE goodness of fit metrics. The results of the goodness of fit criteria showed that the LightGBM model achieved a reliable prediction performance with a higher R2 value and lower error rates for the LightGBM model. In conclusion, the current study demonstrates the potential use of machine learning approaches in estimating body weight in animal science studies. It will support sustainable animal husbandry by contributing to decision-making processes.

Key words: *Black Belly sheep, LightGBM, prediction, machine learning, body weight*

Effect of breed on egg quality traits of South African Potchefstroom Koekoek and Commercial Lohman Brown Chickens

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Abstract

Egg quality is an important feature in egg production, which is affected by many factors that also include the genetic make-up of an animal. However, the influence of the chicken breed on egg quality traits remains poorly understood. Therefore, study aimed to highlight the influence of breed on the external and internal egg quality traits of Potchefstroom Koekoek and Lohmann Brown layers. The Pearson's correlation findings of the external egg quality traits displayed that the egg weight had a positively high significant (P < 0.01) correlation with the egg width and shell surface area in Potchefstroom Koekoek breed. In Lohman Brown, the outcomes revealed that the egg weight had a positively high significant (P < 0.01) correlation with the egg length, egg width and shell surface area. The correlation results of the internal egg quality traits showed that the egg weight had a positively high significant (P < 0.01) correlation with the yolk weight, albumen weight, albumen ratio and egg volume in Potchefstroom Koekoek. In Lohmann Brown, the egg weight had a positively high significant (P < 0.01) association with the yolk weight, albumen weight, albumen ratio and egg volume. The student's t-test findings displayed that all the external egg quality traits were significantly (P < 0.05) affected by the breed, except for the unit shell surface weight (P > 0.05). The outcomes also revealed that the breed significantly (P < 0.05) affected all the internal egg quality traits. Most affected traits favoured the Lohmann Brown than Potchefstroom Koekoek chicken breed. In conclusion, the outcomes displayed significant differences in the external and internal egg quality traits between Potchefstroom Koekoek and Lohmann Brown chicken breeds.

Key words: *Key words: Breed, egg production, egg weight, albumen weight, yolk weight*

Prediction of body weight from body measurements with LightGBM Algorithm

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Abstract

This study aims to use an advanced machine learning algorithm to estimate the body weight (BW) in Black Belly sheep raised in Mexico. For this aim, we used the LightGBM algorithm to get a prediction model. Body measurements were used as explanatory variables. The performances of LightGBM model was evaluated using R^2, RMSE and MAE goodness of fit metrics. The results of the goodness of fit criteria showed that the LightGBM model achieved a reliable prediction performance with a higher R^2 value and lower error rates for the LightGBM model. In conclusion, the current study demonstrates the potential use of machine learning approaches in estimating body weight in animal science studies. It will support sustainable animal husbandry by contributing to decision-making processes.

Key words: *Black Belly sheep, LightGBM, prediction, machine learning, body weight*

INTRODUCTION

Archaeological and genetic studies reveal that sheep were one of the first animals to be domesticated (Tırınk et al., 2022). Sheep breeding is of great importance not only for the development of healthy civilizations, but also for the provision of animal products such as milk, meat and wool in the development processes of rural economies. In line with the value of this importance for humanity, researchers aimed to make sheep farms more profitable, innovative and sustainable.

In addition to all this, sheep farming on a global scale plays a critical role in terms of sustainability and productivity for agricultural economies. Sheep, which can adapt to different geographical and climatic conditions, stands out as an important source of income in many world regions. In this context, Mexico, with its diverse climate and land structure, has great potential for sheep farming and significantly contributes to the country's rural development. In the tropical regions of Mexico, hair sheep breeds have become an important alternative for the efficient use of natural resources and sustainable meat production. Especially Pelibuey and Black Belly sheep breeds are among the widely preferred mother breeds with their environmental adaptation abilities, high reproductive capacity and productivity performance (Muñoz-Osorio et al. 2016; Aguilar-Hernández et al. 2016; Magaña-Monforte et al. 2018; Chay-Canul et al. 2019). Meat production is a critical component of sheep farming for both human health and rural development. Sustainable meat production in tropical regions of Mexico with sheep breeds with high environmental adaptability such as Pelibuey and Black Belly supports the efficient use of natural resources in these regions and makes significant contributions to the rural economy. Meat production plays a major role in meeting nutritional security and protein needs, especially in developing countries. In this context, the expansion of efficient and sustainable meat production methods is of strategic importance for both regional development and global food security. The development of sheep farming with meat-oriented approaches increases Mexico's agricultural production capacity while also directly contributing to the improvement of living standards in rural areas.

Scientific studies within the scope of meat production to meet the demand due to the increasing world population are important in

achieving global food security and sustainable development goals. In this context, the development of innovative breeding methods, improvement of animal welfare and acceleration of genetic improvement studies play a critical role in increasing efficiency in meat production and ensuring the environmental sustainability of production processes.

Especially considering the effects of climate change on agricultural production, research to be conducted on the selection and breeding of sheep breeds that can adapt to different environmental conditions will contribute to the strengthening of food supply chains both locally and globally. In addition, studies to be conducted on nutritional management, disease prevention and integration of modern agricultural technologies to increase quality and efficiency in meat production will improve the profitability of the sector and support sustainable development. Such scientific studies will enable the development of innovative solutions in livestock sectors, such as sheep breeding, while also making significant contributions to strengthening rural economies. In this context, in animal breeding activities, knowing the live weights of animals in the herd is essential in determining the breeding strategy for the herd and in herd management. In this context, knowing the live weights will facilitate the calculation of the optimum amount of feed per animal in the herd, the determination of drug doses, marketing prices more reliably and the determination of the optimum slaughter time of animals (Tırınk, 2022).

In the literature, many biometric measurements have been evaluated within the scope of multivariate statistical methods to provide breed characterization (Khan et al., 2014; Ali et al., 2015; Faraz et al., 2021; Yavuz and Sahin, 2022). Especially in multivariate statistical methods where the classical regression approach is used, the existence of assumptions such as linearity, constant variance, normality, and multicollinearity that must be provided may limit the applicability of these methods. Since these assumptions may affect the accuracy and validity of the model, if they are neglected, they may lead to misleading results. Therefore, it is important to check these assumptions meticulously and use alternative methods, especially assumption-free or more flexible modeling techniques, when necessary (Mendes and Akkartal, 2009; Sahin et al., 2018; Tırınk, 2020; Yavuz and Sahin, 2022). Some data mining and machine learning algorithms suggested to eliminate such problems can also be applied (Tyasi et al., 2021; Kurnaz et al., 2021). In this context, various studies have been carried out within the framework of multivariate statistical methods to estimate characteristics such as birth weight, live weight, weaning weight, and final weight in different breeds and species (Eyduran et al., 2017; Eyduran et al., 2019). The main purpose of estimating these traits is to determine how these traits differ among species and different breeds within the same species. Determining these differences will provide significant convenience in herd management processes such as selection practices and breed characterization.

This study aimed to estimate the live weight from several body measurements with the sex factor. For this aim, LightGBM algorithm was used and examined.

MATERIAL AND METHODS

This study aimed to estimate the live weight of Black Belly sheep using their body measurements such as heart girth (HG), abdominal girth (AG), body length (BL), diagonal body length (DBL), withers height (WH), rump height (RH) and hip-width (HW).

The animals were treated according to the Standards for Ethical Animal Research established by the Scientific Department of Agricultural Sciences of the Autonomous University of Tabasco (CIEI: Folio 1173-2022). The experiment was conducted at the Sureste Ovine Integration Centre (CIOS, 17°78'N, 92°96'W"; 10 masl). It is in the R/a Alvarado Santa Irene 2da Secc, Centro municipality, Tabasco, Mexico. The area has a humid tropical climate. Temperatures range from 15 to 44 °C, with an average of 26 °C.

The LightGBM algorithm used in this study, which was planned to estimate live weight from various body measurements, was proposed by Microsoft's Distributed Machine Learning Toolkit (DMTK) in 2016 (Ke et al.,

2017; Li et al., 2024). The LightGBM algorithm uses histogram-based algorithms that separate continuous feature values into discrete bins (Ranka and Singh, 1998, Li et al., 2007). LightGBM algorithm has fast training speed and lower memory usage. Besides, it supports GPU and parallel learning simultaneously and can process large datasets (Cai et al., 2021; Li et al., 2024). Additionally, a histogram subtraction technique that reaches the target leaf after removing neighboring leaves using the parent of the target leaf can contribute to accelerating convergence (Cai et al., 2021). LightGBM uses the leaf-based tree growth method, which selects the leaf with the highest delta loss during growth (Cai et al., 2021). There are some tuning parameters in the implementation phase of the LightGBM algorithm (Table 1).

Table 1. Hyperparameters of the LightGBM model

Parameters	Description
Bagging Fraction	It determines the data rate to be used in each iteration. Adjusting this rate appropriately can speed up the training process and reduce the risk of overfitting.
Min Data in Leaf	Setting this parameter to a larger value can prevent the resulting tree from growing too deep.
Feature Fraction	Enables subsampling of random features. Setting this reasonably can speed up training and prevent overfitting.
learning_rate	Determines the step size at each iteration as we move towards the minimum of the loss function.

1. Determination of Coefficient

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad (1)$$

2. Root-mean-square error (RMSE):

$$\mathrm{RMSE} = \sqrt{\frac{1}{\mathrm{n}}\sum_{\mathrm{i}=1}^{\mathrm{n}}(\mathrm{y_i} - \hat{y}_i)^2} \quad (2)$$

3. Akaike Information Criteria

$$\mathrm{AIC} = 2\mathrm{k} - 2\ln(\hat{L}) \quad (3)$$

4. Bayesian Information Criteria

$$\mathrm{BIC} = \mathrm{k}\ln(n) - 2\ln(\hat{L}) \quad (4)$$

The aforementioned goodness of fit criteria was used to compare the model performances which were made along with the lowest RMSE, AIC, and BIC. In addition, the model performances should made along with the highest R^2 value (Tatliyer 2020).

All statistical evaluations were made using R and Spyder software (R Core Team, 2020; Raybaut, 2009). Descriptive statistics were used to provide the necessary information about the data. Descriptive statistics for explanatory and response variables were performed using the "psych" package available in R software (Revelle, 2017). Pearson correlation analysis was used with the "corrplot" package in R software to visualize the relationship between explanatory and response variables (Wei et al., 2017). "lightgbm" package was used to apply the LightGBM algorithm used to estimate BW from the body measurements (Shi et al., 2023). Spyder software was used to visualize the 3D surface plots.

RESULTS

Table 2 presents descriptive statistics for explanatory and response variables based on sex factors.

Table 2. Descriptive statistics of the explanatory and response variables for each sex.

Female					
Variables	**n**	**Mean**	**Standard deviation**	**Min**	**Max**
BW	30	24.79	2.95	19.55	32.15
HG		67.90	3.59	60.00	77.00
AG		72.60	4.31	65.00	83.00
BL		45.06	2.22	41.00	50.00
DBL		49.26	2.88	44.00	55.00
WH		63.40	3.30	58.00	70.00
RH		62.00	3.22	56.00	68.00
HW		14.18	1.21	11.50	17.50
Male					
Variables	**n**	**mean**	**Standard deviation**	**Min**	**Max**
BW	25	29.09	3.08	23.50	34.90
HG		70.68	3.21	63.00	77.00
AG		75.16	4.79	65.00	84.00
BL		39.76	2.94	34.00	46.00
DBL		47.20	3.81	40.00	56.00
WH		61.80	2.34	56.00	67.00
RH		62.88	2.40	58.00	67.00
HW		13.464	1.09	11.00	15.40

According to Table 2, various measurements for female and male sample groups were evaluated. Body weight (BW) in female sheep was 24.79 kg on average (n=30) with a standard deviation of 2.95 kg. In male sheep, this value was higher with a mean of 29.09 kg (n=25) and a standard deviation of 3.08 kg. HG was 67.90 cm on average in females and 70.68 cm in males, with a larger mean in males. Similarly, AG was 72.60 cm on average in females and 75.16 cm in males. Differences between sexes were also observed in BL and other parameters. In particular, the mean BL for females was 45.06 cm, while this value was 39.76 cm in males. Measurements such as WH and RH also showed differences between sexes. All these data statistically reveal the variability between genders regarding the morphological characteristics of the samples.

Figure 1. Correlation analysis

Generally, positive correlations are observed between explanatory and outcome variables of "Female" sheep; however, there is a high degree of positive correlation between some variable pairs. Although a similar correlation pattern is observed in "Male" sheep, the distribution and density of correlation values differ for some variable pairs. Correlation coefficients calculated without gender difference are important in terms of evaluating how the relationship structures between different variables may vary according to gender and whether the correlation degrees of some variable pairs are consistent for both sexes. The distribution and density of correlations can help us understand the covariation of related variables and how they act together.

Table 3 shows the hyperparameter optimization results of the LightGBM model. This table includes various hyperparameters optimized to increase the model's performance and their tested value ranges and the optimal values obtained.

According to Table 3, the hyperparameters tested to increase the model performance reached the optimal values as num_leaves (in the range of 30-70) 31, learning_rate (in the range of 0.05-0.2) 0.2, feature_fraction (in the range of 0.8-1.0) 1.0 and bagging_fraction (in the range of 0.8-1.0) 0.8. The min_data_in_leaf parameter was tested between 10 and 20 and was determined as 10, and max_depth was tested between 5 and 15 and was determined as 5. In addition, num_iterations (in the range of 100-300) was optimized as 300.

Table 3. Hyperparameters of the LightGBM model.

Hyperparameters of the LightGBM model		
Hyperparameters	Tried values for optimization	Optimal values
num_leaves	From 30 to 70	31
learning_rate	From 0.05 to 0.2	0.2
feature_fraction	From 0.8 to 1.0	1
bagging_fraction	From 0.8 to 1.0	0.8
min_data_in_leaf	From 10 to 20	10
max_depth	From 5 to 15	5
num_iterations	From 100 to 300	300

These results reflect the carefully selected settings to increase the generalization capacity and prediction accuracy while limiting the complexity of the model. The optimal hyperparameters show that the model maximizes its performance in a way that best fits the data without overfitting.
Figure 2 presents surface plots showing the effect of the learning_rate and num_iterations parameters on the goodness of fit criteria (R^2, RMSE, AIC and BIC) according to the LightGBM algorithm results.

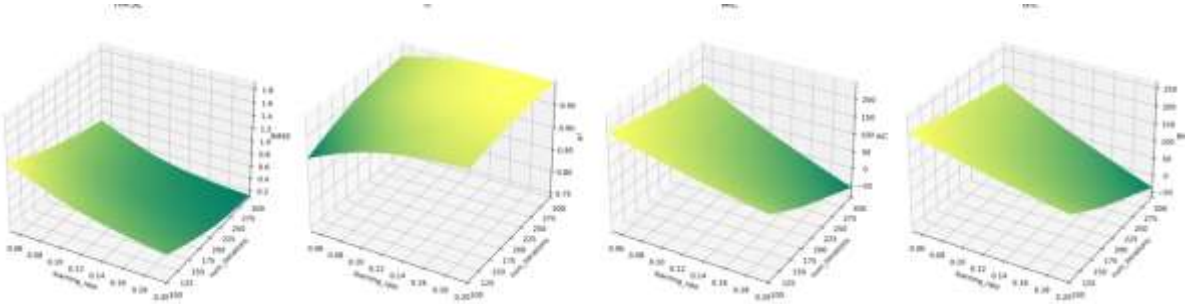

Figure 2. Surface plot for LightGBM algorithm results according to learning_rate num_iterations according to goodness of fit criteria.

According to Figure 2, the first plot shows how the RMSE decreases with the same parameters and low error rates are optimized with high learning rates and iteration numbers. The second plot shows that the R^2 value increases depending on learning_rate and num_iterations and the highest model accuracy is obtained with higher learning rates and iteration numbers. The third and fourth plots emphasize that the AIC and BIC values exhibit negative trends, and the most suitable levels in terms of model complexity and fit balance are obtained again at higher learning_rate and num_iterations values. These results reveal that certain hyperparameter settings should be optimized to increase the accuracy and fit of the model.
Table 4 evaluates the performance of the LightGBM model according to the goodness of fit criteria.

Table 4. Goodness of fit criteria results for optimal LightGBM model

Goodness of fit criteria	
Criterion	Optimal LightGBM model
R^2	0.999
RMSE	0.091
AIC	-86.680
BIC	-66.606

According to Table 4, the R^2 value of the model was calculated as 0.999, which shows that the model explains 99.9% of the total variance in the dependent variable and has a very high prediction accuracy. The RMSE value of 0.091 indicates a low error rate, indicating that the model provides high accuracy in its predictions. The AIC value was determined as -86.680, and the BIC value as -66.606. Both criteria show that the model provides the most appropriate balance between complexity and fit and avoids using unnecessary parameters. These results show that the LightGBM model fits the data well and works with minimal error rates.
Figure 3 shows the importance levels of variables in the sensitivity analysis performed within the LightGBM model.

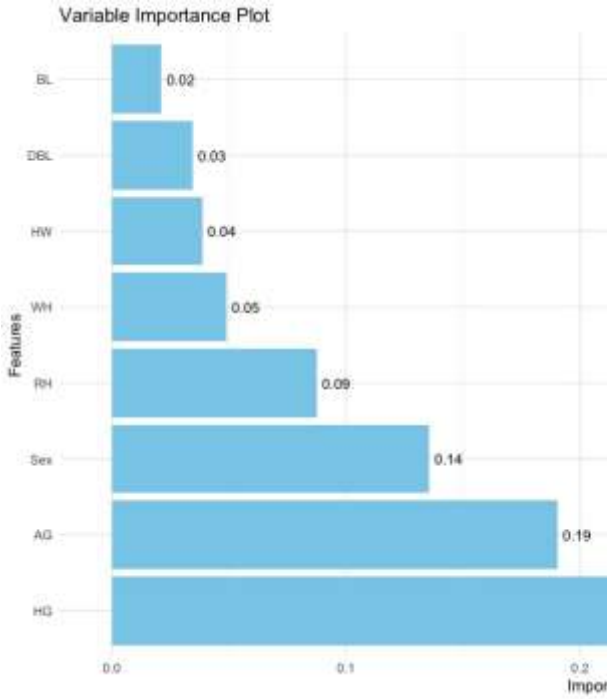

Figure 3. Variable importance values within the scope of sensitivity analysis.

According to Figure 3, the variable that affects the prediction performance of the LightGBM model the most is HG, which contributes to 44% of the total importance. This is followed by AG at 19% and Sex at 14%, respectively, which shows that age and gender make significant contributions to the prediction accuracy of the model. It is understood that other variables such as HW, WH, HG and BL have relatively lower importance and their contribution to the performance of the model is limited. These results indicate which variables are more decisive on the outputs of the model and which variables contribute less to increasing the accuracy of the model, indicating the areas that need to be focused on in the model development and improvement processes.

DISCUSSION

In the present study, the LightGBM algorithm was applied to estimate body weight (BW) in Black Belly sheep. In this context, the obtained LightGBM model aims to improve the computational time and generalizability of the model by allowing it to be trained on fewer, more practical features. In this way, it was aimed to make a more reliable BW estimate.

In the existing literature, there is no study on live weight (BW) estimation using the LightGBM model in the livestock field. This indicates that the LightGBM model offers a potential innovative approach for BW estimation and offers an opportunity to fill the knowledge gap in this area.

Hamadani et al. (2023) used several algorithms, such as the MARS algorithm, Bayesian ridge regression, Ridge regression, support vector machines, Gradient Boosting, Random Rorests, XGBoost algorithm, artificial neural networks, classification and regression trees, polynomial regression, K-nearest neighbours and Genetic Algorithms for predicting weight in sheep. According to the results of this study, the five most reliable methods were MARS, Bayesian ridge regression, Ridge regression, support vector machines and Gradient Boosting algorithms. When the results are compared with the current study, we see that the evaluation criteria used are the same. This is an important criterion for comparing studies. Both studies show similar results.

Xuan et al. (2023) aimed to predict the resilience and cohesion of deep-fried tofu by ultrasonic detection with several machine learning algorithms. For this aim, XGBoost, Random Forest, LightGBM, and ANNs were employed to construct the model, and their prediction performances were compared. The LightGBM algorithm was the best fit within the scope of regression approaches. These results show similar results with the present study.

In this context, the results of the current study show that the LightGBM algorithm offers an innovative and promising approach in the estimation of live weight of sheep. The fact that the LightGBM model has not been used for live weight estimation in the field of animal husbandry in the existing literature reveals that this study makes a significant contribution to the field and has the potential to fill the knowledge gap. In addition, when compared to previous studies, the findings of this study show that the LightGBM algorithm exhibits reliable and general performance in weight estimation. Therefore, this study provides a new perspective on the applicability of data mining and machine learning algorithms in

the field of animal husbandry and provides a basis for future research.

CONCLUSION

In conclusion, the superior performance of the LightGBM model in the estimation of live weight (BW) from body measurements in this study shows that the model can be used as an innovative tool in the field of agriculture and animal husbandry. The findings obtained in the current study show that LightGBM is a suitable and powerful method for estimating live weight based on body measurements in terms of both accuracy and efficiency and has significant potential for future studies in this field. The performance of the model provides more effective results compared to existing estimation methods, thus enabling important applications in the fields of animal husbandry and management.

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Horner´s Syndrome in dogs – overview

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Abstract

Horner´s syndrome is characterized by the symptoms of miosis, anisocoria, ptosis, enophthalmos and protrusion of the third eyelid as a result of an interruption in the sympathetic nervous system extending from the hypothalamus of the brain to the eye. It has been identified in almost every mammal, including humans. The sympathetic nervous system consists of 3 main parts and these are the central, preganglionic and postganglionic neurons. In order to find the interruption on this system, diagnosis and differential diagnosis must be made and the underlying etiology of the disease must be found. For this, an accurate examination and lesion localization is required. Topical application of cocaine to the eyes is considered the gold standard in the pharmacological method of lesion localization. Although there are many underlying etiologies, idiopathic Horner´s syndrome in dogs is more common than other etiologies. Apart from the pharmacological methods, radiology, CT and MRI imaging techniques are also common tools used to determine the etiology. In this article, the anatomy, etiology and pharmacological diagnostic methods for the diagnosis and treatment of Horner´s Syndrome are explained.

Key words: *Anisocoria, enophtalmos, horner's syndrome, miosis, ptosis.*

Lactation records corrected for days open in bulls evaluation

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Abstract

The aim of the present work was to estimate two sets of sire evaluation of Egyptian bulls, kept at Mehalet Mousa Experimental station at karfer El- Shekh Government, belonging to Animal Research Institute, Ministry of Agriculture, Dokki, Cairo, Egypt. The number of sires, buffalos and total number of records used were 42, 596 and 1563, respectively. Two sets of bull evaluation were estimated, set 1 consisted of estimates of ten monthly milk yield after adjusted the data for the main effects of season and year of calving and lactation order, while for set 2 the records adjusted for the main effects of season and year of caving, lactation order and days open. The greatest bull evaluation were 328 kg and 346 kg for set 1 and set 2, respectively for a bull with 15 daughters. The accuracy of bull evaluation increases as the number of daughters per sire increases. Rank correlation among sire transmitting abilities between the two sets of evaluation was a high of 0.99. The average difference in evaluation was small. Also, the correlation of deviations among the two comparisons with the original evaluation was small. Therefore, changes in evaluation were independent for a sire´s genetic value

Key words: *lactation records adjusted Egyptian evaluation*

Genetic parameters and GHR gene polymorphisms related to live weight and body measurements of hair goat kids raised in the Aegean Region

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Abstract

Approximately 96% of Turkey's goat population consists of Hair goats. Despite having such a large goat population, the desired level of meat and milk cannot be obtained from these animals. Many candidate genes affecting the productivity traits of Hair goats have been identified. Therefore, in this study, the environmental factors affecting the live weight and body size traits of Hair goat kids raised in the Aegean region and the polymorphisms in the GHR (Growth hormone receptor) gene affecting these traits were evaluated. The animal material of this thesis study consists of a total of 120 kids raised in two goat farms in Karaburun district of İzmir. As a result of PCR-RFLP analysis in the GHR gene, two genotypes AA and AB were obtained. No effect of these genotypes on live weight and body size was found. The Chi-square test results performed for the Hardy-Weinberg balance control showed that the observed and expected differences between the genotype frequencies of exon 10 were statistically significant ($p<0.05$). This situation indicates that a deviation from the Hardy-Weinberg balance is observed. Deviations from the Hardy-Weinberg balance indicate that selection will be effective in protecting local genetic resources and increasing the productivity of the hair goat.

Key words: Hair Goat, GHR, Polymorphism, Heritability, Genetic Correlation, Selection.

INTRODUCTION

With the rapid increase in the world population, food security and sustainability have become a global priority. In parallel with the population growth, food production is not increasing at a sufficient level, which makes the production and consumption of animal-based foods even more important. Animal products are the main source of high-quality protein and amino acids required in the human diet (Eljagmani & Altuner, 2020). Animal-derived products such as meat and milk provide the essential protein amino acids that humans need (Hoffman & Falvo, 2004).

The World Health Organization (WHO) recommends that individuals should have a daily protein intake of approximately 0.83 grams per kg and that a large portion of this protein should come from animal sources (WHO, 2007). In developed countries, the average daily protein consumption is approximately 100 grams per person, of which approximately 60 grams consists of animal proteins (FAO, 2013). In contrast, these rates are quite low in developing countries, revealing the global inequality in access to animal protein sources.

In small ruminant breeding, the basis of successful breeding studies is the selection of the right breeder. Considering morphological characteristics in breeding selection plays a critical role in evaluating the development and growth potential of animals. Characteristics such as the vitality, live weight, and body size of animals are the key to creating a productive and healthy herd (Smith et al., 2020; Johnson & Thompson, 2019). These criteria are decisive factors in increasing the effectiveness of

breeding programs. Determining genetic factors and polymorphisms affecting growth traits in goats is an important area of research in animal breeding studies. Growth hormone (GH) has a critical role in increasing growth performance and milk yield in farm animals (Etherton & Bauman, 1998). The effects of GH are mediated by the growth hormone receptor (GHR), and this protein has an important place in the growth and development processes of animals (Burton et al., 1994). Various studies have investigated polymorphisms in genes controlling growth parameters in goats (Singh et al., 2009; An et al., 2011; Alakilli et al., 2012; Fu et al., 2013; Bayan et al., 2018; Pandya et al., 2020).

This study aimed to determine polymorphisms in the growth hormone receptor gene (GHR) in Hair goats and to examine the relationships of these genotypes with growth traits and live weight of kids. In addition, the possibility of using these data as a selection criterion was investigated by determining the heritability levels. This study aims to provide a scientific basis for future research on indigenous goat breeds in Turkey.

MATERIAL AND METHOD

MATERIAL

The animal material of this study consists of a total of 120 goats, 61 kids (31 males, 30 females) born in January 2022 on the first farm and 59 kids (30 males, 29 females) born on the second farm from goats aged between 1-3 years in the Karaburun district of Izmir. The goats were kept under extensive breeding conditions, grazed in the pasture for 5-7 hours a day, and feed supplements were given in the morning and evening. The kids were left with their mothers for a short time in the morning and evening and weaned when they were 90 days old. The birth weights of the kids were measured with a digital hand scale with a precision of 5 grams within the first 24 hours following birth and ear numbers were attached. Live weight, chest circumference, rump length, foreshank, hind shank, tail length, ear length, body length, withers height, back height, rump height, chest depth, rump width, hind shank width, hind rump width, forehead width, head length measurements were taken from the kids whose gender and birth dates were recorded between January and June 2022 and at 14-day intervals until the 120th day of birth. Hair samples were taken from the upper area of the nails of the kids for GHR gene polymorphism analyses. Hair samples collected from three regions, two from the fore shank and one from the hind shank of each kid, were stored at -20°C (ICBF, 2022).

DNA Amplification with Polymerase Chain Reaction from Hair Samples

In this study, Polymerase Chain Reaction (PCR) was applied for DNA amplification from hair samples. PCR primers were designed according to the goat sequence of the 10th exon of the GHR gene (AY292282). Forward primer was 5'-TAAGCGACATTACACCAGC-3' and reverse primer was 5'-TTGAGTACGAGGCCCTGT-3'. Primer 3 software was used for primer design (Rozen and Skaletsky, 2000). DNA was isolated from hair samples and the gene region of the GHR gene, coded AY292282 in NCBI gene bank, with a length of 361 bp in exon 10 of chromosome 20 in goats was amplified. Primer pairs used for PCR reaction (An et al., 2011) are given in Table 1.

Table 1. Primer sequences of GHR Exon 10 gene region.

Gene	Primer	T(C°)	bç	Locus	Ref.
GHR	F: 5'-TAAGCGACATTACACCAGC-3' R: 5'-TTGAGTACGAGGCCCTGT-'3	57	361	Exon 10	An, 2011

Statistical Analysis

One-way analysis of variance (ANOVA) was used to examine the effects of sex, herd, and dam age on live weight and body measurements in hair goats. Heritability levels for live weight and body measurements were calculated and for this purpose, WOMBAT software package was used.

In this study, allelic gene frequencies of kids in terms of GHR gene were calculated with popGene package program and it was

examined whether they were in Hardy-Weinberg balance (Yeh et al., 1997). Polymorphism information content (PIC) was calculated from the obtained allele and genotype frequencies. Polymorphism information content is known as an indicator of heterozygosity, a high PIC value means that genetic diversity is high (Erol, 2017). The following multivariate individual model was used to estimate variance components and heritability levels according to morphological body measurements and live weights of goats.

$Y = \mathrm{Xb} + Z_1 a + Z_2 p + \mathrm{e}$

Here, Y: Phenotypic observation values vector for goat body measurements or live weights,

b: Fixed effects vector, a: Additive genetic effect vector due to chance, p: Permanent environmental effect vector due to chance,

X: Pattern matrix for fixed effects, Z_1: Pattern matrix for additive genetic effect, Z_2: Pattern matrix for permanent environmental effect,

e: Random error vector N ~ (0, σ^2) is expressed.

The following model was used to examine the relationships between GHR genotypes and live weight and body traits:

$Y_{ijk} = \mu + s_i + g_j + e_{ijk}$

Here, Y_{ijk}: Body measurement or live weight of the kid from the i. herd, j. genotype, k^{th} observation value,

μ: general mean, s_i: i. herd effect (k = 1 and 2), g_j: j. genotype effect (i = AA and AB),

e_{ijk}: Random error N ~ (0, σ^2) is expressed.

IBM SPSS v25.0 program was used for statistical analysis of the relationships between GHR genotypes and body weight and body traits, and the WOMBAT (Meyer, 2006) program was used for estimating variance components and heritability levels.

RESULTS

It was found that dam age, sex, and herd factors were generally effective on live weight and body measurements in hair goats ($p<0.05$). Additive genetics (σ_a^2), error variance (σ_e^2), heritability (h^2), and standard deviation from the results obtained from hair goat kids are given in Table 2.

Table 2. Heritability estimates for live weight and body measurement traits

	σ_a^2	σ_e^2	h^2	σ_a^2	σ_e^2	h^2	σ_a^2	σ_e^2	h^2
	Body Weight			**Body Length**			**Withers Height**		
Birth	0.22	0.44	0.33±0.5	1.53	3.86	0.28±0.52	0.18	9.51	0.02±0.55
30	0.15	1.33	0.10±0.4	0.18	15.9	0.01±0.60	2.56	2.66	0.49±0.06
60	0.01	3.60	0.003±0.3	0.001	5.12	0.001±0.26	2.71	3.01	0.47±0.06
90	0.001	6.85	0.001±0.25	0.05	8.89	0.006±0.18	0.17	5.59	0.03±0.17
120	0.09	39.52	0.002±0.40	0.68	23.21	0.02±0.18	7.61	9.64	0.44±0.94
	Back height			**Rump height**			**Rump width**		
Birth	0.001	6.69	0.001±0.90	0.001	7.62	0.001±0.44	0.001	0.45	0.002±0.29
30	0.001	5.17	0.001±0.43	0.001	5.39	0.001±0.26	0.08	0.15	0.36±0.57
60	2.06	36.9	0.05±0.8	0.13	8.50	0.01±0.32	0.08	0.15	0.36±0.57
90	0.17	5.59	0.03±0.2	0.28	5.61	0.04±0.18	0.001	0.68	0.001±0.16
120	0.26	13.9	0.01±0.2	0.84	14.78	0.04±0.19	0.84	2.08	0.28±0.59
	Rump length			**Chest circumference**			**Chest depth**		
Birth	0.36	1.89	0.16±0.68	4.29	0.87	0.83±0.82	1.35	1.03	0.56±0.92
30	1.50	1.11	0.57±0.89	0.001	4.37	0.001±0.19	0.04	1.21	0.03±0.34
60	1.46	2.01	0.42±0.78	0.001	8.75	0.001±0.18	0.71	1.40	0.33±0.53
90	0.72	7.02	0.09±0.29	0.80	37.19	0.02±0.86	0.01	3.87	0.003±0.30
120	11.58	32.84	0.26±0.90	0.23	84.10	0.003±0.54	0.001	10.77	0.001±0.25
	Chest width			**Front shin**			**Rear shin**		
Birth	0.30	0.37	0.11±0.53	0.001	0.23	0.004±0.28	0.001	0.32	0.003±0.37
30	0.001	1.10	0.001±0.33	0.04	0.13	0.25±0.54	0.01	0.13	0.12±0.23
60	0.19	1.20	0.14±0.23	0.07	0.16	0.31±0.59	0.03	0.18	0.15±0.24
90	0.002	1.55	0.002±0.15	0.08	0.15	0.34±0.61	0.05	0.17	0.23±0.29
120	0.08	7.79	0.01±0.29	0.001	0.31	0.003±0.17	0.01	0.39	0.03±0.17
	Ear Length			**Forehead Width**			**Head Length**		
Birth	0.77	4.60	0.14±0.67	0.11	0.18	0.37±0.90	0.62	0.65	0.08±0.41
30	0.26	5.21	0.04±0.26	0.001	0.37	0.003±0.43	0.002	1.19	0.001±0.24
60	0.80	5.59	0.12±0.32	0.24	0.33	0.42±0.70	0.001	1.91	0.001±0.23
90	1.92	4.01	0.32±0.54	0.40	0.66	0.37±0.83	0.002	1.81	0.001±0.18
120	0.03	6.44	0.005±0.16	0.68	3.36	0.16±0.51	0.001	6.23	0.001±0.25
	Tail Length								
Birth	0.66	1.06	0.38±0.94						
30	0.001	1.47	0.001±0.16						
60	0.27	2.26	0.10±0.22						
90	0.001	2.04	0.001±0.22						
120	0.001	6.40	0.001±0.15						

In this study, it was observed that environmental effects increased significantly with age on many traits. Especially, the increase in environmental variance with age in live weight, chest circumference, withers height, and other morphological measurements shows that these traits are more affected by environmental factors such as dam age, sex, and herd. While the environmental variance for Chest Width was 0.37 at birth, it increased to 7.79 on the 120th day; while the environmental variance for Croup Height was 7.62 on the birth, it increased to 14.78 on the 120th day. This also shows that environmental effects increased over time. In addition, wither height, rump width, ear length, chest width, and chest circumference stand out as traits with high heritability. Especially, the heritability of withers height on the 30th day was found to be the highest as 0.49 ± 0.06 and the heritability of chest circumference at birth was found to be 0.83 ± 0.82. According to these results, it is understood that withers height and chest circumference are largely dependent on genetic factors.

Table 3. GHR exon 10 genotype frequency, observed, expected, Chi-square, and PIC values.

Genotype	Frequency (%)	Observed	Expected	Chi-Square	PIC
AA	0.62	55	58.17	4.79 p = 0.028	0.26
AB	0.38	34	14.03		
BB	0	0	3.17		
Allel					
A	80.9				
B	19.1				

The 361 base pair gene regions of the 10th exon of the GHR gene obtained from genomic DNA samples of goat kids were successfully amplified by PCR method. The generated PCR products were visualized using 1.5% agarose gel electrophoresis system. The region determined for genotyping was cut using Tag I enzyme. DNA fragments obtained after cutting were separated by 3.5% agarose gel electrophoresis. According to the genotyping results obtained by PCR-RFLP method applied in GHR exon 10 regions in the population, AA and AB genotypes were determined. The frequencies of these genotypes were determined as 62% and 38%, respectively, and Polymorphism Information Content was found as 0.26. A and B allele frequencies were as follows; 80.9% and 19.1%. While the expected value in hair goats is expected to be AA 58.17, AB 14.03, and BB 3.17, respectively, the genotype frequencies obtained in this study are 55, 34, and 0. The results of the Chi-square test performed for the Hardy-Weinberg balance control showed that the observed and expected difference between the genotype frequencies of exon 10 is statistically significant ($p<0.05$). This indicates that a deviation from the Hardy-Weinberg balance is observed. In other words, mutation and selection may cause the goat population to deviate from the Hardy-Weinberg balance. GHR exon 10 genotype frequency, observed, expected, Chi-square, and Polymorphism Information Content (PIC) values are given in Table 3.

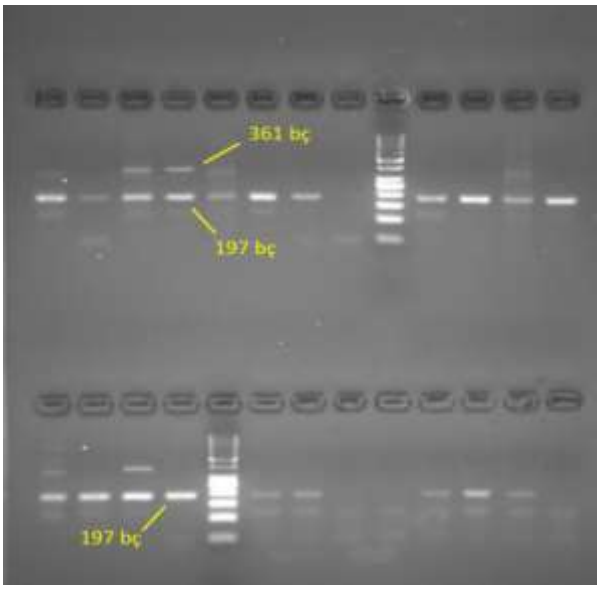

Figure 1. GHR 10. Exon gel image

GHR Exon 10 Effects on Live Weight

The effect of GHR exon 10 on live weights was not found to be significant ($p>0.05$). In this study, the effect of the GHR gene on live weight is explained in Table 4. Here, the average live weights for birth, 30, 60, 90, and 120 days were determined as 4.06±0.09, 8.28±0.16, 11.06±0.20, 15.88±0.32 and 21.14±0.54, respectively (Table 4).

Table 4. Effect of GHR exon 10 on body weight.

	Birth Weight (kg)	30. BW (kg)	60. BW (kg)	90. BW (kg)	120. BW (kg)
μ	4.06±0.09	8.28±0.16	11.06±0.20	15.88±0.32	21.14±0.54
Genotype					
AA	4.07±0.11	8.22±0.20	10.84±0.25	15.64±0.40	20.93±0.67
AB	4.04±0.15	8.33±0.26	11.29±0.32	16.12±0.51	21.36±0.86
p	0.881	0.733	0.284	0.467	0.694

GHR Exon 10 Effect on Body Measurement

The effect of GHR exon 10 was not significant on all body measurements ($p<0.05$). Back height, rump height, rump width, chest circumference, chest depth, chest width, forehead width, and head length were higher in the AA genotype fore and hind shank; but withers height, tail length, rump length, body length, and ear length were higher in the AB genotype (Table 5).

DISCUSSION

In this study, live weight and body measurements were taken from Hair goats raised in Karaburun district of Izmir province. To determine the effect of GHR gene on live weight and body characteristics, hair samples were taken from the kids, and polymorphisms in exon 10 were investigated.

In this study, the effects of gender, herd, and dam age on kid birth weights were found to be significant ($p<0.05$). Erol (2014) reported that the effect of dam age was significant in his study on Ankara goat kids under protection. On the other hand, Tozlu (2006) found the effect of dam age insignificant in his study. According to Thiruvenkadan et al. (2011), the main factors affecting the birth weight of goats were gender, birth period, and birth type. In our study, the birth weight of Hair goat kids was determined as 4.14 kg. In previous studies, the birth weight of Hair goat kids was found to be 2.63 kg by Şengonca et al. (2003), 2.77 kg by Şimşek and Bayraktar (2006), 2.6 kg by Öztürk (2000), 2.85 kg by Kırk (2006), 2.18 kg by Şimşek et al. (2007), 2.58 kg by Odabaşıoğlu et al. (2007), 2.89 kg by Oral and Altınel (2012), 2.89 kg by Erten and Yılmaz (2013), 3.01 kg, which were found to be lower than the value in the present study. It is thought that additional feeding of Hair goats in these two farms in Karaburun may explain this situation. On the other hand, it was observed that the weights in our study were close to the values reported by Darcan (2000) as 3.89 kg, Daş and Savaş (2002) as 3.8 kg, Şimşek (2005) as 2.99 kg, Karadağ (2006) as 3.309 kg and Tozlu (2006) as 3.72 kg. In this thesis study, the birth weights of kids from 1 and 2-year-old dams were found to be heavier than the offspring of 3-year-old dams. The finding that the effect of sex on the birth weight in Hair goat kids was insignificant was compatible with the report of Şengonca et al. (2003). The 30th and 60th day live weights of kids were determined as 8.44 and 11.32 kg, respectively. While the effects of the herd and sex were found to be significant on these weights, the effect of the dam age was not found to be significant. In this study, the live weights of kids on weaning (90th day) and 120th day were found to be the weaning weight was determined as 16.18 and 21.55 kg. The weaning weight was found to be 5.73 kg for the 120th day of the Ankara goat by Odabaşıoğlu (2007) and higher than the value found for the Ankara goat and Hair goat Fı crossbreed by Koyuncu and Tuncel (1996).

The reason for the high value in the present study may be due to the effects of many factors such as genetic structure, environment, care, and feeding. The weaning weights were found to be 11.84 kg by Cengiz et al. (1995). Darcan (2000) 18.0 kg, Şimşek (2005) 17.77, Kırk (2006) 16.75 kg, Şimşek and Bayraktar (2006) 16.05 kg, Tozlu (2006) 16.0 kg, Oral and Altınel (2012) 13.58 kg, Erten and Yılmaz (2013) 12.32 kg were

found to be compatible with the findings. On the other hand, the effect of dam age on weaning weight was not found to be significant. This result was found to be compatible with the report of Şengonca et al. (2003) and inconsistent with the report of Tozlu (2006). The effects of sex and herd on weaning weight were found to be significant, and the finding of a significant effect of sex was found to be compatible with the reports of Şengonca et al. (2003) and Tozlu (2006).

Table 5. Effect of GHR exon 10 on body size.

	Birth (cm)	30.Day (cm)	60.Day (cm)	90.Day (cm)	120.Day (cm)	Birth (cm)	30.Day (cm)	60.Day (cm)	90.Day (cm)	120.Day (cm)
			Body Length					**Withers Height**		
μ	29.59±0.41	38.27±0.26	42.95±0.25	47.55±0.33	52.71±0.56	37.04±0.32	45.73±0.30	49.02±0.29	52.77±0.29	57.08±0.46
AA	29.66±0.51	38.24±0.32	42.85±0.31	47.45±0.40	52.57±0.69	37.39±0.40	45.80±0.37	49.14±0.35	52.77±0.36	56.97±0.56
AB	29.52±0.66	38.31±0.42	43.05±0.40	47.64±0.52	52.85±0.88	36.69±0.51	45.65±0.48	48.90±0.45	52.78±0.46	57.20±0.73
p	0.682	0.899	0.703	0.781	0.804	0.285	0.820	0.678	0.982	0.801
			Back Height					**Rump Height**		
μ	35.24±0.30	43.96±0.29	46.99±0.28	51.37±0.28	56.32±0.47	36.23±0.31	44.96±0.29	48.15±0.27	52.36±0.31	57.11±0.49
AA	35.63±0.37	44.03±0.36	47.06±0.34	51.40±0.35	56.24±0.57	36.52±0.38	45.05±0.39	48.22±0.34	52.30±0.36	56.95±0.60
AB	34.86±0.48	43.89±0.47	46.93±0.44	51.34±0.45	56.40±0.74	35.93±0.49	44.87±0.50	48.07±0.43	52.41±0.46	57.27±0.78
p	0.802	0.815	0.818	0.925	0.868	0.349	0.783	0.795	0.847	0.750
			Rump Width					**Croup Length**		
μ	5.78±0.11	7.08±0.05	7.78±0.11	9.08±0.05	11.78±0.11	18.24±0.24	21.35±0.17	23.82±0.82	26.51±0.30	30.62±0.43
AA	5.78±0.14	7.05±0.06	7.65±0.06	9.44±0.87	11.54±1.82	18.17±0.30	21.18±0.21	22.38±0.06	26.34±0.37	31.00±0.53
AB	5.78±0.18	7.12±0.08	7.85±0.08	9.51±0.77	11.55±1.90	18.25±0.39	21.52±0.28	23.±0.19	26.51±0.48	30.23±0.68
p	0.991	0.476	0.184	0.347	0.387	0.476	0.991	0.151	0.581	0.379
			Chest Circumference					**Chest Depth**		
μ	36.13±0.31	44.16±0.30	49.87±0.37	58.17±0.47	66.99±0.77	14.31±0.14	19.00±0.14	21.79±0.15	25.30±0.22	29.47±0.41
AA	36.13±0.38	44.33±0.37	49.49±0.46	58.23±0.58	67.59±0.94	14.42±0.17	18.91±0.17	21.78±0.18	25.34±0.27	29.88±0.50
AB	36.12±0.49	43.98±0.47	50.24±0.59	58.12±0.75	66.39±1.22	14.21±0.22	19.09±0.22	21.80±0.24	25.26±0.35	28.18±0.65
p	0.984	0.565	0.318	0.908	0.440	0.445	0.537	0.945	0.853	0.479
			Chest Width					**Ear Length**		
μ	8.33±0.16	11.18±0.13	12.73±0.13	14.06±0.17	15.68±0.28	9.79±0.22	11.95±0.25	13.46±0.27	13.98±0.26	15.05±0.28
AA	8.31±0.19	11.10±0.16	12.78±0.17	13.92±0.17	15.41±0.44	9.88±0.27	12.27±0.31	13.86±0.33	14.11±0.32	14.91±0.34
AB	8.36±0.25	11.26±0.20	12.67±0.22	14.19±0.22	15.95±0.34	9.70±0.35	11.62±0.40	13.06±0.42	13.84±0.41	15.20±0.44
p	0.884	0.546	0.673	0.358	0.884	0.701	0.207	0.145	0.610	0.611
			Forehead Width					**Head Length**		
μ	5.84±0.09	8.94±0.08	11.53±0.08	12.36±0.10	13.47±0.19	11.44±0.08	15.22±0.11	17.66±0.15	19.57±0.15	21.65±0.27
AA	5.88±0.11	9.07±0.10	11.53±0.10	12.56±0.13	13.73±0.24	11.42±0.10	15.36±0.13	17.75±0.18	19.60±0.19	21.64±0.43
AB	5.80±0.14	8.80±0.12	11.53±0.13	12.16±0.17	13.21±0.31	11.45±0.14	15.09±0.17	17.58±0.23	19.54±0.24	21.66±0.32
p	0.684	0.102	0.980	0.069	0.190	0.871	0.227	0.567	0.834	0.971
			Front Shin					**Rear Shin**		
μ	6.00±0.03	6.44±0.05	6.88±0.05	7.06±0.06	7.33±0.07	6.50±0.05	6.89±0.05	7.37±0.06	7.58±0.06	7.86±0.08
AA	5.98±0.04	6.43±0.06	6.91±0.07	7.11±0.07	7.36±0.09	6.53±0.05	6.88±0.06	7.40±0.07	7.61±0.07	7.89±0.10
AB	6.01±0.06	6.44±0.07	6.84±0.09	7.03±0.09	7.30±0.12	6.47±0.07	6.90±0.08	7.33±0.09	7.55±0.10	7.84±0.13
p	0.707	0.930	0.586	0.536	0.703	0.569	0.843	0.537	0.604	0.795
			Tail length							
μ	10.36±0.16	12.53±0.13	14.22±0.16	15.50±0.16	17.07±0.28					
AA	10.45±0.19	12.70±0.16	14.29±0.20	15.55±0.19	17.13±0.35					
AB	10.26±0.25	12.36±0.21	14.15±0.26	15.46±0.25	17.01±0.45					
p	0.551	0.200	0.658	0.765	0.835					

In this study, the findings obtained for the body measurements of Hair goat kids at birth were found to be similar to those of Eser (1998); Barıtcı and Eliçin, (2001); Şimşek et al. (2007) and Yıldırım et al. (2013). However, Erduran and Yaman (2012), in their

study on Hair goat kids, the body measurements between birth and 6 months of age were as follows, respectively; body length 31.30, 52.81 and 62.68 cm, withers height 32.82, 55.30, 64.65 cm, rump height 33.10, 55.48, 65.49 cm, rump width 8.40, 12.50 cm, 15.12 cm, chest circumference 35.93, 59.07, 70.22 cm, chest width 8.48, 12.96, 15.77 cm, chest depth 13.26, 20.60, 25.73 cm and shank circumference 6.41, 9.54, 10.86 cm and Bolacalı and Küçük (2012), Body measurements of kids at birth, weaning (90) and 180th day withers height: 34.7, 48.8 and 56.7 cm; back height: 33.9, 47.9 and 55.8 cm; rump height: 35.7, 50.0 and 57.7 cm; coccyx height: 33.9, 48.3 and 54.2 cm; body length: 33.3, 50.5 and 58.3 cm; chest length: 19.7, 26.5 and 30.1 cm; chest depth: 13.3, 21.6 and 25.1 cm; chest circumference: 36.0, 53.5 and 60.1 cm; thigh circumference: 33.3, 49.4 and 55.9 cm; foreshin circumference: 5.5, 6.5 and 7.1 cm; chest width: 7.0, 9.8 and 11.3 cm; reported the front rump width as 6.2, 9.9 and 11.5 cm; and the middle rump width as 8.8, 12.1 and 13.7 cm. These values were found to be close to the current study values. For the goat kids, the heritability of birth weight was determined as 0.335±0.502, 0.104±0.355 for the 30th day, 0.003±0.257 for the 60th day, 0.001±0.254 for the weaning (90th day) and 0.002±0.403 for the 120th day. This value was found to be 0.13 ± 0.06, 0.08± 0.04 higher than the value found by Zhang et al. (2008; 2009) in the Boer goat. It was below the values reported by Hasan and Gunawan (2014), and Mohammed et al. (2018) in the Damascus goat as 0.54±0.12 and 0.410. The heritability of body length was found to be 0.285±0.529, 0.011±0.606, 0.001±0.266, 0.006±0.182 for birth, 30, 60, 90 and 120 days. In the study of Akpa et al. (2009), body length (1, 3, 6, and 9 months) was reported as 0.70, 0.70, 0.72 and 0.70, respectively. For withers height, the heritability was found to be 0.020±0.555, 0.490±0.067, 0.474±0.069, 0.030±0.174, 0.441±0.949 for birth, 30, 60, 90, and 120 days, respectively. In the study conducted by Akpa et al. (2009), it was found to be 0.78, 0.80, 0.75, and 0.71 for (1, 3, 6, and 9 months), respectively. Heritability of back height at birth, 30, 60, 90, and 120th days was found as 0.001±0.900, 0.001±0.432, 0.053±0.879, 0.030±0.174 and 0.019±0.168. Also, heritability of rump height at birth, 30, 60, 90, and 120th days was found as 0.001±0.442, 0.001±0.268, 0.015±0.322, 0.048±0.186 and 0.054±0.191. For rump width, 0.002±0.293, 0.362±0.571, 0.362±0.571, 0.001±0.164 and 0.287±0.593 were found. For rump length, 0.160±0.680, 0.573±0.897, 0.420±0.784, 0.094±0.295 and 0.261±0.902 were found. When chest circumference was examined, the heritability and standard errors at birth, 30^{th}, 60^{th}, 90^{th}, and 120^{th} days were; were found to be 0.830±0.823, 0.001±0.198, 0.001±0.180, 0.021±0.867 and 0.003±0.542. In the study by Akpa et al. (2009), chest circumference (1, 3, 6, and 9 months) was reported as 0.74, 0.83, 0.68, and 0.80, respectively. The heritability degree of chest depth was found to be 0.567±0.928, 0.033±0.347, 0.338±0.532, 0.003±0.302 and 0.001±0.254, respectively. The heritability values of chest width in kids were determined as 0.112±0.535, 0.001±0.331, 0.141±0.238, 0.002±0.153 and 0.011±0.291. When examined as fore and hind shanks, the heritability values were 0.004±0.282, 0.259±0.541, 0.316±0.591, 0.347±0.615 and 0.003±0.171 for the fore shank, while they were 0.003±0.375, 0.124±0.232, 0.153±0.248, 0.230±0.293 and 0.037±0.178 for the hind shank. The heritability and standard errors of ear length are 0.144±0.673, 0.049±0.269, 0.126±0.325, 0.324±0.540 and 0.005±0.160. The heritability of forehead width was found to be 0.376±0.905, 0.003±0.437, 0.421±0.700, 0.378±0.831 and 0.169±0.511 from birth to 120th day, respectively. For head length, it was found to be 0.087±0.414, 0.001±0.244, 0.001±0.232, 0.001±0.187 and 0.001±0.250, while for tail length, it was found to be 0.384±0.942, 0.001±0.165, 0.108±0.220, 0.001±0.226 and 0.001±0.153. Long-term and meticulous studies are required to understand the genetic basis of yield traits under multiple gene control. Some traits, such as eye and skin color, are almost completely unaffected by environmental factors and heritability is evident. On the other hand, traits such as live weight and milk yield are largely affected by environmental factors, and heritability is seen to be below 0.5. Therefore, it is important to analyze the genetic component

in detail to provide genetic progress in a quantitative trait in any animal species. A large number of well-maintained data is needed to better understand these relationships. In this study, it is thought that the heritability is not at the desired level due to the small number of animals, inbreeding, and environmental factors. There are limited studies in the literature on the heritability of body measurements in hair goats.

Many genes and hormones affect the growth performance of farm animals. Growth hormone (GH) plays an important role in milk yield and animal growth as one of them. Etherton and Bauman (1998), Growth hormone carries out its various effects in the body by using specific binding proteins Burton et al. (1994), the most important of these proteins is GHR. They are defined as candidate genes for production traits such as meat quality, growth, and milk yield in hair goat kids. A limited number of studies have been conducted on hair goats in terms of the GHR gene.

In this study, when the analysis was performed using PCR-RFLP technique in order to determine the diversity in exon 10 of GHR gene of hair goat kids, two alleles A and B genotypes were obtained. Here, the frequency of the A allele is 80.9 and B allele is 19.1 and the genotype frequency is AA 0.62, AB 0.38. Dettori et al. (2019) detected two SNPs in their study of Sarda goats. Bai et al. (2011) found two alleles A, G, and three genotypes AA, AG and GG in their study. Allele frequencies are 0.70 and 0.30 and genotype frequencies are 0.53, 0.34, and 0.12, respectively. An et al. (2011) obtained two alleles, G and T, and two genotypes (GG, GT) in the GHR gene in Saanen and Boer goats. As a result of the study, they reported that AA and GG genotypes were associated with growth characteristics in 1, 2, and 3-month-old individuals. Pandya et al. (2020) found monomorphic PCR results in the GHR gene of Surti goat. Singh et al. (2018) in their study on Jamunapari goat, as a PCR result in exon 10 of the GHR gene, the frequencies of two alleles A and G, and three genotypes (AA, AG, and GG) were 0.53 and 0.47, respectively, and the frequencies of the genotypes were 0.16, 0.74 and 0.10. It was observed that the AG genotype had a higher live weight than other genotypes in different growth periods. AG genotype reported that it can be used as a marker in Jamunapari goats.

CONCLUSION

Today, global challenges such as increasing population and food demand increase the importance of modern techniques of animal breeding. It is a fact that with the increase in population, food demand also increases and access to reliable food becomes difficult. This situation shows that more effective and efficient solutions are needed in the livestock sector. Modern breeding offers the potential to obtain more animal products at lower costs by increasing the productivity of animals. Especially in countries such as Turkey, goat breeding has an important place. However, hair goats, which constitute a large part of the goat population, are insufficient to provide the expected meat and milk yield. This situation is an important problem, especially in terms of increasing productivity on farms. Determining the genetic factors affecting the productivity traits of hair goats is one of the main goals of modern breeding. In this study, the environmental effects of Hair goat kids raised in the Aegean region on live weight and body measurements were examined. In the study conducted on goats selected from two different herds, it was observed that various environmental factors play an important role in the growth and development of the animals. It was revealed that factors such as geographical conditions, pasture quality, and the care and feeding practices of the breeder are important factors affecting the productivity of the animals. The examination of polymorphisms in exon 10 of the GHR gene is also important in understanding the genetic structure of hair goats. The fact that the obtained findings were not statistically significant indicates that more comprehensive studies are needed. However, the least squares mean in the SNPs in the GHR gene revealed a tendency. It was concluded that larger data sets should be used at this point. In addition, the results found in the 4th exon of the GHR gene were found to be monomorphic. The PIC value of 0.26 obtained in this study indicates a moderate level of genetic diversity.

Deviations from the Hardy-Weinberg equilibrium indicate that selection to be made in goats will be effective. It is thought that such studies are important in terms of protecting local genetic resources and increasing the productivity of hair goats. Increasing breeders' awareness of breeding and continuing economic support will increase the success of breeding studies and contribute to increasing milk and meat yields in goats. It is thought that the findings obtained from this study are an important step for a better understanding of the genetic potential of goats and more effective breeding.

This study was supported by Ege University Scientific Research Projects Coordination Unit. Project Number: 23472 (16/12/2021, 06/06/2024).

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Repeatability model application in Anatolian Buffalo lactation milk yield by using Wombat software

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Abstract

The repeatability of traits, defined as the correlation between an individual´s performance across different years, serves as a crucial parameter in animal breeding. This parameter not only indicates the probability that traits measured in a given year will be consistently observed in subsequent years but also informs decisions regarding the necessity of multiple records for selection purposes. In this study, we aimed to demonstrate the estimation of repeatability for lactation milk yields in Anatolian buffaloes utilizing Wombat software, with data derived from the Çorum region. The dataset encompassed 2210 lactation records from Anatolian buffaloes collected between 2014 and 2022, as part of the community-based animal improvement project (TAGEM/19/MANDA2012-01). The results showed that estimated repeatability for milk yield were 0.695±0.017. These findings underscore the significance of repeatability in repeated measures, such as lactation yields, suggesting that targeted selection based on these traits holds substantial potential for enhancing production performance in Anatolian buffaloes.

Key words: *Anatolian buffalo, growth traits, (co)variance components, repeatability, lactation milk yield*

Growth curves of local and commercial chicken layers at 10 weeks old

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Abstract

Growth is an economically important trait in the animal production industry and is one of the subjects that can be justified mathematically. The development of growth curves is a powerful tool for establishing feeding programs and other management recommendations to improve feed conversion efficiency and increase productivity. However, the growth pattern of chickens is poorly understood. The study´s objective was to determine an appropriate non-linear model to describe the growth curve and examine whether there are breed differences in the growth parameters of Lohmann Brown (LB) and Potchefstroom koekoek (PK). Four (n = 40) unsexed chickens (20 per breed) were used. The body weight of chickens was collected once a week from hatching till 10 weeks of age The Gompertz, Richard's and Logistics were used to describe the growth curves. The goodness of fit criteria such as coefficient of determination (R2), root mean square error (RMSE), Bayesian information criterion (BIC) and Akaike information criterion (AIC) were used to observe the best model. The results showed that there was no significant difference (p > 0.05) between the body weight of LB and PK at hatching, week 1, 6 and 8. However, LB and PK had a significant difference (p < 0.05) at 2, 3, 4, 5, 7, 9, and 10 weeks. The growth curve results in PK, Gompertz model provided the best fit (R2 = 0.969, RMSE = 3872.98, AIC = 218.83, BIC = 221.81) followed by Logistics model (R2 = 0.969, RMSE = 3921.56, AIC= 219.08, BIC = 222.06). While in LB, the Gompertz model was the best fit (R2 = 0.952, RMSE= 4557.57, AIC = 221.13, BIC = 224.12) followed by Richard's model (R2 = 0.951, RMSE= 4656.72, AIC= 223.47, BIC = 227.44). The study suggests that at 10 weeks of age, the Gompertz model might be used to describe the growth of PK and LB chickens. Further studies need to be conducted on the growth patterns of the Lohmann Brown and Potchefstroom koekoek chickens till they reach maturity.

Key words: *Growth curves, Gompertz model, Richard's model, Logistics model*

Assessment of non-genetic factors affecting milk production in goats

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Abstract

Milk production in goats is influenced by both genetic factors along with non-genetic variables. The objective of the current study was to find out how non-genetic factors affect goats' characteristics related to milk production in southern Punjab province of Pakistan. The data about weekly milk yield of goats were collected along with non-genetic factors like parity, birth type, flock, and age of lactating goats up to drying off (ten weeks). A fixed effect model has been used for data analysis in which the average weekly milk yield and lactation milk yield were the dependent variables. The birth year, parity, birth type, and birth season, were independent variables. Co-variables included the lactation length and age of animals. There is a significant ($p \leq 0.001$) influence on weekly milk production in sixth, seventh, eighth, ninth, and tenth week as well as the overall yield on the flock. The effects of parity on the production of milk in each week were found to be non-significant ($p > 0.05$). In the first week, there was a significant ($p \leq 0.05$) correlation between the production of milk and TOB (type of birth) and during the next weeks, there was no significant ($p > 0.05$) correlation. The third, fifth, ninth, and overall weeks' milk production were analyzed when compared to the dam's age, which was found to have a significant ($p \leq 0.01$) effect. However, for this characteristic, the non-significant ($p > 0.05$) effect on all other weeks was shown by linear effect of age. In the third, fifth, and ninth weeks, the quadratic effect of age on the production of milk was studied for significant ($p \leq 0.05$) effects; in the other weeks, the effects were non-significant ($p > 0.05$).

Key words: *Goats, non-genetic factors, milk production, goat milk.*

INTRODUCTION

Milk is essential because it's healthy for the body. sixty-five percent of milk produced globally comes from goats. The annual milk production of goats worldwide is 15,510,411 tons. India is the country that produces the greatest quantity of milk. It makes up 18% of the milk produced worldwide. 94% of goats are situated in poor nations, and 73% of goat milk is produced there (Devendra, 1987). The number of days in milk, which indicates the lactation stage, determines the amount of milk produced (Swalve, 1995). For those who are allergic to cow's milk or other foods, goat milk has been used as a replacement. Patients with cow milk allergies can tolerate goat milk in 40–100% of cases. Thus, the value of goats as a milk supply is becoming more widely recognized. (Mia and others, 2014) The lactation curve,

which illustrates the milk yield rate, is a curve. From one kidding to next kidding, goats remain in lactating conditions. More milk was produced by crosses between Alpine and Saanen. Goats having two or more offspring and producing more milk showed substantial effects from year of kidding, season of kidding, and age (Marete et al., 2014). Factors both genetic and non-genetic affected milk production. Analysis was done using the effects of non-genetic variables on milk production. Analysis indicates that the month of calving has a major impact on milk production. Flock exhibits significant effects as well. Milk production was high up until parity, from first to second parity, and thereafter it became steady (Kunaka and Makuza, 2005). Mother milk is considered to be an essential component for the growth of the children (Zujovicet al, 2011). Goat milk is special because of its nutritional and commercial value. Breeding is an internal element, and food, age, seasonal fluctuations, body weight, parity and lactation stage and environmental variables are external factors that influence it. Goat milk's components and qualities are highly significant (Vondraskova et al., 2012).

As the research in this field was scarce in the region, therefore, the objective of the current study was to predict how non-genetic factors might affect goat milking.

MATERIALS AND METHODS

The current study was carried out on goat flocks found in southern areas of province Punjab, Pakistan. The other relevant information was recorded including identification number of goats, sex of kids born, date of kidding, parity and age of dam. ASREML software was used for the data analysis (using restricted maximum likelihood procedure). The study gave us an understanding of the impact of non-genetic factors on goat milk production in the context of the local environment. The following mixed effect models were used to further analyze the data after they had been analyzed for inconsistencies:

$Y_{ijkl}=\mu+YOK+SOK+TOB+sex+parity+age+\epsilon_{ijkl}$

Where Y_{ijkl} is collected data on milk production, Population mean=μ, Type of Birth=TOB, Kidding Season=SOK, Year of Kidding=YOK, Male or Female=Sex, Determination of age by dentition, One or Two=Parity, ϵ_{ijkl}= Each observation has a random error that is normally distributed.

RESULTS AND DISCUSSION

The current analysis indicates that the current flock of goat showed a significant ($p\leq0.001$) impact on milk production in 7^{th} 8^{th}, 9^{th}, and 10^{th} weeks as well as for the overall week. Additionally, a significant ($p\leq0.01$) influence was shown by the 6^{th} week in this characteristic for the flock. However, for remaining weeks, a non-significant ($p>0.05$) impacts were noted. The effects of parity on milk yield in every week were assessed to be non-significant ($p>0.05$). The first week's results for milk TOB (type of birth) showed substantial ($p<0.05$) effects on milk supply; for the next weeks, the results were non-significant ($p>0.05$). The third and fifth weeks of the study evaluated the significant ($p<0.05$) effects of age on milk yield, whereas the ninth and final weeks examined the significant ($p<0.05$) effects. However, the linear effect of age on this trait over all other weeks is non-significant ($p>0.05$). Three, five, and nine weeks of the study evaluated the significant ($p<0.05$) impacts of the quadratic effect of age on milk yield; the remaining weeks indicate a non-significant ($p>0.05$) impact for this characteristic.

Table 1. Analysis of variance

Milk yield for different weeks MY (milk yield)	Non-Genetic Factors affecting milk production in goats				
	Flock	Parity	TOB (type of birth)	Linear effect of Age	Quadratic effect of Age
Week1	NS	NS	*	NS	NS
Week2	NS	NS	NS	NS	NS
Week3	NS	NS	NS	**	*
Week4	NS	NS	NS	NS	NS
Week5	NS	NS	NS	**	*
Week6	**	NS	NS	NS	NS
Week7	***	NS	NS	NS	NS
Week8	***	NS	NS	NS	NS
Week9	***	NS	NS	*	*
Week10	***	NS	NS	NS	NS
Week (overall)	***	NS	NS	*	NS

NS=non-significant; *=significant; **=highly significant; ***=very highly significant

Effects of Flock on milk yield

Maldonado-Jaquezet al. (2018) found that the traits related to milk production for the Flock in the community were extremely significant (p≤0.01). The Laxta breed's overall milk production was significantly (p≤0.01) impacted by flock (Ruiz et al., 2000). Milk production was studied in a foundation flock consisting of 125 young Nubian female goats from Sudan. Bauer et al. (2012) reported that there was a highly significant (p≤0.0001) effect on test day milk production. A significantly significant flock effect was noted for the Chios sheep's milk production features (Mavrogeniset al, 1988). Waheed et al. (2011) and Waheed & Khan (2013) observed a significant flock effect (p≤0.01) on all of the lactation curves in Beetal goats. Important effects of Flock were reported that were comparable to our results (Goetschet al, 2011).

Impact of parity on goat milk production

The findings on the goat farms in Croatia and Sloveni shown the parity's significant impact on composition of milk and milk yield at test day (Klirzet al, 2015). A significant (p≤0.01) impact of the parity on the daily milk produced by Black Bengal goats when the genetic and phenotypic factors were analyzed (Mia et al., 2014). Parity significantly (p≤0.01) affected the overall production of milk (Ruiz et al., 2000). The mountain goat's parity number was noted. According to Kakous et al. (2015), parity number increased daily milk production. According to Komprejet al (2012), all milk characteristics showed a significant (p≤0.01) influence on parity. According to Gonzalo et al. (1994), the effect of parity on the production of milk was highly significant (p≤0.001). Parity was found to have a significant (p≤0.01) impact on daily milk output (Mia et al, 2014). Parity has a major impact on the production of milk (Crepaldiet al., 1999). In the second parity, there was a high production of milk (Ayyatet al, 2010). Parity had an impact on each factor taken into consideration. Goats in the fourth parity, in particular, had longer lactation periods and produced more milk as a result (Carnicella et al., 2008). Typically, milk production peaks at a parity of three or four and then decreases gradually from there (Goetschet al, 2011). Parity of does has significant effect for milk yield. Does of first parity produce less milk than those of 2nd and 3rd Parity (Zeng and Escobar 1995).

Present research Data showed the non-Significant effect of Parity upon weekly milk yield in goats. Among this Improper Feeding, Poor Body conditions Score, any Disease or other non-genetic factors might be the possible reasons for effect of non-Significant (p>0.05) Parity on milk yield.

Effects of TOB on milk yield.

The type of birth has a significant (p≤0.01) effect on the milk production that Beetal goats produce during lactation (Waheed et al., 2011; Waheed & Khan, 2013). According to analysis, the number of kidding's milk production traits were very significant (p≤0.01) (Maldonado-Jaquezet al, 2018). The type of kid's birth in Sudanese Nubian goats significantly affected the overall milk production (p<0.05). Black Bengal goats' parameters for daily milk yield was analyzed

to evaluate the genetic and phenotypic parameters where milk yield at daily basis was significantly ($p \leq 0.01$) influenced by kidding season (Mia *et al*, 2014). According to Muhammad et al. (2007), triplets do produce a lot of milk. According to Carnicella et al. (2008), the milk production was significantly ($p \leq 0.001$) influenced by goats giving birth to twins. Compared to twins, the single child's does have considerably higher total solid and fat percentages ($p < 0.05$). According to Merkhan and Alkass (2012), the effect of lactation stages was significant ($p \leq 0.01$) for every component of milk. According to Bagnick et al. (2015), nanny goats with multiple kids produce more milk than goats with just one or two kids. According to Gonzalo et al. (1994), there is a significant milk production observed with twin births. Early-year kidding produced a large production of milk (Crepaldiet al., 1999). The overall milk supply was significantly affected by the birth type (Kominakiset al., 2000). Lacteal yields were notably ($p < 0.01$) influenced by the size of the litter and the kidding season. According to Mahalet al. (2013), non-genetic elements should be used as a strategy to enhance the productive qualities.

Linear and Quadratic effect of age of dam on milk yield.

The results of the current study show that linear impact of age have highly significant effects on milk production in the third and fifth weeks. Milk production showed a significant ($p < 0.05$) influence on in the ninth week and in the overall week. The milk production trait was not influenced significantly by all other weeks. Analysis shows that the quadratic effect of age has a significant influence on milk yield in the third, fifth, and ninth weeks. All other weeks' quadratic effect of age was non-significantly ($p > 0.05$) influenced by milk yield. In this research, on several parameters of the lactation curve shape, significant interactions between genetic group age and genetic group season have been identified. The findings indicate that each genotype responds differently physiologically to the influences of age and season (Montaldo*et al*, 1997). Linear and quadratic Effects of age of cow and year of calving were significant and positive for Daily milk yield (Chew et al, 1981).

ACKNOWLEDGMENTS

The authors are thankful to NRPU HEC for providing funding opportunity under project #9977.

Statement of conflict of interest

Authors have declared no conflict of interest.

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Potential side effects of nitrogenous fertilizer on the Grey Worm (aporrectodea. spp) as a bioindicator species: morpho-histological approaches

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Abstract

The huge augmentation of food demand that accompanied along with the continuous human population growing and the emerging of mortal epidemic (Covid-19) recently. Make the reaching to global food demands sufficiency is an impossible matter. However, the only unique and faster solution that seems to become a decisive step is the usage of agrochemical products excessively such as; pesticides and chemical fertilizers in agricultural land as well as to increase crop yields. The overuse of chemical fertilizer in Algerian agricultural humid zone has resulted in adverse effect on soil properties over a long period. The effects are clearly seen in soil environment including soil biodiversity; which has undergone a major decrease in farmlands over the last decades. Therefore, much consideration should be paid to soil health and environmental safety of such humid areas. Invertebrates such as earthworms are used as a bio-indicator species, for monitoring the impact of soil pollutants; they are the most important actors in soil formation, maintaining its structure and fertility. The present study was undertaken to assess the potential hazards of nitrogenous inorganic fertilizer, commonly used in agricultural lands, on the morpho-histological level of the earthworm A. trapezoides. The sub-acute toxicity test (28 days) was conducted according to OECD guideline 222. Four increasing concentrations of chemical fertilizer were tested, based on 10, 30, 40, and 50% of 14 days's 50 LC value. Therefore, transverse sections from the preclitellar, clitellar, and postclitellar regions were performed. There were various morphological behavior abnormalities of tested earthworms after exposure (14/28 days). Integumentary lesions, clitellar swelling and loss of pigmentations were found to be major morpho-pathological changes in the worms. In addition, significant changes in the histological structure of tested earthworms were obviously noticed. Severe injuries along the whole region included damage of epidermal layers with vacuolation, damages and breakdown in both circular and longitudinal muscles with increasing concentration and time exposure, atrophied chloragogenous tissue of the midgut, and focal profusion of blood plasma with spread cell debris turning around the pharynx. These findings demonstrated that the histopathological data could be used to evaluate the environmental risk assessment of agrochemicals. As a result, it might be a useful biomarker for diagnosing and providing early warning of soil pollution.

Key words: *Histology, earthworm, ecotoxicology, chemical fertilizer, bio-indicator, morphology.*

Comparative analysis of commonly used statistical package programs

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Abstract

Statistical package programs are used for data analysis, statistical calculation, and graphic operations. In this study, SPSS, SAS, MINITAB and JMP statistical package programs were examined. SPSS can easily perform data entry, analysis, and reporting operations with its user-friendly interface, SAS is a program used in commercial companies and universities to provide data management and security and offers various tools to perform operations such as data storage, processing, analysis, and reporting. MINITAB is mainly used in areas such as production and quality control. JMP is used for data discovery and analysis with dynamic and interactive graphics and has a user-friendly interface for data visualization and discovery operations. The researcher or the person who will perform the analysis should first choose the most appropriate program for his/her needs. In this study, SPSS, SAS, MINITAB, and JMP statistical package programs were examined comparatively and the differences between them were revealed.

Key words: *SPSS, SAS, MINITAB, JMP, Statistical package programs*

INTRODUCTION

Statistics is a branch of science that is widely used in data analysis and conclusion-making processes in many areas. Statistical analysis is of great importance especially in many areas such as scientific research, industrial applications, economics, social sciences and health. Statistical analysis provides meaningful interpretation of large amounts of data and statistical support of the results.

Statistical packages are important tools widely used by researchers and analysts for conducting statistical analyses. These packages provide a range of statistical functions, graphing tools, and reporting features that facilitate data analysis. They also provide great convenience to users in data processing processes such as preparing, cleaning, and transforming data sets.

This study aims to provide a review and comparative analysis of widely used statistical packages.

MATERIALS AND METHODS

In this study, SPSS, SAS, MINITAB and JMP programs, which are widely used statistical package programs, were examined comparatively. The basic features of these package programs such as functionality, ease of use, data analysis capabilities, graphing tools, and reporting features were compared.

SPSS is a statistical software package developed for data management, advanced analytics, multivariate analysis, business intelligence, and crime investigation. Although it is a program designed for statistical analysis in social sciences, it is also a frequently used program in health and science. Being familiar with its interface, ease of use, flexibility and scalability make SPSS accessible to users of all skill levels (Anonymous, 2024a).

MINITAB is a software that specializes in statistical analysis and quality control analysis. It is software designed to facilitate statistical analysis and is used in many areas such as health, economy, marketing, production, supply chain management, R&D, sports, and data analysis control. MINITAB Statistics Software is designed to help students perform statistical calculations more easily and thus to be able to concentrate more on statistical applications (Karaokur et al., 2019; Anonymous, 2024b).

SAS is a software that has a wide range of analyses and can be used for various analyses. It is a software that provides statistical analysis, data extraction and

transformation, data updating and modification, and graphical reports. SAS is a software package that can extract, manipulate, manage, and perform statistical analysis on data from various sources. SAS provides a point-and-click graphical user interface and more for non-technical users through the SAS language (Keeling and Pavur, 2007; Anonymous, 2024c).

JMP is a software that focuses specifically on visual analysis. JMP software focuses in part on exploratory data analysis and visualization. It is designed for users to explore data to learn something unexpected rather than to validate a hypothesis. JMP combines statistical data with graphs that represent it, so users can drill down to explore the data and various visual representations. JMP is the statistical analysis program of JMP, a subsidiary of the SAS Institute (Anonymous, 2024d).

RESULTS

An analysis performed with a sample data set in the SPSS program is shown in Figure 1-3.

Figure 1. Data Entry in SPSS Program

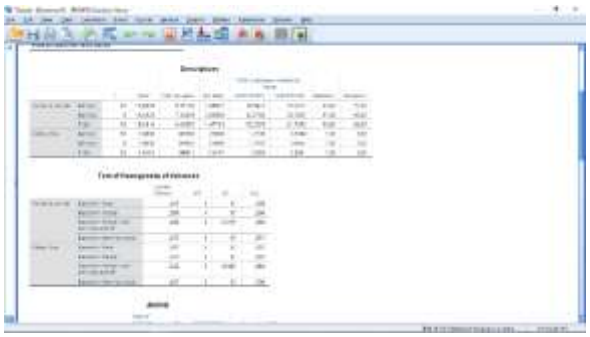

Figure 2. SPSS Analysis Results

Figure 3. SPSS Analysis Results (Cont.)

An analysis performed with a sample data set in the MINITAB program is shown in Figure 4-6.

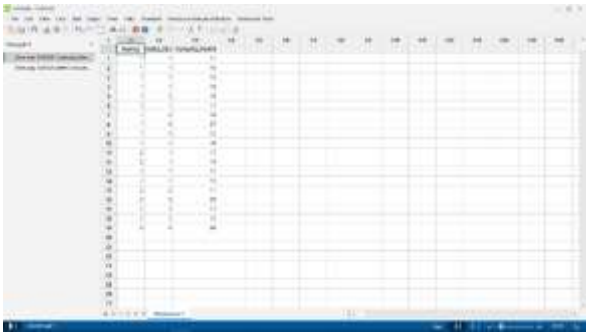

Figure 4. Data Entry in MINITAB Program

Figure 5. MINITAB Analysis Results

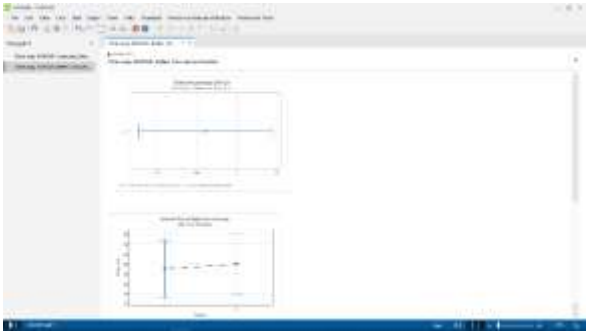

Figure 6. MINITAB Analysis Results (Cont.)

An analysis performed with a sample data set in the SAS program is shown in Figure 7-10.

Figure 7. Data Entry in SAS Program

Figure 8. SAS Analysis Results

Figure 9. SAS Analysis Results (Cont.)

Figure 10. SAS Analysis Results (Cont.)

An analysis performed with a sample data set in the JMP program is shown in Figure 11-12.

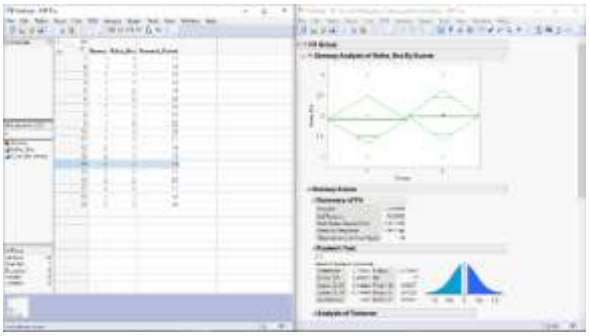

Figure 11. Data Entry in JMP Program

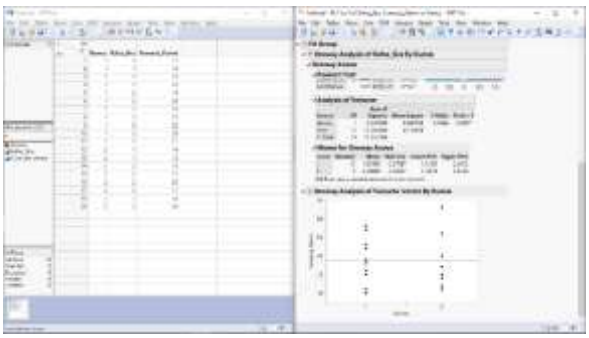

Figure 12. JMP Analysis Results

The differences between the different features of the programs are presented in Table 1-3.

Table 1. Supported operating systems.

	SPSS	MINITAB	SAS	JMP
Windows	+	+	+	+
MacOS	+	+	-	+
Linux	+	-	+	-
BSD	-	-	-	-
Unix	+	+	+	-
Cloud	+	+	+	-

(Wikipedia, 2024)

Table 2. Various regression methods are supported.

Method	SPSS	MINITAB	SAS	JMP
OLS	+	+	+	+
WLS	+	+	+	+
2SLS	+	-	+	-
NLLS	+	+	+	+
Logistic	+	+	+	+
GLM	+	_	+	+
LAD	+	-	+	-
Stepwise	+	+	+	+
Quantile	+	-	+	+
Probit	+	+	+	+
Cox	+	-	+	+
Poisson	+	+	+	+
MLR	+	+	+	+

(Wikipedia, 2024)

Table 3. Other Supported Features.

Analysis	SPSS	MINITAB	SAS	JMP
Descriptive Statistics	+	+	+	+
Non-parametric Tests	+	+	+	+
Quality Control	+	+	+	+
Survival	+	+	+	+
Discriminant	+	+	+	+

(Wikipedia, 2024)

Within the scope of the study, the basic features and analysis options of each program were examined. SAS proved to be a powerful tool for complex data analysis by offering a wide range of analyses. MINITAB stands out as a preferred program, especially for quality control analysis. SPSS was determined as a widely used option for statistical analysis with its wide user base and user-friendly interface. JMP is known for its visualization and data exploration capabilities (Keeling and Pavur, 2007).

DISCUSSION

As a result of this study, it was concluded that when deciding which statistical package program to use, the researcher should consider factors such as the research purpose, the complexity of the data set, and the analysis requirements. Each program has its advantages and limitations. Therefore, it is recommended that researchers choose the most appropriate program by considering their analysis needs. This study guides researchers in making the right choice among these programs.

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Validation of molecular tools for sex determination in Coho salmon using Crispr/Cas12.

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Abstract

Sex determination is a vital aspect when managing aquaculture populations. This is particularly crucial in species with delayed sexual maturation, where no signs of external sexual dimorphism are evident in the early stages. The main goal of this research is to validate small indels at the pseudogene GH2 on chromosome 10 at the whole-genome level using genome-wide association analysis. A custom array for Coho salmon was developed, and whole-genome sequence data from independent commercial and natural populations were utilized for validation. It was discovered that chromosome 30 exhibited the highest number of significant associations between SNPs and sex. Many genes on chromosome 30 displayed differential expression between male and female immature gonads. A series of SNPs in coding regions on a single gene, si:dkey-181m9.8, an ortholog of Salmo salar, E3 ubiquitin-protein ligase (RNF31) located in the telomere of chromosome 30, were found. These SNPs demonstrated the expected segregation of the sex determination system in this species, with genotypes following the XY/XX (male/female) pattern. Interestingly, the segregation pattern of the SNPs mirrored the patterns observed in GH2 on a different chromosome. While the gene did not exhibit differential expression between immature female and male gonads, it is part of a cascade known to play a central role in ubiquitination that regulates spermatogenesis by regulating DAX. Several other genes in various chromosomes suggested the polygenic nature of sex determination in this species. A rapid test using CRISPR/Cas12 was developed for classifying male Coho salmon using indels within the pseudogene GH2 on chromosome 10. This CRISPR/Cas12 test outperformed high-resolution melt (HRM) analysis as it does not necessitate the use of specialized software to model HRM curves and control for each sex every time the samples are processed. It is believed that this type of test can be swiftly adopted in other species of the genera due to its simplicity and ease of use, and similar tests could be used to determine sex in other closely related species that have been studied using genomic data.

Key words: *Seriola genomics*

Study of the toxicity of iron oxide nanoparticles and Al2o3 NPS in a bioindicator of environmental pollution, the Snail Helix Aspersa «L´Escargot Helix Aspersa»

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Abstract

The current study aimed to evaluate the hazardous impact of Fe2O3 NPs and Al2O3 NPs on bio-accumulating organisms and bio-indicators of land pollution. Specifically, the focus was on Helix aspersa, a species that is consumed by humans and has direct exposure to the environment. The therapy involved administering escalating dosages of Fe2O3 NPs and Al2O3 NPs (100, 500, 1000, 5000 µg/g) in the feed (wheat flour) for a duration of 4 weeks alongside a control group. Their effects have been examined using a focused methodology in the laboratory, where many parameters were assessed, including the biochemical metabolites (carbohydrates, lipids, and proteins), as well as the evaluation of specific biomarkers of oxidative stress (GST, GSH, CAT, and GPx) in the hepatopancreas. The results demonstrated that both treatment types had toxic effects. Specifically, the biochemical analysis revealed an elevation in protein, carbohydrate, and total lipid levels. Additionally, the antioxidant analysis indicated the presence of oxidative stress and activation of the detoxification system, as evidenced by increased levels of GSH, GST, GPx, and catalase activity. There is a variation in the level of effect, but it can be stated that the toxicity of iron oxide at the nanoscale is equally severe as its toxicity at the nanoscale of Al2O3.

Key words: *Comparison, Helix aspersa, Fe2O3, Al2O3, nanoparticles.*

Some reproductive defects in farm animals

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Abstract

The importance of animal products for human health and development cannot be ignored. It is important for the sustainability and profitability of the enterprises that the animals raised do not have problems in terms of reproduction, as well as in terms of meeting the demand for animal products regularly. Depending on the type of animals used in production, the number of offspring obtained in a production period varies according to the reproductive activities of the animals and the care and management methods of adding the obtained offspring to the economy. In terms of reproductive efficiency in animal production enterprises, few or many reproductive problems seen in animals do not completely prevent the realization of reproductive performance at the optimal level. The formation of a new individual in farm animals covers the process until birth, such as the formation of gamete cells, fertilization, embryo implantation and fetal development. At the same time, the postnatal factors that take place from the birth of the new individual to its entry into the economy are also part of this process. Depending on the animal species, rates of embryo losses, genetic defects, losses due to disease and losses due to care and feeding errors can vary. The care that owners take in the selection of animals and the care and management methods they apply to them is decisive in reducing possible losses, obtaining more offspring and ensuring the profitability and sustainability of the business. In this review, it is aimed to explain the problems such as embryo losses, fetal losses, loss of offspring during and after birth and the precautions that can be taken against these problems in the process from the beginning of the formation of a new individual in livestock breeding until the new individual is brought into the economy.

Key words: *Reproductive disorders, Embryo losses, Care-management errors, Offspring losses*

INTRODUCTION

Reproductive health of farm animals is one of the cornerstones of the livestock sector. The reproductive health of farm animals is important for the sustainability and profitability of farms and for meeting the demand for animal products regularly. However, reproductive defects are a serious problem that is frequently encountered in farm animals and can occur for various reasons. Reproductive defects reduce the reproductive efficiency of animals, decrease the survival rate of offspring and may adversely affect their general health status. This situation causes economic losses in farm enterprises and threatens animal welfare.

Reproduction in farm animals is a vital trait for sustainability, product continuity, business profitability and animal breeding studies. As a result of increasing the amount of product obtained per unit animal, reproductive results have started to experience disruptions.

In animal production, the main objective is to ensure that reproductive activities are carried out regularly and smoothly, that each gamete cell, which has the potential to be born alive as a new individual, develops without loss and is brought to the economy. In order to achieve this goal, losses should be avoided or minimized at all stages of reproduction and from the birth of a healthy individual until it is brought into the economy. When reproductive losses are analyzed, it is reported that the highest rate (20%-50%) occurs during the acceptance of the embryo by the maternal (Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Campanile et al., 2021). In developing reproductive biotechnologies, it is reported that losses in in-vitro embryo production are up to 60%-70%, and pregnancy rates in in

vitro produced embryos vary between 10%-40% (Ealy et al., 2019).

Embryo losses prior to maternal acceptance vary by species and within species by breed. The lack of satisfactory progress in reducing pre-implantation embryo losses is due to a poor understanding of the functioning of this period (Campanile et al., 2021).

Reproductive defects can be caused by genetic factors (Berry et al., 2017; 2018), environmental factors, inadequate care and feeding conditions and infections (Ali et al., 2015; Akbarinejad and Robert, 2024). Genetic factors include structural abnormalities and hereditary diseases. Environmental factors that cause reproductive defects include hygienic conditions of the environment, climate change and stress factors. Nutrition is an important factor that directly affects reproductive health. Nutritional deficiency can impair the functions of reproductive organs and reduce reproductive performance. Infections, on the other hand, can cause reproductive disorders and infertility by directly affecting reproductive organs (Ali et al., 2015; Akbarinejad and Robert, 2024).

The formation of the new individual in livestock includes processes starting from the production of gametes and continuing until parturition, such as fertilization, embryo implantation and fetal development (Uju and Unniappan, 2024). At the same time, postnatal factors, which continue until the parturition of the new individual and its introduction into the economy, are also part of this process. Depending on the animal species, rates of embryo losses, genetic defects, losses due to disease and losses due to care and feeding errors can vary (Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Ealy et al., 2019; Campanile et al., 2021). The care of the owners in the selection of the animals and the care and management methods they apply to them is decisive in reducing possible losses, obtaining more offspring and ensuring the profitability and sustainability of the business.

In this review, it is aimed to present the problems such as embryo losses, fetal losses, loss of offspring during and after birth and the measures that can be taken against these problems in the process from the beginning of the formation of a new individual until it is brought into the economy.

FEMALE REPRODUCTIVE SYSTEM AND OFFSPRING YIELD

The formation of a new individual in farm animals begins with the coming together of mature female and male gamete cells in a suitable environment. The production of mature female and male gamete cells takes place in healthy individuals that reach sexual maturity and do not have anatomical and physiological problems in the reproductive system (Lea and England, 2019). In normal production systems, errors in the care and feeding conditions of male and female individuals affect sexual maturity and cause a decrease in the number of offspring to be obtained from an individual for a lifetime. In the process following the fusion of gametes (fertilization, embryo), the primary responsibility for the survival, development and birth of the new individual depends on the female individual who carries it as a zygote, embryo and fetus (Lonergan et al., 2023). Here, the possible situations that can be encountered in the process starting from the gonad structures responsible for the production of gametes related to each sex and until the age of the offspring's economicization after birth will be evaluated.

Dysfunction of the ovaries

Ovarian dysfunctions in farm animals are important health problems affecting the reproductive ability of female animals. These disorders reduce fertility rates by preventing the production of sufficient and healthy eggs from the ovaries. Ovarian dysfunctions can occur as a result of the combined effects of one or more of several causes such as disease, infection (Davis, 2019), nutritional deficiencies and imbalances (Ali et al., 2015), including temperature increase due to climate change (Chen et al., 2021).

Ovarian Cysts

Ovarian cysts are a common ovarian disorder in farm animals. These cysts are fluid-filled sacs that grow abnormally in the ovary and adversely affect the reproductive cycle. Clinical signs vary depending on the

number of cysts and the degree of luteinization. Ovarian cysts are more common in dairy cattle and reproductive problems in dairy cattle triple when milk production doubles (Lonergan et al., 2023; Steeneveld et al., 2024). Ovarian cysts are rare in sheep and goats. Ovarian cysts cause impaired ovarian function due to disruption of the hormonal mechanism (Viana et al., 2021), frequent and irregular estrus and constant desire to mate in animals (Kaymakçı, 2012; Roy et al., 2024).

Ovarian Hypoplasia

Ovarian hypoplasia is a condition in which the ovaries are smaller and underdeveloped than normal. Ovarian hypoplasia can be found in the ovaries of farm animals, either unilaterally or bilaterally. Depending on the degree of hypoplasia and whether it is unilateral or bilateral, the animal may be infertile or sterile. This is a serious condition that negatively affects the animal's reproductive capacity and general health. It may occur due to genetic factors, embryo development, hormonal imbalances and nutritional deficiencies (Kaymakçı, 2012; 2016; Rhoads, 2023; Akbarinejad and Robert, 2024).

Freemartinismus

In twin or triplet pregnancies, when one of the offspring is male and the other female, various developmental anomalies can be observed in the female offspring's reproductive organs. This is due to the more rapid development of the male offspring's testicles and the hormonal effect on the development of the female offspring's ovaries and other reproductive tracts. Female offspring in this situation are called freemartins (Kaymakçı, 2016; Bozkurt et al., 2024).

Freemartinismus is especially common in cattle, but similar conditions can also be seen in other farm animals (Kaymakçı, 2016; Özhan et al., 2012). The incidence of freematinusmus in sheep ranges from 0.23% to 1.23% (Davis, 2019).

Fertilization Disorders

Fertilization is the first phase of reproduction and takes place when the sperm cell and the egg cell successfully unite. Disorders that occur in this process are serious problems that negatively affect reproductive efficiency and lead to economic losses (Hafez and Hafez, 2013; Lonergan et al., 2023; Steeneveld et al., 2024). Fertilization disorders are generally divided into two main categories: failed fertilization and abnormal fertilization.

Fertilization failure can be caused by the death of the egg before it meets the sperm, structural and functional abnormalities in the egg, poor sperm quality or obstruction of the oviduct. Abnormal fertilization, on the other hand, can result from a variety of causes that interfere with the normal fertilization process and prevent the development of a healthy embryo (Hafez and Hafez, 2013).

White heifer disease

White Heifer Disease is an anatomical defect characterized by various degrees of developmental delay of the Müller ducts (Ishiyama et al., 2019). This condition manifests itself in anomalies such as a perforated or partially closed hymen, absence of the cervix or the cranial part of the vagina. In addition, other developmental disorders such as unilateral development of the uterus can be observed (Kaymakçı, 2012; Ishiyama et al., 2019). In a study conducted with Holstein cattle, it was reported that defects related to Müller duct (fusion, obstruction) development occurred at a level of 2.09% (Ishiyama et al., 2019).

Failure of oviduct development

It is defined as a developmental disorder related to the reproductive system in farm animals. It has been observed that some or all of the oviduct of animals in this condition does not develop (Kaymakçı, 2012; Ishiyama et al., 2019). As a result of breeding efforts for high milk yield in dairy cattle, it has been reported that problems related to ovarian tract development deficiency have increased (Mee, 2012; Ishiyama et al., 2019; Steeneveld et al., 2024). This result reveals the necessity to implement production models that provide a balance between productivity increase, health and welfare in animals (Galioto et al., 2017; Segerkvist et al., 2020; Whatford et al., 2022).

Vulva Atresia

The vulva is markedly reduced in size, creating an impediment to copulation. This usually makes it impossible for even pregnant individuals to give birth. Such

animals cannot give birth normally even if they become pregnant (Kaymakçı, 2012).

Delay of ovulation

In normally cycling animals, the time of ovulation can be altered due to many factors such as temperature stress (Rhoads, 2023), sudden changes in nutrition, dehydration, disease and sudden hormonal imbalance. Such situations can lead to failure of inseminations. Delayed ovulation is mostly hormonal and is due to insufficient secretion of LH. In some cases, oval-bursal adhesions may completely cover the ovary and prevent ovulation (Kaymakçı, 2012; Rhoads, 2023).

Embryonic losses and Fetal losses

Most reproductive defects in farm animals result in pregnancy loss. Pregnancy loss can be divided into embryonic and fetal (Hafez and Hafez, 2013).

Embryonic death is one of the important phenomena that usually cause temporary infertility in farm animals (Kaymakçı, 2012). Embryonic death refers to the death of fertilized eggs and embryos until the end of implantation. In farm animals, approximately 25% to 40% of embryos are lost depending on the species. These embryonic losses are often unrecognized by breeders and the dead embryo is usually resorbed by the organism and absorbed into the body (Hafez and Hafez, 2013).

The fetal period refers to the period during which the embryo implants and continues to develop in the uterus until it leaves the uterus at birth. Losses that occur during this period occur at a later stage than embryo losses and are usually characterized as miscarriage or premature birth. Fetal losses can significantly reduce the reproductive efficiency of animals and cause serious economic losses on farms. Fetal mortality is lower than embryonic losses and varies between 3-5% depending on the species (Çam et al., 1998; Dixon et al., 2007; Abdel-Mageed and Abd El-Gawad, 2015; Koyuncu and Duymaz, 2017; Ealy et al., 2019; Campanile et al., 2021).

Perinatal and postnatal losses of offspring

Losses of offspring during the perinatal period

Perinatal losses are defined as the death of offspring shortly before birth, during birth or within 7 days of birth. Perinatal losses, including stillbirths, account for a large proportion of losses between birth and weaning. Offspring mortality during birth can be as high as 30%, and 80-90% of deaths occur within the first 7 days after birth (Celi and Bush, 2010; Koyuncu and Duymaz, 2017; Hafez and Hafez, 2013; İder and Ertürk, 2023). Offspring losses during and after birth on farms are an indicator of animal welfare problems and represent a significant economic loss (Koyuncu and Duymaz, 2017).

The birth process is a stage that carries vital risks for both maternal animal and offspring. Complications that occur during parturition can lead to the loss of the female animal during parturition or stillbirth of the offspring. This can vary depending on the difficulty of labor, the structure of the birth canal, the size of the offspring or the general health of the female animal.

Difficult parturition (dystocia) in animals is defined as a situation in which parturition does not take place within a certain, species-specific period of time, is delayed or cannot take place without any intervention (Atasever et al., 2017). Dystocia can be caused by fetal and maternal factors.

Fetal dystocia can be caused by various reasons such as fetopelvic incompatibility, offspring position problems, offspring abnormalities, birth canal problems, maternal health problems, inadequate delivery assistance and multiple litters (Hafez and Hafez, 2013).

Maternal dystocia in farm animals is a condition in which the birth process becomes difficult due to functional disorders of the maternal birth canal or reproductive system during labor. This can be caused by factors such as narrowing of the maternal birth canal, inadequate contractions, birth position problems, genital infections or uterine abnormalities (Hafez and Hafez, 2013; Jacobson et al., 2020).

Postpartum litter losses are often closely associated with low or high condition scores, poor maternal behavior, inadequate colostrum intake, infectious diseases and environmental factors. Against these factors, the breeder is more likely to intervene in offspring survival (Çam et al., 1998; Celi and Bush, 2010; İder and Ertürk, 2023; Hafez and Hafez, 2013). The pre-weaning mortality rate

after live birth varies worldwide between 8% and 30% in lambs and 11.5% to 37% in kids (İder and Ertürk, 2023).

Postnatal offspring losses

Postnatal offspring losses are deaths from the end of the perinatal period until weaning (Koyuncu and Duymaz, 2017). The losses of offspring during this period are usually caused by factors such as postnatal trauma, environmental conditions, inadequate colostrum intake, poor maternal behavior, infectious diseases and malnutrition.

MALE REPRODUCTIVE DEFECTS

Male reproductive efficiency in farm animals is associated with several phenomena. These are sperm production, sperm viability and fertilization capacity, sexual desire and mating ability. Infertile males can be easily detected, but males with low reproductive efficiency can pose serious problems and cause economic losses for breeders and the artificial insemination industry (Hafez and Hafez, 2013).

Male reproductive defects in farm animals are caused by various genetic, anatomical and environmental factors that can significantly reduce fertility. These disorders negatively affect not only animal welfare but also production efficiency.

Cryptorchidism

It is a condition in which one or both testicles do not descend to their normal position but remain in the abdomen. It is a hereditary defect seen in farm animals. This condition negatively affects spermatozoite production and can lead to infertility (Hafez and Hafez, 2013; Olğaç and Sabuncular, 2023).

Since body temperature prevents the formation of viable spermatozoites, an animal with both testes in the abdominal cavity is infertile. If one of the testes has descended into the scrotum, this male animal is capable of fertilization. It has been observed that sexual desire persists in animals with cryptorchidism even if both testes have not descended into the scrotum (Kaymakçı, 2016).

Testicular Hypoplasia

It is a condition in which the testicles do not reach normal size or do not develop fully. Testicular hypoplasia, which is a congenital defect, is seen in all farm animals, especially in bulls of various breeds. This condition may cause a decrease in sperm production or infertility (Kaymakçı, 2016; Hafez and Hafez, 2013).

Testicular Degeneration and Atrophy

They are common reproductive disorders in farm animals and can negatively affect the fertility capacity of the animal. The germinal epithelial cells in the testis are highly sensitive to various factors such as temperature, infection and trauma. Due to this sensitivity, testicular degeneration and atrophy can be seen in male animals in different sizes according to their etiologies (Ladds, 1993; Roberts, 1986; Watt, 1972). Depending on the etiology, testicular degeneration may be mild or severe, focal or diffuse, unilateral or bilateral. Depending on the duration, severity and type of this condition, degeneration may have temporary or permanent negative effects on reproductive functions (McEntee, 1990; Watt, 1972; Younguist, 1997).

Impotence

Impotence refers to the situation in which spermatogenesis takes place in some farm animals, but the testicles do not produce enough testosterone so that the animals show no desire to mate. This situation manifests itself in the animals avoiding the search for females in heat or remaining indifferent to them (Kaymakçı, 2016; Hafez and Hafez, 2013; Pickett et al., 1977).

CONCLUSIONS

Reproductive health in livestock is of paramount importance for the sustainability and profitability of the livestock sector. Genetic, environmental and nutritional reproductive defects can adversely affect male and female reproductive efficiency and cause embryo, fetal and offspring losses. The causes and effects of reproductive defects can differ at each stage of this process.

Fertilization is the first stage of the reproductive process, resulting from the successful union of spermatozoon and oocyte. However, poor sperm quality, inadequate sperm motility or reduced sperm count in male animals and ovulation

problems and reduced fertilization ability of the oocyte in female animals can negatively affect this process. These problems can be caused by genetic factors, hormonal imbalances, infections or environmental stressors. In particular, extreme temperatures, nutritional deficiencies or poor care conditions can negatively affect fertilization success. In order to prevent fertilization problems, it is necessary to optimize the nutritional and care conditions of the animals and to perform genetic evaluation and reproductive health checks regularly.

After fertilization, embryo development begins, but various defects and losses can occur during this process. One of the most common causes of embryo loss is genetic disorders. In addition, hormonal imbalances, infections and nutritional deficiencies can also lead to embryo loss. In order to reduce embryo loss in farm animals, infections affecting reproductive health should be controlled, animals should be properly fed and protected against stress.

The fetal period refers to the period during which the embryo continues to develop in the womb. Losses that occur during this period occur at a later stage than embryo losses and usually manifest as miscarriage or premature birth. Fetal losses can be caused by genetic abnormalities, nutritional deficiencies, infections, trauma or hormonal imbalances. It is common in farm animals and can be associated with uterine infections or nutritional deficiencies during pregnancy. To prevent fetal loss, close monitoring of animals during pregnancy, appropriate care and nutrition programs, and prevention and treatment of infections are essential.

Parturition is a life-threatening stage for both maternal and offspring. In order to prevent losses during birth, it is necessary to closely monitor the birth process and intervene when necessary. Veterinary supervision plays a major role in reducing the risks. In addition, prenatal care can be effective in preparing the animal for delivery and preventing complications.

The postpartum period is a critical period for the health of the newborn offspring and the maternal. Protection of newborn offspring in the postpartum period can be achieved by strengthening their immune system and proper nutrition. Therefore, careful postnatal care and monitoring the health of the maternal play a critical role in preventing losses.

As a result, careful and informed management of all stages of the reproductive process in farm animals is essential to protect animal welfare and minimize production losses. By optimizing reproductive health, minimizing genetic and environmental risks, improving nutrition and care conditions, losses before and after birth can be significantly reduced. In this way, the sustainability of farms can be ensured by improving animal welfare, while at the same time the profitability and productivity of enterprises can be maintained in the long term.

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Morphophysiological characterization of ZnO nanoparticle toxicity in the Snail Helix Aspersa, an environmental pollution bioindicator

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Abstract

This study aimed to evaluate the influence of zinc oxide (ZnO) metal nanoparticles on the body of Helix aspersa snails, which are known as bioaccumulators and bioindicators of environmental pollution and human health. This study investigates the harmful effects of a substance over a period of 28 days, focusing on its absorption through the digestive system. The toxicity of ZnO is assessed in the snail Helix aspersa by a bioassay done in laboratory animals subjected to escalating quantities of ZnO (500, 1000, 5000, 10000, 15 000 µg / g). The initial findings indicate that the presence of metallic nanoparticles ZnO resulted in a dose-dependent inhibition of growth. The estimated concentrations that reduced the growth rate by 50% (EC50), 75%, 90%, and 100% were calculated for a duration of 4 weeks. The values are as follows: During the first week, the EC50 value was 3842.33 µg/g, the EC75 value was 9134 µg/g, the EC90 value was 12309 µg/g, and the EC100 value was 14425.67 µg/g. In the second week, the EC50 value was 1727.11 µg/g, the EC75 value was 4560.44 µg/g, the EC90 value was 6260.44 µg/g, and the EC100 value was 7393.78 µg/g. Moving on to the third week, the EC50 value was 2624.75 µg/g, the EC75 value was 4529.75 µg/g, the EC90 value was 5669.75 µg/g, and the EC100 value was 6429.75 µg/g. Finally, in the fourth week, the EC50 value was 2403.3 µg/g, the EC75 value was 4308.3 µg/g, the EC90 value was 5448.3 µg/g, and the EC100 value was 6208.3 µg/g. Furthermore, the NOEC (No Observed Effect Concentration), which is the greatest concentration with no discernible impact, is 1000 µg/g. Conversely, the LOEC (Lowest Observed Effect Concentration), which is the lowest concentration that had an effect, is 5000 µg/g. The growth inhibition in the snail Helix aspersa is manifested by both weight loss and a decrease in shell weight. This effect is directly proportional to the dosage of the treatment and is accompanied by a greater death rate.

Key words: *Helix aspersa, ZnO, metal nanoparticles, bioaccumulation, health, status indicator, growth.*

Genetic differentiation of edilbay sheep breed

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Abstract

Domestic sheep Ovis aries Linnaeus, 1758 is widely recognized and economically important livestock species that have been domesticated for thousands of years ago. They are valued for their versatile purposes, including wool production, meat consumption, and as a source of dairy productions. Kazakhstan is the largest Central Asian country leading in sheep farming, which is since historical times has been part of the national economy, providing raw materials and food products, and which is matching the harsh climatic conditions and national lifestyle of the country. Edilbay fat-tailed sheep characterized by well-adapted year-long grazing abilities to accommodate different types of vegetations, and being an only descendant of the most ancient representatives of Ovis living in Central Asia. Nowadays, Kazakhstani fat-tailed sheep breeds representing a blank spot in genomic study, as well as lacking of genetic characterization on a population level throughout the country. Current work represents the primary genomic data based on sequences of cytochrome b (cyt b) gene segment of mitochondrial DNA (mtDNA) obtained from the population of Edilbay breed from North Kazakhstan region (LLP "Birlik" farm). Neighbor-joining haplotype network and Bayesian phylogenetic analysis involved the individuals of Ovis aries available from GenBank (n=27) and data from this study (n=30) and outgroups: Ovis ammon and Ovis vignei. The results showed the subdivision of the Edilbay breed from European and East Asian haplogroups (PP=87%), which may evidence of the separate evolutionary history. Haplotype diversity (0.867±0.013), and nucleotide diversity (0.0143±0.0011) was registered for the studied population respectively. Neutrality test yielded the significant statistical values (Tajima`s D = -1.987; Fu`s Fs = -18.129; P≤0.05), while mismatch distribution analysis demonstrated a unimodal curve with non-significant values of Raggedness index Rg = 0.018, and sum of squared deviations SSD = 0.001 respectively. Overall, the Edilbay breed of fat-tailed sheep characterized by relatively high genetic diversity and recent population expansion, which can be explained by the extensive breeding measurements in the agricultural segment of the country. Moreover, this work will contribute to the sheep husbandry of Kazakhstan by mitigating the genetic data of breed, as well as will improve the understanding of phylogenetic relationships within sheep breeds on a large scale. This study was supported by a project: Analysis of phylogenetic relationships and evolutionary demographic events of fat-tailed coarse-wool sheep breeds raised in Kazakhstan (project no: AP19577108).

***Key words**: Ovine, mtDNA, Kazakhstan, genetic variability*

Effectiveness of fish oil and microalgae additives on Eicosapentaenoic Acid and Docosahexaenoic Acid concentrations in chicken eggs

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Abstract

Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA, 22:6) are necessary fatty acids in the human brain, cerebral cortex, retina, and skin components, and they can be formed from omega-3 fatty acids in the body. EPA and DHA can be obtained from marine fish and microalgae. This study aimed to reveal the effects of fish oil (FO) and microalgae EPA and DHA on laying hen diets on the egg EPA and DHA fatty acid compositions. For this purpose, 36 Super Nick Chick white laying hens at 54 weeks of age were divided into 3 treatment groups with 12 replicated. The experimental groups were as follows; T1: Control (sunflower oil, 3%), T2: Fish oil 3%, and, T3: Microalgae (MALG) group 1.5% +sunflower oil 1.5% were added to laying hen diets. Egg samples were collected at 28, 56, and 84 days, and egg yolk fatty acid composition was analyzed using gas chromatography. On d 28, the EPA ratio in the C group was higher than in fish oil and MALG-added groups and MALG groups EPA ratio was highr than FO group (P<0.01) and there were no significant differences at 56 and 84 in terms of EPA ratio %. The FO and MALG groups the DHA ratios were higher than that of the C group at 28, 56, 84, and in the overall period. (P<0.01). In the overall period, the C group's EPA ratio was higher than those of the FO and MALG-added groups (P<0.01). There were no significant differences among the FO and MALG groups in terms of EPA and DHA concentrations in egg yolks. In conclusion, FO and MALG addition can increase the DHA ratio but is not effective in increasing the EPA ratio in hen egg yolk.

Key words: *chicken egg, microalgae, fatty acids, DHA, EPA, sustainability*

INTRODUCTION

The ω-3 FAs are important for the body and must be received through diet because the body is unable to produce them. Dietary ω-3 FAs are integrated into the cell membranes of all tissues. Different ω-3 FAs are essential in a well-maintained ratio for a variety of developmental, physiological, biological, and health-promoting activities (Richardson, 2006). The ω-3 FAs are important nutrients that support the growth health and production of poultry (Simopoulos, 2011; Arias-Rico et al., 2018; Cherian, 2015; Lee et al., 2019).

Marine-derived oils which rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, 22:6) content and they may be used efficiently for egg fortification, rather than alpha linolenic acid (ALA). Previous research revealed that EPA and DHA concentration in eggs may increase with 120 mg EPA and DHA supplementation from fish oil (FO) for 21 days when compared to 120 mg α-ALA supplementation from flaxseed per 100 g feed (Lemahieu *et al.*, 2015). Zhao *et al.* (2021) noted that the inclusion of 15 g/kg FO and 15 and 30 mg/kg krill oil inclusion to the diet caused an increase in egg's approximately 300 mg of EPA and DHA. Compared to krill oil addition, FO supplementation increased the retention of EPA and DHA in eggs. Another experiment showed that a 4 g/kg FO addition could increase 200 mg/kg of DHA in egg yolk (Cachaldora et al., 2014).

This study was conducted to determine the effects of adding FO, and DHA-rich

microalgae to laying hen diets on the egg yolk fatty acid composition of laying hens.

MATERIALS AND METHODS

Animals, experimental design and diets

This project's ethical issues have been assigned by the Erciyes University Institutional Animal Care and Use Committee (protocol 03.05.2023, no 23/101). In the experiment, a total of 36 Super Nick Chick white 54-week-old egg-laying hens were used. Animals were allocated into 3 different groups with 12 replicas in individual cages. The experimental groups were as follows; T1: Control (C, sunflower oil, 3%), T2: Fish oil 3% (FO), and, T3: Microalgae (DHA Gold) 1.5% Sunflower oil 1.5% (MALG) were added to laying hen diets for 98 days.

Before the experiment began, the animals were given a 14-day adaptation period. The body weight and egg production of animals was recorded and considered to minimize differences among the treatment groups.

The experimental feeds were produced from a similar nutrient composition (protein, energy, vitamins and minerals, and amino acids) for laying hens. Two ratios were calculated and differences in oil types (sunflower, fish oil) and microalgae+sunflower oil. The feed material and chemical components are provided in Table 1. Feeds were sealed with plastic caps and stored in a shaded area with the lid closed.

The hens were housed in individual laying hen cages (L45xW32xH35 cm) with 4 floors and 3 cages in every floor (4*3= 12 cages each block). The partitions were placed between the feeders to prevent the next chick feed consumption. The bird's room was a semi-controlled environment. The cages have contained one nipple drinker in each.

Table 1. Composition of diets

	Groups		
	Sunflower oil	Fish oil	Microalgae
Yellow corn	500.00	500.00	500.00
Wheat	33.97	33.97	30.00
Sunflower meal	120.00	120.00	120.00
Soybean meal 46	151.69	151.69	150.48
Vegetable oil[§]	30.00	30.00	26.00
Salt, NaCl	3.19	3.19	3.2
Vitamin and mineral premix*	2.00	2.00	2.00
Di-calcium phosphate	4.8	4.8	4.3
Limestone	94.15	94.15	94.47
Methionine	0.55	0.55	0.55
Multi-enzyme	0.5	0.5	0.5
Wheat bran	59.15	59.15	58.5
Microalgae	0	0	15
Total			1000
Chemical composition**			
Dry matter, %	92.02	92.16	92.61
Crude protein, %	17.00	17.00	17.00
Crude fat, %	5.37	5.38	5.38
Methionine, %***	0.36	0.36	0.36
Lysine, %***	0.77	0.77	0.77
Calcium, %***	3.70	3.70	3.70
Available phosphorus, %***	0.36	0.36	0.36
Metabolic Energy Kcal ME/kg****	2730	2730	2730

*Vitamin-mineral premix per kilogram of the diet; Vitamin A, 15,000 IU; Vitamin D3 2000 IU; Vitamin E, 40.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin K, 5.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Vitamin B2 (riboflavin), 6.0 mg; Biotin, 0.1 mg; Niacin, 30.0 mg; Folic acid, 1.0 mg, Calcium D-pantothenate, 12 mg; Choline chloride, 400 mg, Iron, 35.0 mg; Manganese, 80.0 mg; Zinc, 50.0 mg; Iodine 2.0 mg; Cobalt, 0.4 mg; Copper, 5.0 mg; Selenium, 0.15 mg assures. **Compositions are calculated according to NRC feedstuff tables (1994). ****ME values calculated according to TSE (1994).

The fatty acid (FA) profiles of treatment diets and eggs were determined according to the Shimadzu application catalog. The FA composition of eggs was analyzed determined at 28, 56, 84, and 98 days. In every period, a total of 24 eggs from all groups were used (8 eggs for each group). Every 2 egg yolks from same groups were put in the bag, then cooked with boiling water, after cooling they were stored at −20°C until analysis. The FA composition was analyzed by gas chromatograph

(Shimadzu GC-2010 Plus, Japan) equipped with a flame ionization detector and a 100 m × 0.25 mm ID HP-88 column. The results were expressed as g FA/100 g total FAs.

RESULTS

The effect of fish oil and microalgae supplementation on egg yolk EPA and DHA concentrations are presented in Table 2. On d 28, the EPA ratio in the C group was higher than FO and MALG added groups and the DHA ratio was lower in the C group compared to these groups (P<0.01). MALG addition to diets did not affect the EPA ratio on days 56 and 84, the DHA ratio FO and MALG groups were higher than the C group's egg yolk (P<0.01). In the overall period, in the C group, the EPA ratio was higher than FO and MALG-added groups, and the DHA concentration FO and MALG-supplemented group was significantly higher than that of the C group (P<0.01). There were no significant differences among the FO and MALG groups in terms of EPA and DHA concentrations in egg yolks.

Table 2. Effect of fish oil and microalgea supplementation on egg yolk EPA and DHA concentrations

	Oil sources			
	SFO	FO	MALG	P
Day 28				
EPA	0.20±0.03^{a}	0.05±0.01^{c}	0.12±0.04^{b}	0.001
DHA	0.23±0.06^{b}	0.78±0.06^{a}	0.90±0.02^{a}	0.001
Day 56				
EPA	0.073±0.004	0.018±0.003	0.048±0.002	0.153
DHA	0.0675±0.05^{b}	0.43±0.10^{a}	0.40±0.08^{a}	0.001
Day 84				
EPA	0.12±0.04	0.08±0.01	0.07±0.01	0.601
DHA	0.08±0.00^{b}	0.83±0.08^{a}	0.67±0.02^{a}	0.001
Overall period				
EPA	0.19±0.03^{a}	0.09±0.03^{b}	0.09±0.01^{b}	0.002
DHA	0.17±0.04^{b}	0.68±0.04^{a}	0.61±0.02^{a}	0.001

^{a,b,c}: Values with different superscripts in a row differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FO: Fish oil, MALG: Microalgae, EPA: C20:5n3 Eicosapentaenoic acid, DHA: C22:6n3 Docosahexaenoic acid

DISCUSSION

In the current study, the composition of yolk fatty acids was significantly affected by dietary omega-3-rich oil treatments. The yolk DHA content increased with addition FO and MALG. The EPA concentration did not increase in FO and MALG groups, in contrast to decreased to the control diet. Kralik et al. reported that the addition of soybean and fish oil (0.-1.5%) to the diet significantly increased the EPA and DHA levels as the fish oil content increased. Świątkiewicz et al. (2020) found that the addition of 2% of algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil did not affect performance traits. However, microalgae supplementation significantly increased egg yolk DHA and EPA values. Kim et al. (2016) Flax seed oil and microalgae, Huang et al. (1990) 1,2,3% menhaden fish oil, and Wen et al. (2019) 1%, 2%, 4%, and 8% microalgae, additions to the laying hen's diet was increased the DHA and EPA content on egg yolk. Kim et al. (2016) also noted that the Flax seed oil inclusion increased the ALA content of eggs more than microalgae.

The α-linolenic acid (**ALA**) is a precursor of n-3 PUFA and ALA is metabolized into EPA and DHA in the body. However, the synthesis of DHA may limit depend linoleik acid LA and ALA ratio and best convertion ratio is up to 4% (Shahidi and Ambigaipalan, 2018). Today, researchers are focusing on enriching eggs with EPA and DHA because of their benefits to human health. It can be changed by supplementing n-3 PUFA through non-feeding. It has been shown that the fatty acid profile of the eggs of chickens can be changed by adding a traditional n-3 PUFA source, α-linolenic acid sources, to the diet of chickens. It has been recommended that either fish oil rich in these sources or microalgae should be added to increase EPA and DHA in the eggs of chickens (Fraeye et al., 2012). In this study, both fish oil and microalgae supplementation provided a significant increase in DHA levels. Therefore, it was demonstrated in this study that both FO and MALG supplementation can be used effectively in making eggs functional in order to contribute to human health.

CONCLUSIONS

In conclusion, FO and MALG addition can increase the DHA ratio, but not effective in increasing the EPA ratio in chicken egg yolk.

ACKNOWLEDGEMENTS

Authors thanks Erciyes University Scientific Research Projects (BAP) Coordination Office for support with project number 2023-13011. This project was supported by the

Erciyes University Scientific Research Projects Coordination Office with project number 2023-13011. This paper was produced from Qali Mohamed Yusuf's MSc. thesis.

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Helix aspersa land snails´ biomarker responses to prolonged metal pollution in the field and laboratory

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Abstract

The terrestrial land snail Helix aspersa was used to study the effects of metal exposure in both field and lab settings. In order to preserve human health, we were interested in evaluating the impacts of (Fe2O3) based metal nanoparticles and their effects on body bioindicators of environmental pollution and a health status indicator, Helix aspersa. This kind of intestinal absorption is called subchronic (lasting 28 days). Using a bioassay on lab animals exposed to varying concentrations of NPs (100, 500, 1000 µg/g, 5000 µg/g), the toxicity of NPs is assessed in the snail Helix aspersa. According to preliminary findings, metallic NPs inhibited (Fe2O3) growth in a dose-dependent manner. The estimated concentrations that inhibit 50, 75, 90, and 100% of growth were determined over a period of four weeks and are as follows: The following results are for the Fe2O3-treated group: in the first week, EC50 = 1620,48 µg/g, EC75 = 3814,08 µg/g, EC90 = 5130,24 µg/g, EC100 = 6007,68 µg/g; in the second, EC50 = 844,51 µg/g, EC75 = 2673,66 µg/g, EC90 = 3771,15 µg/g, EC100 = 4502,81 µg/g; in the third, EC50 = 1527,33 µg/g, EC75 = 2619,73 µg/g, EC90 = 3275,16 µg/g, EC100 = 3712,12 µg/g et g; and in the fourth, EC50 = - 205,08 µg/g, EC75 = 746,73 µg/g, EC100 = 1698,55 µg/g. To further elaborate, the concentration at which there is no effect (NOEC) is 500 µg/g, whereas the concentration at which there is an effect (LOEC) is 1000 µg/g. Weight loss in snails is accompanied by a drop in shell weight and diameter. Additionally, a decrease in the weight of the snail, a reduction in the dose-dependent consumption rate, the dry weight of the waste, and behavioral abnormalities were observed, along with a reduction in the weight of soft tissues and organs, particularly the kidney and hepatopancreas. In reference to the histological analysis, which manifestly demonstrated significant tissue alterations, particularly in the hepatopancreas, kidney, lung, and foot.

Key words: *Helix aspersa, metal NPs, Fe2O3, indicator of health status, bioaccumulation, growth, pollution.*

Investigation of runs of homozygosity and genomic inbreeding of Turkish Holstein dairy cattle

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Abstract

This study aimed to investigate genomic homozygous regions by using Runs of Homozygosity (RoH) in the Turkish Holstein cattle population. Model-free and model-dependent approaches, in three different methods, were used to identify the RoH regions. The genomic inbreeding coefficient was calculated as the ratio of the sum of the length of RoH blocks and the total length of the genome. A total of 18172 Holstein cow genotypes were obtained from the Turkish Dairy Cattle Genomic Selection program. Holstein cows originated from Turkey (TR, n = 17381), Italy (IT, n = 131), Austria/Germany (AT/DE, n = 387), Denmark (DK, n = 34), Estonia (EE, n = 129), and Slovakia, Bulgaria, and Ireland were classified as one group namely other countries (Other, n = 110). In addition to the cows´ origin, year of birth (YOB), from 2010 to 2019, and geographical regions of farms were also evaluated. Selection signatures of 4 RoH islands were determined by at least two different methods. The significant RoH islands were located on chromosomes BTA-4, BTA-21, BTA-1, and BTA-17. The detected RoH islands were included previously associated genes with fitness, fertility traits (ABCB8, ASIC3, CDK5, SLC4A2, FASTK, AGAP3, ABHD2, RLBP1, POLG, FANCI, TRNAR-UCG) and horn status (OLIG1, OLIG2, C1H21orf62). The mean genomic inbreeding level of the population was estimated with three different methods 0.102±0.0003, 0.060±0.0002, and 0.104±0.0003, respectively. According to cows' origin, mean genomic inbreeding coefficients ranged from 0.058 to 0.105 for AT/DE, 0.059 to 0.099 for DK, 0.0628 to 0.108 for EE, 0.064 to 0.107 for IT, and 0.068 to 0.111 for Other countries. Thus, these findings propose novel insights worldwide for Holstein Dairy Cattle genomic selection programs.

Key words: *Inbreeding, Dairy Cattle, Runs of Homozygosity, Holstein*

Using multivariate adaptive regression splines for estimating honey yield

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Abstract

In this study, a model was developed to estimate the Honey Yield (HY) variable, which is an important production parameter in the beekeeping sector. MARS (Multivariate Adaptive Regression Splines) method was used in the creation of the model. Hygiene (HI), Aggressiveness (HI), Brood Frame (YV) and Total Frame (TC) were among the independent variables used in the analysis. In order to better reflect the non-linear structure of the model, the interactions of the independent variables with each other were also included in the model. The performance of the model was examined using critical evaluation criteria such as R^2, Pearson correlation coefficient (PC), root mean square error (RMSE) and Akaike Information Criterion (AIC). For the training set, R^2 was calculated as 0.677, PC as 0.823, RMSE as 6.307 and AIC as 544.389. These results show that the model can estimate the HY variable quite well on the training data. However, R^2 0.567, PC 0.760, RMSE 7.060 and AIC 197.715 values were obtained in the test set. A decrease in the performance of the model was observed in the test set, indicating that the generalization ability of the model may be limited. According to the model results, one of the most effective factors on HY is the HI variable. In cases where HI is above 1.25 units, a significant decrease in HY was observed. Similarly, positive and negative effects were observed on HY in the ranges where the TC value varied between 5.17 and 8.83. In addition, the effects of the interactions of HI and TC variables with YV on HY were also observed clearly. The model reveals that low hygiene and medium total frame count are important factors in reaching the highest levels of HY. As a result, while the MARS model exhibits a strong performance in the training set, it is observed that its performance decreases slightly in the test set. This suggests that the model requires further improvement. However, in general, it shows that the MARS model is a usable tool for HY estimation and can contribute to the development of strategies to increase HY in beekeeping.

Key words: *MARS, Honey, Aggressiveness, Brood*

INTRODUCTION

Beekeeping is a critical activity that ensures the sustainability of ecosystem services in the agricultural sector and supports biodiversity. Honey production is one of the most important parameters that determine the economic return of beekeeping activities. Honey yield is shaped by the combination of many factors such as various environmental factors, colony health and management practices. In this context, predicting the performance of bee colonies and developing strategies to increase honey yield is of great importance for the beekeeping sector.

Factors affecting honey yield include variables such as hygiene (HI), aggressiveness (HI), number of brood frames (YV) and total frame number (TC). Hygiene defines the capacity of bee colonies to be resistant to diseases and parasites; aggressiveness is a feature that affects colony defense behaviors and human-bee interactions. Hygiene is critical as it reflects the colony's ability to resist diseases and parasites, which can directly impact honey production (Seltzer et al., 2021). Aggressiveness influences not only the colony's defense mechanisms but also the interactions between bees and beekeepers, potentially affecting management practices and honey yield (Rittschof et al., 2019). The number of brood frames and total frame number are considered indicators of colony size and production capacity (Padilha et al., 2013). Each of these variables can directly or indirectly affect honey yield. However, the complexity of the effects of these factors on honey yield and their nonlinear relationships make it difficult to model accurately with

traditional estimation methods. Each of these factors can have direct or indirect effects on honey yield, and their complex, often nonlinear relationships complicate traditional modeling approaches.

To address the challenges posed by these nonlinear relationships, the Multivariate Adaptive Regression Splines (MARS) algorithm has been employed as a robust tool in data mining. MARS is particularly advantageous for modeling complex data relationships due to its flexibility and ability to adapt to various data structures (Andonov et al., 2019). In this study, MARS (Multivariate Adaptive Regression Splines) algorithm, known as a powerful tool in the field of data mining, was used to determine and estimate the factors affecting honey yield. MARS algorithm is a flexible method that provides advantage especially in modeling complex and non-linear data relationships. In our study, factors such as hygiene (HI), aggressiveness (HI), number of brood frames (YV) and total frame number (TC) were evaluated as variables related to honey yield (HY).

This research aims to evaluate the usability of MARS algorithm in honey yield estimation and to provide a data-driven approach for the management of bee colonies. As beekeepers face increasing challenges from environmental changes and pest pressures, understanding the intricate dynamics of colony health and management practices becomes ever more critical (Haddad et al., 2015). The results of the study will contribute to the development of strategies to optimize honey production for beekeepers.

MATERIAL AND METHODS

The data was taken from an old study which conducted in 2017 and wasn't published. honey yield (HY) was determined as response variable and the variables such as hygiene (HI), aggressiveness (HI), number of brood frames (YV) and total frame number (TC) were selected as explanatory variables from 191 colonies form Syrian bee genotype.

MARS (Multivariate Adaptive Regression Splines) algorithm is a nonparametric regression technique proposed by Friedman (1991) that allows for the effective interpretation of nonlinear and interactive effects between dependent and independent variables. This algorithm draws attention by not making any assumptions for the functional relationships between variables (Aksoy et al., 2019). MARS creates more understandable and interpretable models by using piecewise linear regressions. The MARS algorithm, which consists of a two-stage process, includes forward pass and backward pass stages (Arthur et al., 2020).

In the forward pass stage, random nodes are determined within the change range of each independent variable while creating basic function pairs. This stage provides a good fit to the model, but in terms of the generalization ability of the model, overfitting problem may occur when applied to a previously unseen data set. In order to solve this problem, in the backward pass stage (or also known as the backward pass stage), the model is optimized by eliminating the basis functions that contribute least to the model (Zaborski et al., 2019; Arthur et al., 2020).

The equation of the MARS algorithm, which aims to estimate HY (honey yield) using explanatory variables, can be given below:

$$y_{ip} = \beta_0 + \sum_{m=1}^{M} \beta_m \prod_{k=1}^{K_m} h_{\mathrm{km}}(X_{v(k,m)}) \quad (1)$$

where: $y_{ip}$is the predicted value of the dependent variable (HY), β_0 is a constant (intercept), β_m is the coefficient of basis functions, h_{km} ($X_{v(k,m)}$) is the basis function, in which $v(k,m)$ is an index of the independent variable in the m^{th} component of the k^{th} product, K_m is the parameter restrictive the order of interaction. After producing the extremely complicated MARS model in forward pass stage, the basis functions reducing the obtained model performance were eliminated in the pruning procedure in the backward pass, contingent on the following generalized cross-validation error (*GCV*) (Eyduran et al. 2019; Zaborski et al. 2019):

$$\mathrm{GCV}(\lambda) = \frac{\sum_{i=1}^{n}\left(y_i - y_{\mathrm{ip}}\right)^2}{\left[1-\frac{M(\lambda)}{n}\right]^2} \quad (2)$$

where: n is sample size, y_i is the observed value of the dependent variable (HY), y_{ip} is

the predicted value of the dependent variable (HY), $M(\lambda)$ is the penalty function for the convolution of the model covering λ terms.

All the statistical computations are made with R statistical software (R Core Team 2020). Descriptive statistics of the quantitative characteristics in the present study are performed using psych package (Revelle 2020). For specifying MARS algorithm in HY prediction, earth and caret packages accessible in R environment were used (Milborrow 2020; Kuhn 2020). Predictive performances of the CART and MARS algorithms were measured based on ehaGoF package (Eyduran 2020).

RESULTS

Descriptive statistics presented in Table 1 summarize the basic characteristics of the variables used in the study. The mean value for honey yield (HY) variable was calculated as 17.23 with a standard deviation of 11.03. Honey yield ranged from 0 to 43.78. Hygiene index (HI) was found to have a mean of 86.5 and a standard deviation of 20.18; the minimum and maximum values of this variable were 50 and 100, respectively. The mean value for aggressiveness (HI) variable was 2.71 and the standard deviation was 1.58, and the values of this variable ranged from 0 to 6.75. The mean of the brood frame (HF) number variable was 3.75, the standard deviation was 1.2, and the value ranged from 0 to 6.17. The mean value for the total number of frames (TC) variable was calculated as 6.24, the standard deviation was 2.1, and the values of this variable varied between 0.67 and 10.5. These data indicate different levels of distribution and variability among the variables.

Table 1. Descriptive statistics

	n	mean	sd	min	max
HY	191	17.23	11.03	0	43.78
Hİ		86.5	20.18	50	100
HI		2.71	1.58	0	6.75
YC		3.75	1.2	0	6.17
TC		6.24	2.1	0.67	10.5

Figure 1 presents a correlation matrix showing the correlations between the variables included in the study. This matrix was used to determine the strength and direction of linear relationships between the variables.

A high positive correlation (0.82) is observed between the honey yield (HY) variable and the hygiene index (HI), indicating that increased hygiene positively affects honey yield. Similarly, a strong positive correlation (0.85) is also found between honey yield and the number of brood frames (YC), suggesting that increased brood frames may increase honey yield. A moderate negative correlation (-0.55) is found between the aggressiveness (HI) variable and honey yield, indicating that increased aggressiveness may decrease honey yield.

In addition, a very high positive correlation (0.94) is found between the total frame (TC) number and honey yield, indicating that increased total frame numbers significantly increase honey yield. A moderate positive correlation (0.22) was observed between hygiene index (HI) and aggressiveness (HI) and a strong positive correlation (0.88) was observed between hygiene index (HI) and number of frames with brood (FR). The correlation between total number of frames (TC) and number of frames with brood (FR) was found to be very high (0.96), indicating that total number of frames was strongly related to number of frames with brood.

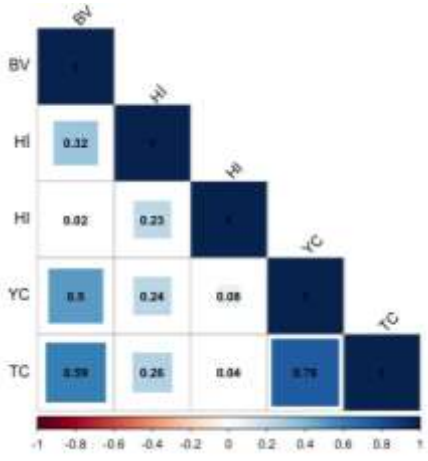

Figure 1. Correlation matrix

According to MARS (Multivariate Adaptive Regression Splines) model, the coefficients

and relationships used to estimate the honey yield (HY) variable are presented in Table 2 above.

Table 2. Constructed MARS model

	coefficients
(Intercept)	11.0247873
h(1.25-HI)	-7.2494389
h(TC-5.17)	5.3821899
h(TC-8.83)	-13.19077
h(HI-1.25)*h(YC-4)	-4.0454854
h(HI-1.25)*h(TC-7.83)	6.5417143
h(Hi-67.5)*h(1.25-HI)*h(TC-5.17)	0.1561499

The model's intercept value is determined as 11.02, which indicates the basic level of honey yield when independent variables are not taken into account. In the model, if the aggressiveness (HI) variable is greater than or equal to 1.25, a decrease of 7.25 units in honey yield is expected (h(1.25-HI) coefficient -7.25). While the total number of frames (TC) is greater than 5.17, a 5.38 unit increase in honey yield is associated (h(TC-5.17)), a decrease of 13.19 units is observed when the total number of frames exceeds 8.83 for (h(TC-8.83)). In addition, the model is sensitive to the interaction between aggressiveness (HI) and the number of frames with brood (YC); When aggressiveness is above 1.25 and the number of frames with brood is above 4, a decrease of 4.05 units in honey yield is expected (h(HI-1.25) * h(YC-4)). Similarly, when aggressiveness (HI) is above 1.25 and the total number of frames (TC) exceeds 7.83, an increase of 6.54 units in honey yield is observed (h(HI-1.25) * h(TC-7.83)). The model also predicts a small increase (0.16 units) in honey yield when hygiene index (HI) is above 67.5, aggressiveness is below 1.25 and the total number of frames (TC) is above 5.17 (h(HI-67.5) * h(1.25-HI) * h(TC-5.17)). These coefficients reveal the complex and non-linear relationships affecting honey yield and model the combined effects of these factors.

The goodness of fit criteria presented in Table 3 evaluate the performance of the MARS model on the training and test datasets. This table includes four main criteria used to determine the accuracy and generalization ability of the model: root mean square error (RMSE), Pearson correlation coefficient (PC), performance index (PI) and coefficient of determination (R^2).

Table 3. Goodness of fit criteria

criterion	Train	Test
Root mean square error (RMSE)	6.307	7.06
Pearson's correlation coefficients (PC)	0.823	0.76
Performance index (PI)	19.868	24.08
Coefficient of determination (Rsq)	0.677	0.567

The root mean square error (RMSE) for the training dataset was 6.307, while this value was calculated as 7.06 for the test dataset. This result indicates that the model shows a slightly lower prediction error on the training data compared to the test data. The Pearson correlation coefficient (PC) was found as 0.823 on the training dataset and 0.76 on the test dataset, indicating that the model represents a strong linear relationship on both datasets, but the correlation on the training set is slightly higher than on the test set.

The performance index (PI) was calculated as 19.868 for the training data and 24.08 for the test data; This shows that the performance of the model is better on the training set but slightly decreases on the test set. The coefficient of determination (R^2) was determined as 0.677 for the training data set and 0.567 for the test data set. These results show that the model can explain 67.7% of the variance in the training set and 56.7% of the variance in the test set, meaning that the model fits the training set slightly better. In general, it can be concluded that the performance of the model is better on the training data set, but it has a reasonable generalization ability on the test data set.

According to the results of the MARS model, it is clear that the complexity of the factors affecting honey yield and the interactions of these factors should be taken into account. In particular, the effects of the relationships between variables such as hygiene, aggressiveness, number of brood frames and total number of frames on honey yield were effectively explained by this model.

These findings provide valuable information for the management of bee colonies and strategies to optimize honey production. It is recommended that these findings be further strengthened with larger data sets and different modeling approaches in future studies.

These results provide a scientific basis for developing strategies to increase honey yield and for decision-making processes in the beekeeping sector.

DISCUSSION

The Multivariate Adaptive Regression Splines (MARS) algorithm has emerged as a powerful tool for predicting honey yield, particularly due to its flexibility in modeling complex relationships between various predictors and the response variable. Honey yield prediction is inherently challenging as it is influenced by a multitude of factors, such as climatic conditions, flower availability, and beekeeping practices. With the ability to handle nonlinearities and interactions among variables, the MARS algorithm provides a robust framework for such predictions.

MARS works by creating piecewise linear models that adaptively select the most relevant predictors from a potentially large set of variables. This adaptability is crucial in the context of honey yield prediction, where relationships between environmental factors and honey production can vary significantly across regions and seasons. For example, traditional models often fail to generalize across geographic boundaries due to unique climatic conditions affecting honey yield in different regions (Rocha and Dias, 2017). MARS addresses this limitation by allowing localized modeling, which can be especially useful when dealing with heterogeneous datasets.

Moreover, MARS has been shown to outperform other predictive modeling techniques in various applications. For example, MARS outperforms traditional regression methods regarding predictive accuracy, as evidenced by its application in environmental modeling (Al-Shourbaji et al., 2021). This is particularly important for honey yield prediction, where accurate predictions can significantly impact agricultural planning and market strategies. MARS's ability to capture complex interactions and nonlinear relationships makes it a suitable choice for modeling multifaceted effects on honey production.

In addition to its predictive capabilities, MARS also provides insights into the relative importance of different predictors. This feature is important for beekeepers and agricultural planners, as it allows them to identify which factors most significantly affect honey yield. For example, analysis of honey production traits showed that while the number of beehives increased, the yield per hive showed a slight decrease (Novković, 2022). By applying MARS, stakeholders can better understand the underlying causes of these trends and make informed decisions to increase honey production.

Moreover, the integration of MARS with other data mining techniques can increase its predictive power. For example, the study discusses the combination of MARS with other methods for prediction in different contexts, highlighting the potential benefits of hybrid approaches in improving prediction accuracy (Shao and Tsai, 2018). This hybrid approach may be particularly useful in the context of honey yield prediction, where both historical data and real-time environmental variables play critical roles.

CONCLUSION

In conclusion, the MARS algorithm represents a significant advance in the field of honey yield prediction. Its ability to model complex relationships, adapt to changing data conditions, and provide insight into predictive significance makes it an invaluable tool for both beekeepers and agricultural researchers. As the demand for honey continues to increase, utilizing advanced predictive models such as MARS will be important to optimize production and ensure sustainability in the beekeeping sector.

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Hygienic quality assessment of minced meat and beef sausages sold in the boumerdes region, algeria

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Abstract

Meat and meat product are one of the most common food stuffs in the world, exposing consumer to the risk of out breaks of Staphylococcus aureus, Escherichia coli and Salmonella spp with serious economic consequences. Although many researchers have already reported the presence of these bacteria in various foods. Our study aimed to assess the level of initial bacterial contamination to these different bacterial species, minced meat and sausage types "Merguez", in order to help assess the bacteriological quality of the product chosen and study its possible impact on public health. For this purpose, 80 samples were taken randomly in some butcher shops of the wilaya of Boumerdes, fresh minced meat (n = 30), frozen minced meat (n = 30), fresh sausage (n = 10) and frozen sausage (n = 10). The results show that 100% of the samples are microbiologically contaminated, and a global number of samples were contaminated by S. aureus with an important rate of E. coli, and a total absence of Salmonella spp. It is to be reminded that the quality of the finished product depends on the quality of the meat used, so, to produce high quality meat products, it is necessary to follow high technology procedures and safety measures during the processing, handling, transport and sale, these parameters must be controlled by a program of self-control, in order to ensure better health safety for the consumer.

Key words: *Minced meat, Sausage, Hygiene,Bacteriological quality*

Efficiency of the developed feed with highly nutritious and easily digestible components in feeding laying quails

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Abstract

Quail farming in Kazakhstan is a rapidly developing branch of poultry farming. The consumer chooses meat and quail eggs because of their high nutritional value. The farmer needs fodder designed specifically for quail satisfying the needs of the biological features of this species. Kazakhstan has the capacity to fully meet its domestic feed needs by producing them. This article presents the effectiveness of the developed food enriched with highly nutritious and easily digestible components, when feeding quail hens during the first 120 days of egg laying. For the experiment, two groups of quails of 25 heads in each (control - CG and experimental - EG) were formed, the keeping system of birds was the same, the differences were only feeding, EG fed developed food. Our experiment showed that the egg-laying intensity was higher in quail EG - 53.62%, which is higher than in CG by 4.1%. The gross yield of eggs was correspondingly higher in EG - 2574 pieces. With less ingestion of EG - 23.8 1.7 g per head per day, quail of this group showed higher rates (P≤0.05). The results of the study conducted in the commercial farm conditions prove the effectiveness of the feed and are recommended for use.

Key words*: Quail, Laying, Egg productivity, Extrusion, Manchurian breed*

INTRODUCTION

aspect of production and requires science-based approaches. The solution to food security of the population of any region depends on the creation of its own production of high-protein plant feed (Fedorova, 2021).

Considering that feed costs make up a significant portion of the price of eggs and meat, optimizing feed use becomes a priority for the poultry industry. Efficient feeding it is not only reducing costs, but also improves the health and productivity of birds, which is important for breeding a variety of breeds and achieving high performance levels (Bagno, 2018; Batkowska, 2014).

Kazakhstan is a grain producer and is one of the top ten exporters in the world, due to which it has its own potential for feed production. However, the trend in the feed production market indicates that the quail farmer is not able to find feed that would fully meet the needs of quail laying hens. Most of the feed presented on the market in Kazakhstan and neighboring countries is

Proper feeding of poultry is a key produced for young and meat production quails.

The nutritional value of the alternative feed should be able to replace some of the traditional ingredients without compromising the performance of quail farming (El-Azeem, 2019). Therefore, nutritionists and feed manufacturers are looking for non-traditional ingredients or processing to reduce feed costs (Yang, 2021).

Kazakhstan is a Muslim country, which means that the feed should not contain such components as bone meal (because it is mainly made from the remains of pig products, and in some countries is prohibited). One of the non-traditional technologies for producing quail feed is extrusion. Extrusion has been shown to significantly improve bird weight gain, feed consumption and feed conversion (Arija, 2006).

Enriched extruded feed allows for more efficient use of nutrients and, accordingly, increases the productivity of birds (Borovsky,

2021). In this regard, we developed a feed enriched with highly nutritious and easily digestible components for laying quails and determined its effectiveness in comparison with commercial feed.

DETAILS EXPERIMENTAL

Materials and Procedures

The scientific work was carried out during the period from 06/08/2023 to 11/4/2023 at the commercial farm. The production of food for quails was carried out in the production and testing workshop of NFT-KATU LLP (https://biofeed.kz) at the Faculty of Veterinary and Livestock Technology of Seifullin KATRU. When developing the recipe, the recommendations of Nutrient requirements of poultry (https://www.nationalacademies.org) were considered. The developed feed for laying quails consists of the following components: crushed and extruded corn, extruded wheat, soybean and rapeseed meal, fish meal, tricalcium phosphate, feed yeast, shell rock, premix for poultry and amino acids, salt. The composition of commercial feed is not distributed by the manufacturer; the packaging indicates only the main components (corn, wheat, soybean meal, bone meal premix, etc.) and the energy value of the feed is 2500-2600 kcal. We carried out a feed analysis in the accredited laboratory "Zootechnical analysis of feed and milk" (Seifullin KATRU).

To conduct the experiment, two groups of birds were formed, 25 birds each. The keeping of the birds was the same, the differences were only in feeding: the control group (hereinafter CG), whose diet consisted of commercial feed; the experimental group (hereinafter EG) was fed the developed food. The daily feed supply according to Nutrient requirements of poultry is 35-40 g per head, however, at the beginning of the experiment we noted that this breed did not consume this amount of feed, the average consumption in both groups was different, so this feature was also object research. Egg production was recorded daily from the 1st to the 120th day of laying; at the end of the experiment, the feed intake was calculated during the entire experiment. Statistical analysis was carried out using the SSPS 25.0 application.

RESULTS AND DISCUSSION

In order to provide poultry with all the necessary nutrients for their health and productivity, the actual nutritional content of the feed used on the farm must be known. The nutritional content of the feed used for the experiment is provided in Table 1.

Table 1. Nutritional content of feed, %

Components, %	Feed	
	Commercial	Developed
DM	89,3±10,3	90,4±8,6
CP	18,2±2,3	20,3±1,9
CK	4,7±1,2	4,5±0,9
CF	5,6±0,7	3,9±0,6
Starch	37,3±3,4	36,7±4,2
Ash	5,3±0,9	5,4±0,6
ME, kcal	2257±56,9	2723±67,3

According to Nutrient requirements of poultry, laying hens with 90% dry matter requires 2700-2900 kcal ME. The developed feed meets the needs of quails in this indicator: 2723±67.3 kcal. In commercial feed, the manufacturer stated 2500-2600 kcal, however, calculations showed a low amount of ME 2257±56.9 kcal. The CP requirement for laying quails is 20%; commercial feed does not meet the crude protein requirements. Thus, the feed contains 18.2±2.3, or 2.1% less than the developed feed. High fiber content in poultry feeds makes it more difficult for quails to digest other nutrients, thereby reducing the nutritional value of the feed. With a norm of this indicator of no more than 5% according to the recommendation, in the commercial amount of crude fiber was at the level of 5.6±0.7%, while in the developed food it was almost 1.5 times less – 3.9±0.6%. For other indicators, the differences were insignificant. Our research has proven to the farmer that any feed should be checked for its actual composition and nutritional value. At the request of the farmer, although this feed did not meet the energy and protein needs of the poultry, we continued research with exactly this commercial feed.

Quail's egg productivity is presented in Table 2, for 120 days of egg laying.

Table 2. Egg productivity of quails for 120 days of laying

Feature	Unit	CG	EG
Gross egg collection	pcs	2472	2574
Oviposition intensity	%	51,5	53,62
Feed consumption for the entire period per layer	g	3099,2	2872,2
Feed consumption per day per layer	g	25,7±2,4[a]	23,8±1,7[b]
Feed consumption for 1-unit products	g	1,25±0,09	1,12±0,23
Survival rate	%	100	100

a,b P≤0.05

Gross egg harvest is the total number of eggs collected from all birds over a given period. According to the results of collecting eggs, 2574 eggs were collected in the experimental group, which is 4.1% more than in the control group, where 2472 eggs were collected. Laying intensity is an indicator that evaluates how actively birds lay eggs. The higher the intensity, the more eggs they produce over a period. In this case, the EG demonstrated a higher intensity of oviposition compared to the CG. The intensity of oviposition in the EG reached 53.62%, exceeding the level of the CG, which was 51.5%. One of the main economic factors is the feed consumption per gained productivity, and for a farmer this is the main economic indicator. During 120 days of laying, 2872.2 g of feed were consumed per head of EG; in the CG this figure was 3099.2 g, or 227 g more. In terms of one unit of production, the EG spent 1.12±0.23 g, while the CG spent 1.25±0.09 g. A statistically significant difference between the groups showed the indicator of daily feed consumption per hen, for the EG 23.7 ±1.7 g, and for the CG it is almost 2 g more than 25.8 ± 2.4 g (P≤0.05). The survival rate of quails in both groups was 100%. Thus, the developed food turned out to be more effective in all studied objectives.

Our results are like the studies of foreign scientists. Depending on the composition of the feed and other factors, their results vary. According to Schneider et al., complete feed and various additives can be used to ensure optimal mineral nutrition for birds (Schneider, 2017). Research conducted by Ratriyanto et al. confirms that feeding a feed containing 2800 kcal/kg and 20% protein provides the best egg production from 12 to 14 weeks as well as average overall production (Ratriyanto et al. 2014). According to studies conducted by Li et al., using feed containing 19.95% protein in quail eggs results in more protein production than those containing 17.75%, 21.85% and 24.08% protein (Li, 2011).

CONCLUSIONS

The use of the developed feed helps to improve the egg production of laying quails. EG quails with less food consumed per day 23.8±1.7 g (P≤0.05) had higher gross egg collection and egg laying intensity. Over 120 days of oviposition, the EG per hen was 102.96 pcs, and the CG was 98.88. For this eggs amount, the laying hen EG consumed 2872.2 g of feed, while the CG consumed 3099.2 g. Experienced quail consumed less feed to achieve the same number of eggs. As a result of the experiment to prove the effectiveness of the developed feed in comparison with the commercial feed used on the farm, there is a recommendation from the farmer for the use of this feed.

ACKNOWLEDGMENTS

The scientific work was carried out within the framework of the scientific project AP13068280 with funding from the Ministry of Science and Higher Education of Kazakhstan under the Grant funding of young scientists for scientific and (or) scientific and technical projects for 2022-2024.

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Challenges and New Developments in Technology of Duck Incubation

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Abstract

The requirements of the duck embryo incubation environment differ significantly from those used for chickens. The main differences concern: the size of the eggs and their chemical composition (significantly higher fat content in the yolk than in hen broods), and consequently high heat production by the embryo, as well as the presence of a thick mucin cover on the surface of the eggshell. For this reason, during artificial broods, the key factors are the improvement of gas exchange and control of the incubation temperature. Research conducted as part of the POIR.01.01.01-00-1010/17 project in 2018-2022 allowed us to propose solutions: Storing duck eggs at a temperature of 8-12ºC allows them to maintain their correct physicochemical properties for up to 28 days, while storing eggs at a higher temperature should not exceed 14 days. "Dry" disinfection (by ozonation) gives similar results to washing eggs with benzalkonium chloride, and at the same time is more efficient, faster and cheaper. That is why it was decided to use it in the further part of the project. Additional fumigation by a preparation containing 2-phenylphenol and ammonium nitrate definitely improves the results of duck hatching. However, it should be noted that vertical infections related to the health of the flocks, the quality of the litter and compliance with biosecurity rules on the parent farm have a very large impact on these results. It is advisable to improve the egg sprinkling and cooling system. It was found that the eggs cooled unevenly depending on the position of the hatching tray on the trolley and the eggs on the tray itself and the shelf. On the upper shelves, the better cooled area (EST average 34.4°C) is located closer to the medial edge of the trays, while on the lower trays it is in the middle of the trays (EST average 34.6°C). The less cooled area is usually located at the outer edge of the tray and is characterized by an EST of about 36.2°C. There was found that the hatchability of Pekin ducks strongly depended on the origin of the eggs (reproductive flock) and the current health situation. A factor that was difficult to verify in experimental conditions was the health problems of the flocks (mainly mycoplasmosis), which required antibiotic treatment. In the case of obtaining full-quality material (hatching eggs), the incubation parameters used allowed for achieving hatching results exceeding 80% from fertilized eggs. The hatchability during final stage compared to initial stage of project increased in the Pekin duck from 70.0±9.98 to 82.2±6.6%, while in the Mulard hybrid duck from 70% to 79.4±7.9%. Providing the right parameters in storage, duck eggs can be stored for up to 3 weeks without losing hatching value. This applies in particular to eggs obtained from reproductive flocks at the peak of laying. The factor influencing hatchability and chick quality is mainly the age of the flock. The prevailing opinion that the quality of chicks decreases with extended storage has not been confirmed.

Key words: *Pekin duck, incubation, egg fertilization, embryo mortality, microclimate*

Comparison of different selection indexes for milk traits in friesian cows by using different methods of relative economic values

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Abstract

A total of 2181 normal lactation records of Friesian cows kept at Sakha Experimental Farm, belonging to Animal Production Research Institute, Ministry of Agriculture, Dokki, Cairo, Egypt were used to construct several selection indexes by using two relative economic values , method 1 actual relative economic values (set1) and one phenotypic standard deviation (set2). Variables studied were 305 day milk yield (MY), fat yield (FY), Protein yield (PY), age at first clving (FC) and calving interval (CI). Data were analysis by using MTDFREMAL Software Boldman et al. (1995). The model includes the fixed effects of month and year of calving, parity and lactation length as covariate and random effects of animals, permanent environmental and errors. The overall means of MY, FY and PY were 2570 kg, 99 kg and 77 kg, respectively. Direct heritability estimates for MY, FY and PY were 0.23, 0.24 and 0.31, respectively. Phenotypic and genetic correlations among different variables were positive and significant. Comparison of the two methods for estimating relative economic values revealed no differences in any of the two methods for the expected genetic gain per generation for each variable. Therefore, the two methods were succeeded in predicting the genetic gain per generation for the three variable studied. However, the second method (one phenotypic standard deviation) was recommended based on ease of calculation.

Key words: *Selection indexes milk traits Friesian cows*

Nutritional quality of milk from two cattle breeds (local and ımported) from the Souk Ahras Region (north-eastern algeria)

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Abstract

Milk is an excellent product, consumed daily and with a large quantity, food safety for the dairy industry recommends that only the milk that has the required sanitary characteristics enters the collection and processing circuit, otherwise it is removed from the food chain, in order to guarantee a better quality to the consumer. Today, like all foods, milk is subject to certain uncertainties in its quality. Our study was carried out on the physico-chemical and microbiological quality of raw cow´s milk from two cattle breeds (Locale and Imported) high at the region Souk Ahras cows during the lactation period of strong (March to May). The results of physico-chemical analyzes show that the parameters: density, acidity, are close to the standards for imported breed, by cons in the local race, only the pH and density are close to standard. The fat is standard in the imported breed, it depends mainly on the race factor.

Key words: *Key words: analysis, feeding, fat, hygienic quality, local cow, milk, Souk Ahras.*

Factors affecting growth during the grazing and development periods in central Anatolian merino

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Abstract

Türkiye holds a significant position in the region and globally in small ruminant farming with a population of 56 million small ruminants (79.4% sheep, 20.6% goats). In sheep farming, there are different geographic and climatic conditions, with the breeding of 91.14% native sheep breeds and 8.86% Merino crossbreeds adapted to these conditions. This study aims to determine characteristics such as initial turnout weight, return-from-pasture weight, and average daily live weight gain in Central Anatolian Merino sheep raised in the vicinity of Ankara province, as well as to identify the effects of certain environmental factors on these traits. For this purpose, a linear mixed model was used to reveal the impact of factors, and the least squares means for the desired traits were calculated. Additionally, a multiple comparison test was applied to determine the differences between groups. In the study, the influence of factors on first time grazing weight (FTG), return period from grazing weight (RPG), and average daily weight gain (ADWG) was found to be significant for most factors. In the obtained results, the initial turnout weight, return-from-pasture weight, and average daily live weight gain are 22.77 kg, 34.01 kg, and 269.27 g, respectively. In the study, the time spent in the farm until turnout, the time spent on grazing, and the time spent from birth to grazing end are 65.99, 45.94, and 111.92 days, respectively. Additionally, the most significant proposal made in our study is to evaluate the heritability of these variables using full pedigree and to improve the breed genetically in addition to improving environmental conditions in order to analyse the grazing time attributes of Central Anatolian Merino sheep in more detail.

Key words: *Grazing performance, Environmental factors, Central Anatolian Merino.*

INTRODUCTION

Türkiye holds a significant position in the region and globally in small ruminant farming with a population of 56 million small ruminants (79.4% sheep, 20.6% goats). In sheep farming, there are different geographic and climatic conditions, with the breeding of 91.14% native sheep breeds and 8.86% Merino crossbreeds adapted to these conditions (TUIK, 2023). In Türkiye, where genetic diversity is rich in terms of native and crossbred sheep breeds, the most commonly raised sheep breeds with fat tails are Akkaraman, Morkaraman, and İvesi, while those with thin tails are Central Anatolian Merino, Kıvırcık, and Karayaka breeds (Şirin et al., 2017).

The Inner Anatolian region, which is rich in genetic diversity, provides a favorable environment for small ruminant farming due to factors such as its climate, geographical conditions, traditional livestock culture, and local market demands (Ceyhan et al., 2012). Therefore, in this region, sheep and goat farming is carried out as a significant agricultural and labor-intensive activity. The Central Anatolian Merino sheep, developed in the early years of the Republic to improve meat and wool production, is widely bred in the region as a breed adapted to adverse climate conditions (Yalcin, 1986).

The Central Anatolian Merino sheep is an important breed in Ankara region for lamb breeding and makes significant contributions to the economy through meat and wool production. It can be said that factors influencing the preference of breeders in using this breed include a good twinning rate, adaptation to the region's arid climate and poor pasture conditions, and the ability of lambs to reach early slaughter weight. Breeders typically feed lambs with

maternal milk from the time they are born until they are taken to the pasture. During the grazing period, they join their mothers in the pasture to benefit from both maternal milk and natural grazing resources. At the end of the grazing period, breeding animals are carefully selected for the next generation, while non-breeding male and female lambs are separated for short-term fattening and sold as meat.

Since the start of the Central Anatolian Merino sheep community-based sheep breeding program under Project I-II in 2012 - 2016, Both initiatives are still working properly in Ankara. It is carried out as a sub-project of the Ministry of Agriculture and Forestry's "National Community-Based Small Ruminant Breeding Programme" in partnership with several universities, research institutions, sheep and goat breeder organizations, and breeders. The weights of lambs were recorded after their first entry onto the pasture in this study to assess the influence of pasture and maternal milk on their weights throughout the grazing season. Daily live weight gains were measured after returning from pasture, and the environmental effects on these traits were investigated.

MATERIALS AND METHODS

Animals and Phenotype

The animal material from this study was used in the investigation. Lambs were utilized in the research from 16 Central Anatolian Merino sheep breeding farms in the project in Ankara province and its regions, which raise lambs with mother's milk until grazing time. Data from lambs born in 2018 were utilized in Ankara Central Anatolian Merino breeding, a sub-project of the National Community-Based Small Ruminant Breeding Programme. During the spring and summer months (March to November), the small ruminants grazed on low-quality pastures, while during the winter, they were fed an average of 0.7 kg/day of concentrated feed per animal in their enclosure. Lambs, at an average age of 65.99 days, are introduced to the pasture alongside their mothers in March, April and May benefiting both from maternal milk and natural grazing resources. Lambs live 45.94 days on average in the grazing together with their mothers. Breeding animals are carefully selected for the generation that follows at the end of the grazing period, while non-breeding male and female lambs are separated for short-term fattening and sold as meat. Before their mating, male sheep (rams) were separated from the herd and fed concentrated diet for two months.

Table 1. Descriptive statistics of grazing period growth traits.

Trait	FTG (kg)	RPG (kg)	ADWG (g)
Number of observations	5972	5972	5972
Mean	22.77	34.01	269.27
Standard error	0.08	0.10	1.02
Minimum	9.98	12.63	1.21
Maximum	35.42	55.35	499.68
Coefficient of Variation	29.79	22.06	29.13

FTG: First Time to Grazing; RPG: Return Period from Grazing; ADWG: Average Daily Weight Gain.

As attributes, 5972 observations of first time to grazing (FTG), return period from grazing (RPG), and average daily weight gain (ADWG) were acquired. Additionally, first time to grazing, return period from grazing and average daily weight gain dates, sex, birth type (singlets/twins) and first time to grazing month (March/April) were regularly recorded. The animals' age at grazing emergence day was roughly 65.99, their age from birth to the end of the grazing day was approximately 111.92, and their grazing time was approximately 45.94 days. Average daily weight gain (ADWG) was obtained via linear statistics by using FTG and RPG. Tables 1 and 2 provide a detailed explanation of the data structure as well as the sample size after removing outliers.

Table 2. Descriptive statistics of grazing period days.

Parameters	ALGE(day)	ALBEG(day)	GT(day)
Number of observations	5972	5972	5972
Mean	65.99	111.92	45.94
Standard error	0.23	0.23	0.18
Minimum	16	54	31
Maximum	117	164	82
Coefficient of Variation	26.91	29.90	16.12

ALGE: Age of Lambs at Grazing Emergence; ALBEG: Age of Lambs from Birth to End of Grazing; GT: Grazing time

Statistical Analyses

We removed outliers from the data by deleting observations with values greater than the mean 3 times the standard deviation. Normality of the responses were tested with Shapiro-Wilk test. In addition, the variance homogeneity was assessed visually using a plot created from the residual vs fitted value of the answers. For the construction of the final linear mixed models, we first investigated the impact of environmental factors (such as sex, birth type, mother age, herd size, first time to graze month, and district). The R statistical environment's core packages were used for managing data and initial analysis (R Core Team, 2020). The "SPSS 26" program was utilized for linear model analysis. Before fitting the final models for the traits, generalized linear models were used to investigate the impact of environmental factors. Using these mixed models, evaluated the mean least square differences of the components, with herd impact included as a random factor in the model. Following that, Duncan's Test was used to assess the differences between groups for the important factors. The following is a description of the final linear mixed model that was applied to the traits:

Model: $y_{ijklma} = \mu + h_i + d_j + m_k + t_l + s_m + r_a + Zh + e_{ijklma}$

Where **y** are the observations of the dependent variables (i.e., FTG, RPG and ADWG); **μ** is the intercept; **h** is the fixed effects of herd size (3 levels); d is the fixed effects of district (7 levels); **m** is the fixed effects of Month of going out to grazing (3 levels); **t** is the fixed effects of birth type (2 levels); **r** is tı is the fixed effects of Rams age (6 levels); **s** the fixed effects of sex and **h** is the random herd effect where **e** is the residual error of observations in the models and **Z** is the incidence matrix.

RESULTS AND DISCUSSION

In this study, the effect of environmental parameters such as sex, birth type, district, mother age, herd size, and month of grazing on FTP, RPP, and ADWG of Central Anatolian Merino lambs was explored. For this objective, linear mixed models were fitted to quantify the effect of variables and give mean least square estimates for the features. Diagnostic tests and factor interactions were performed in addition to the models' diagnostic tests. Finally, multiple comparison tests were employed to establish the significance of changes between component groups.

Table 3 outlines the components that have a significant impact on the characteristics after generating the appropriate final models. The diagnostic tests confirmed that the data was distributed normally with homogenous variance. In general, factor pairwise interactions were not significant.

Table 3. The least square mean value (± SE) of the grazing time growth traits with the relevant p-values and sample sizes.

	FTG (kg)			RPG (kg)			ADWG (g)		
Fixed Effects	**n**	**LSM ± SE**	**p-value**	**n**	**LSM ± SE**	**p-value**	**n**	**LSM ± SE**	**p-value**
Sex						***			
Male	3075	24.21±0.18		3075	37.01±0.21^{a}		3075	281.49±2.20	
Female	2897	24.20±0.18		2897	36.45±0.21^{b}		2897	279.05±2.39	
Birth type			***			***			
Single	3438	24.68±0.16^{a}		3438	37.19±0.20^{a}		3438	280.49±2.20	
Twin	2534	23.77±0.19^{b}		2534	36.28±0.23^{b}		2534	280.38±2.57	
District			***			***			***
Gölbaşı	1059	27.12±0.27^{e}		1059	40.98±0.32^{c}		1059	296.90±3.61^{d}	
Güdül	813	25.56±0.31^{c}		813	37.63±0.38^{a}		813	292.51±4.24^{bc}	
Haymana	1827	23.23±0.27^{c}		1827	36.58±0.32^{b}		1827	289.61±3.59^{bcd}	
Kızılcahamam	226	22.36±0.63^{d}		226	34.88±0.76^{b}		226	251.49±8.54^{cd}	
Polatlı	1333	23.92±0.28^{b}		1333	35.80±0.33^{b}		1333	281.41±3.77^{b}	
Sincan	714	23.02±0.33^{a}		714	34.55±0.40^{a}		714	270.71±4.49^{a}	
Dam age			***			***			***
2	1353	22.73±0.20^{a}		1353	35.07±0.24^{a}		1353	277.86±2.72^{ab}	
3	1295	24.87±0.22^{bc}		1295	37.03±0.26^{a}		1295	277.61±2.98^{a}	
4	600	24.26±0.27^{d}		600	37.27±0.32^{c}		600	293.46±3.61^{c}	
5	1134	25.53±0.22^{e}		1134	38.16±0.27^{c}		1134	276.51±3.03^{ab}	
6	471	23.39±0.30^{ab}		471	35.58±0.36^{a}		471	273.07±4.05^{a}	
7 and up	1119	24.53±0.23^{cd}		1119	37.30±0.28^{b}		1119	284.09±3.15^{b}	
Herd size			***			***			***
0-150	811	25.10±0.23^{a}		811	37.06±0.28^{b}		811	280.38±3.11^{b}	
150-300	4300	21.45±0.16^{b}		4300	34.27±0.19^{a}		4300	277.72±2.14^{a}	
>300	861	26.11±0.27^{c}		861	38.89±0.32^{b}		861	283.21±3.60^{b}	
Lamb turn out to pasture			***			***			***
March	1340	21.06±0.26^{a}		1340	38.40±0.31^{b}		1340	270.30±3.47^{a}	
April	4319	23.36±0.20^{b}		4319	33.79±0.24^{a}		4319	261.48±2.70^{a}	
May	313	28.23±0.56^{c}		313	38.01±0.67^{b}		313	309.53±7.51^{b}	
Intercept	5972	28.78±0.81		5972	37.81±0.97		5972	304.08±10.90	

Notes: The mean values which have different superscript are significantly different. ***P <0.001. **P <0.01. *P <0.05. SE = standard error; N=number of observations

All fixed variables evaluated were shown to have a substantial influence on first time to grazing (FTG). Table 3 shows that the sex mean for male and female lambs was 24.21 ± 0.18 and 24.20 ± 0.18, respectively. Also, there was a statistically insignificant difference between the male and female groups. (Aktaş et al., 2018) discovered that the mean 75-day weight of male and female Central Anatolian Merino lambs born between 2007, 2008, 2009 and 2010 was 18.4, 17.9, 19.1 and 18.9 kg, respectively. They also found in their studies that the weight of female lambs on the 75th day was 18.0 kg, and that of male lambs was 19.2 kg. In another study, they found that the difference between the groups in the 75th day weight of males and females in Central Anatolian Merino lambs was significant (Ünal and Akçapınar, 2001). In this study, it is seen that the difference between male and female lambs with an average age of 66.99 days is insignificant, unlike other studies, and is higher than the results obtained from other studies. The absence of distinction between males and females in Central Anatolian Merino lambs suggests that breeders do not treat men and females differently throughout the growing phase and that they grow entirely under the influence of the mother. The first time to graze weights of singlet and twin lambs were found to be 24.68 0.16 and 23.77 0.19 kg, respectively, in the research, and the differences were statistically significant (P-value0.001). Many investigations in the same and other breeds indicated that the weight of single born lambs at 60 and 75 days old was larger than that of twin born lambs, which was statistically significant. (Aktaş et al., 2018; Yağcı et al., 2018; Tüfekçi, 2023; Türkmen, 2021). The difference between singleton and twin lambs is observed in research done on the same and related breeds, and the results

gained from these studies are identical to our study. The disparities in weight acquired from birth to pasture and the discrepancies with other research can be attributed to changes in the care and feeding circumstances of mothers between farms. In our study, mean first time to grazing weights of lamb's district in Gölbaşı, Güdül, Haymana, Kızılcahamam, Polatlı and Sincan were found to be 27.12±0.27, 25.56±0.31, 23.23±0.27, 22.36±0.63, 23.92±0.28 and 23.02±0.33 kg respectively. Moreover, the difference between the groups was found to be significant (P-value <0.001) in the multiple comparison test between these districts. When we look at the findings of this study, we can see that the highest weight was assigned to the Gölbaşı area and the lowest weight was assigned to the Kızılcahamam district. It is possible to argue that nutritional variations between mothers during the lamb rearing phase are more beneficial in lambs maintained in closed barns from birth to pasture than district differences. In this study, mean first time to grazing weights of lamb's mother age in 2, 3, 4, 5, 6, aged 7 and up were found to be 22.73±0.20, 24.87±0.22, 24.26±0.27, 25.53±0.22, 23.39±0.30 and 24.53±0.23 kg respectively. Moreover, the difference between the groups was found to be significant (P-value <0.001) in the multiple comparison test between these mother age. The highest lamb weight was found in lambs born to 5-year-old moms, while the lowest was found in lambs born to 2-year-old mothers, according to a study that looked at the influence of maternal age. It is thought that the reason for the low rate of 2-year-old mothers may be due to their first motherhood experience and the milk yield of the mothers being lower than the mothers in other groups. This study is comparable to the one conducted by (Aktaş et al., 2018). When the influence of herd size was considered, the mean first time to graze weights of lambs' herd size 0-150, 150-300, and >300 were found to be 25.10±0.23, 21.45±0.16, and 26.11±0.27 kg, respectively. In addition, the difference between the groups was confirmed to be significant (P-value 0.001) in the multiple comparison test between these herd sizes. First time to grazing weights of lambs were found to be highest on farms with flock sizes of 300 heads and above, and lowest in farms with flock sizes of 150-300 heads. It is possible to believe that the management system of large livestock firms is superior to that of other enterprises. The other effect month of going out to grazing mean first time to grazing weights of lamb's March, April and May were found to be 21.06±0.26, 23.36±0.20 and 28.23±0.56 kg respectively. Month of going out to grazing It is observed that the difference between the groups is significant (P-value <0.001). It was determined in the study that lambs that went to pasture in May had a higher weight at pasture than lambs that went to pasture in other months. This condition is influenced by elements such as moms, care, nutrition, and sickness owing to variances between farms throughout the breeders' lamb raising phase. This study's findings suggest that feeding and other herd management practices are crucial when it comes to grazing weight of lambs for the first time.

In this study, the effect of all environmental factors on the live weight of return period from grazing (RPG) lambs was found to be significant. Table 3 contained detailed information and RPG methods. According to Table 2, the average time spent on pasture is 45.94 days, while the average time spent returning from pasture is 111.92 days. The least squares mean of RPG of male and female lambs are 37.01±0.21 and 36.45±0.21 kg, respectively. It was determined that the difference between groups based on sex was significant (P-value <0.001).

Tüfekçi (2023) and Aktaş et al., (2018) observed that male and female Akkaraman lambs weighed 31.51 and 31.07 kg, respectively, whereas Central Anatolian Merino lambs weighed 29.5 and 27.0 kg. In comparison to this study, the 120th day weight is lower in the study done. This circumstance may be caused by the influence of grazing between the 60th and 120th day, the effect of breast milk, and variances in additional care and feeding.

The least square means return period from grazing of RPG of single and twin lambs are 37.19±0.20 and 36.28±0.23 kg, respectively. The difference between groups based on birth type was shown to be significant. (P-value <0.001). When compared with the

other study Tüfekçi, (2023), the result obtained showed that the effect of birth type on the 120th day weight was insignificant, unlike this study. Similar to Aktaş et al., (2018) study on Central Anatolian Merino lambs, single lambs are heavier than twin lambs and the difference between groups is significant. It can be said that the most important factor in this situation is that singlet lambs benefit more from mother's milk than twin lambs during the grazing period.

In this study, return period from grazing (RPG) of lamb's mother age in 2, 3, 4, 5, 6, aged 7 and up were found to be 35.07±0.24, 37.03±0.26, 37.27±0.32, 38.16±0.27, 35.58±0.36 and 37.30±0.28 kg respectively. Moreover, the difference between the groups was found to be significant (P-value <0.001) in the multiple comparison test between these mother age. According to a study that examined the impact of maternal age, lambs born to 5-year-old mothers had the greatest weights and lambs born to 2-year-old mothers had the lowest weights, which was similar to the weight at grazing Because they are experiencing parenthood for the first time, and because they produce less milk than moms in other groups, it is believed that the low rate of 2-year-old mothers is related to these factors. This investigation is similar to that by (Aktaş et al., 2018).

In this study, Moreover, means of the district in Gölbaşı, Güdül, Haymana, Kızılcahamam, Polatlı and Sincan are 40.98±0.32, 37.63±0.38, 36.58±0.32, 34.88±0.76, 35.80±0.33 and 34.55±0.40 kg, respectively. It has been determined that the difference between the districts in weight at the end of the grazing period is significant (P-value <0.001). While the result obtained in Gölbaşı district was higher than other regions, the lowest was found in Sincan district. It can be said that the differences in lamb weights between regions are due to the changes in pasture areas and geographical structure.

The last square means effect of herd size, return period from grazing weights of lamb's herd size 0-150, 150-300 and>300 were found to be 37.06±0.28, 34.27±0.19 and 38.89±0.32 kg respectively. Additionally, the multiple comparison test between these herd sizes indicated that the difference between the groups was significant (P-value 0.001) on farms with flock sizes of 300 heads or more, return time from grazing weights of lambs were found to be greatest, while on farms with flock sizes of 150–300 heads, lowest. The high weight of large breeders can be attributed to the fact that their pastures and breast milk are superior than those of other breeders.

Effect month of going out to grazing mean return period from grazing weights of lamb's March, April and May were found to be 38.40±0.31, 33.79±0.24 and 38.01±0.67 kg respectively. Month of going out to grazing It is observed that the difference between the groups is significant (P-value <0.001). It has been determined that lambs that go to pasture for the first time in March have higher live weight than those that go out to pasture in April and May. Due of differences between farms throughout the breeders' lamb-growing phase, factors including moms, care, nutrition, district, and disease have an impact on this condition. As in this study, other studies show that environmental factors such as sex, type of birth, year and month of birth are effective on 90th day and 120th day weight. (Ceyhan et al., 2019; Çolakoğlu & Özbeyaz, 1999; Gül et al., 2020).

In the lambs of the Central Anatolian Merino, factors other than sex and birth type such as district, mother's age, flock size, and the timing of pasture turnout have been found to have a significant impact on daily live weight gain. The least square means of ADWG for male lambs and female lambs were 281.49±2.20 and 279.05±2.39 g, while they were 280.49±2.20 and 280.38±2.57 g for single-born lambs and twin-born lambs, respectively. In the study, it was found that lambs born to ewes with an age of 4 had a higher daily live weight gain compared to lambs from other ewe age groups. The ADWG of district in Kızılcahamam were lower than those grazing time other district. ADWGs by herd size are 280.38±3.11, 277.72±2.14 and 283.21±3.60 g, respectively. Effect month of going out to grazing mean the ADWG month March, April and 270.30±3.47, 261.48±2.70 and 309.53±7.51 g, respectively. In the study conducted by Ceyhan et al. (2019) on Akkaraman lambs Aktaş et al., (2018) on Central Anatolian

Merino it was reported that the ADWG of male lambs were higher than that of females and ADWG of single-born lambs were higher than twin-born lambs. It appears that the findings of this study are consistent with those of other investigations.

CONCLUSIONS

In the study, it was determined that all environmental factors except for sex had an effect on FTG, all environmental factors were effective on RPG, and all environmental factors except for sex and birth type were effective on ADGW. Furthermore, single-born lambs had a longer return period from pasture growth features than twin-born lambs. The weights of lambs first introduced to pasture in March were found to be higher in all RPG and ADWG characteristics compared to April and May. Lambs born to 5-year-old mothers exhibited higher growth data in terms of FTG, RPG, and ADWG characteristics compared to lambs born to mothers in other age groups. Greater than those of lambs born in other areas are the growth statistics for lambs born in the Gölbaşı district. Furthermore, it has been found that lambs born in flocks of 300 or more sheep have better growth traits than lambs born in other flocks. Lamb first time to grazing (FTG), return period from grazing (RPG), and average daily weight gain (ADWG) have all been proven to be significantly influenced by herd and nutrition management, climate, and pasture quality. The study discovered that a variety of environmental factors affected the development characteristics of the grazing period. As a result, it has been found that enhancing the habitat can increase animal productivity. Calculating the birth months according to the feeding strategy may yield the most benefit.

The most important recommendation made in our study is to use full pedigree to assess the heritability of these variables, improve the breed genetically, and improve the environment in addition to grazing time attributes of Central Anatolian Merino sheep.

ACKNOWLEDGEMENTS

The study population, Central Anatolian Merino Breeding Programme of the National Community-Based Small Ruminant Breeding Programme, with the project batch numbers of (TAGEM/06OAM2012-TAGEM/06OAM2016). The author kindly acknowledges the General Directorate of Agricultural Research and Policies (Ministry of Agriculture and Forestry) of the Republic of Türkiye, who fund and run the National Community Based Small Ruminant Breeding Programme for all contribution.

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Pigeon breeding in Kahramanmaras province

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Abstract

The purpose of the study was aimed to collect information about pigeon breeding in Kahramanmaras province in Turkey. Pigeon breeding is a socio-cultural and folkloric breeding that has preserved its importance from history to the present. It has always had an important place in Turkish culture. Although it is cultivated in every region of Turkey, it also has an important role in the culture of the people living in Kahramanmaras. From past to present, guinea pigs have been bred for purposes such as experimental animals, ornaments, communication, hobby, competitions and portion consumption. Many breeds are fed. Damascus, Çarşılı, Meverdi, Embroidered, Maraş White, Urfalı, Netherlands, Museyid, Carnation, Olive, Ehlez, Roving, İstanbullu, Bağdadadı, Pied Forty Tail, Güllü, Denmark, Wing Post, Mail, İspir, Red Back, Şafra, Sticky , Harduni, Şinasi, Hünkari and Derviş Ali, these pigeon species are important pigeon types in pigeon breeding in Kahramanmaras province. These pigeons are grouped as Filo, Elvan, Posta and game bird (twistler). There are breeders who keep a large number of pigeons and regularly participate in competitions in the province.

Key words: *Pigeon, Kahramanmaras, culture, breeing*

INTRODUCTION

B.C. Pigeons, known as the first domesticated bird species in the 4500s, were bred for various purposes. (Yılmaz at al.,2014, Garip., 2017). With the discovery of writing, letters were written by pigeons. With the discovery of writing, letters were written by pigeons. Today, guinea pigs have been bred from past to present for purposes such as experimental animals, ornaments, communication, hobby, competitions and portion consumption. Pigeons are symbols of both religion and some concepts. Pigeons symbolize peace. The word pigeon is mentioned in Divanü Lügat-it Türk, the first written dictionary in its history. Pigeons are large, plump birds with stubby tails and plenty of feathers. It is from the Pigeon family (Columbidae) and has more than 300 species. Kahramanmaras province has a developed and ancient culture in terms of pigeon breeding.(Sales and Janssens, 2003; Garip., 2017; Yılmaz and Boz., 2012; Çelik at al., 2021; Çakmak ve Iş ın 2005; .Yılmaz at al., 2012; Yılmaz at al., 2014)

In Kahramanmaras region, "Şamı, Çarş ılı, Meverdi, Nakış lı, Maraş Beyazı, Urfalı, Hollanda, Museyid, Karanfilli, Zeytinli, Ehlez, Fitilli, İ stanbullu, Bağ dadadı, Alaca Kırk Kuyruk, Güllü, Denmark, Kanat Posta, Posta, İ spir, Sırtı Kızıl, Ş afra, Yapış kan, Harduni, Ş inasi, Hünkari and Derviş Ali" pigeon species. In particular, maintenance and feeding are carried out on the roofs of detached houses.

The Fleet Pigeon Festival is held every year in the province in order to maintain the love of pigeons and to keep young people and those engaged away from bad habits.

With this study, it was aimed to bring the verbal and visual data about pigeon breeding in Kahramanmaras to the literature.

MATERIALS AND METHODS

The sample of the study and the data collection work formed the parameters taken from people living in Kahramanmaraş and devoted to pigeon breeding.

The members of Kahramanmaras Province Pigeon Breeders Association and Kahramanmaras Pigeon and Songbirds Association were formed. Issues such as care, feeding, reproduction, and shelter were discussed.

RESULTS AND DISCUSSION

The findings related to pigeon breeding in the province were presented under the headings.

Care and Housing

In Kahramanmaraş , it is usually made in shelters called bird coops in the terraces or gardens of detached houses. It is usually made using briquette, wood, iron and cage wire materials. Maintenance is done in the morning and evening hours. Those who keep pigeons are also called "Mırtikçı" in the province.

A period of at least 3 hours/day is allocated throughout the province for pigeon care. During this period, operations such as cleaning the shelters, flying the pigeons and watching with pleasure are carried out. As with all poultry, hygiene is of great importance in pigeons. For this reason, importance is given to shelter cleanliness. In the litter part of the poultry house, litter cleaning is done every 2-3 months. It is done once a week during the breeding period.

Pigeons coming from outside are not taken into the cage immediately in order to be protected from the epidemic and they are kept under observation for a day.

It is also important to pay attention to the cleanliness of the drinking water of the pigeons. Distilled mains water is used, and attention is paid to the temperature of the water in summer. Otherwise, the possibility of diarrhea increases. Various disinfectants are used to purify the bath water from external parasites. Members of the association reported that the average feed consumption of pigeons is 15-20 kg/year throughout the province.

Perforated beak stones are presented in the cage, and when it is insufficient, beak and nail are cut with the help of nail clippers.

Figure 1. Poultry area

It is necessary to be more careful in the breeding cages with the emergence of the fry, and the feed specially prepared for the feeding of the fry in the morning and evening hours accelerates the development of the fry. (figure 1)

Spring months are preferred as the breeding season in Kahramanmaraş . Thus, the puppies are prevented from being affected by the cold. Temperatures where the weather is close to the end of comfort are preferred, which are the temperatures where the air temperature rises above 25 °C on average.

Pigeons need to feather depending on the time. This period is called feke entry among the people.

Feeding

A special feed is prepared in Kahramanmaras and this feed is a mixed feed consisting of many seeds being brought together.Raw materials such as wheat, corn, millet, sunflower, culban, vetch, and lentils are generally used in the content of this feed. Members of the association reported that the average feed consumption of pigeons is 15-20 kg/year throughout the province.

Breeding pigeons vomit the food (crop milk) they have extracted from their crops into the crops of their offspring in order to feed their young. This behavior is also called colostrum (bird's milk) among the people. This period lasts for about 2 weeks. While the vomiting process continues for an average of 6 weeks,

the grain size increases day by day for the development of the baby's crop.
From the 7th week, the baby pigeon feeds itself. This process of taking feed is called hitting the feed among the people.

Breeding

Pigeons are monogamous and mate together in the spring to mate. Movements such as fluffing of feathers, chasing the female, snuggling with the female, turning around, scratching the underwing feathers, knitting a nest are seen, and these movements are signs of anger.
Among the people, this phenomenon is called curing. After the mating of the male pigeon with the female pigeon, fertile eggs are taken from 7-8 days.
It is called the main spawning period when the eggs begin to be taken. There are pigeons that do not hatch, and the problem is solved by placing the eggs of these pigeons in gold by other pigeons. This event is also called as stepmother, porter pigeon feeding, egg laying. Afterwards, the period of feeding the colostrum (bird's milk) offspring begins. The offspring, which are not taken care of by the mother pigeons, are fed to the other porter (sitter) pigeons as a plowing process.

Vaccination

Diseases that are common in pigeons, which are common in pigeons in the world and in our country, and the kinds of diseases they cause have been found. Although the way to prevent most of them is hygiene, vaccination is done to ensure that they have a protective effect and live a healthy life.
Example of vaccination program made by the members of the association;
- On the 40th day, mixed vaccines such as plague, newcastle, adenovirus are made from the nape of the neck,
Pigeon breeding in Kahramanmaraş province is gaining importance day by day and it is drying up as an occupation where both young and middle-aged people value their time.
For this reason, conscious breeding is being carried out gradually. In line with the mists obtained from the oral and visual materials made with the members of the association, important steps are taken in terms of care, feeding and reproduction day by day.
In the construction of animal shelters, the welfare of animals is considered and hygiene is given importance. Even vaccination programs have been developed for animals to protect them from various diseases.
The high interest of the local government in Kahramanmaraş also ensures that the fleet pigeon festival is held in the province every year.

CONCLUSIONS

As a result; although pigeon breeding in Kahramanmaraş preserves its traditional culture, it maintains its place as a city with exemplary behaviors for pigeon breeding.

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A Machine learning approach to exploring the complex mechanisms underlying milk traits in murciano-granadina goats

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Abstract
The Murciano-Granadina goat (MUG) is a renowned dairy breed, known for its adaptability and resilience, as well as for its exceptional milk traits characterized by high protein and fat content, along with low somatic cell counts. These traits are governed by complex biological processes, crucial in shaping phenotypic diversity. Thus, it is imperative to explore the factors regulating milk production and lactation for this breed. In this study, we investigated the genetic architecture of seven milk traits in MUG, employing a two-step analysis to examine genotypephenotype associations. Initially, a Random Forest algorithm identified the relative importance of each SNP in determining the traits of interest. The second step applies an information theory-based approach, Mutual Information-Based Detection of Epistatic SNP Pairs, to explore the complex genetic architecture of quantitative milk traits, focusing on epistatic interactions that might be overlooked in the first step. These approaches allowed us to identify an almost distinct set of candidate genes for each trait. In contrast, by analyzing the promoter regions of these genes, we have revealed common regulatory mechanisms among the milk traits under study. These traits include dry matter percentage, length of lactation/milk production days, fat percentage, protein percentage, lactose percentage, milk yield at 210 days, and somatic cell count. These findings are crucial for understanding the molecular mechanisms underlying gene regulation and highlight the pivotal role of transcription factors (TFs) and their preferential partner choice in the development of these traits. Notably, TFs such as DBP, HAND1E47, HOXA4, PPARA, and THAP1 were consistently identified for all traits, highlighting their important roles in immunity within the mammary gland and milk production during lactation.

Key words: *Murciano-Granadina, Machine Learning, Milk Traits, Random Forest, Transcription Factors, Mammary Gland*

Predicting the course of delivery in Polish Merino Sheep based on zoometric dimensions of their progeny

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Abstract
A disturbing phenomenon regarding sheep farming in Poland is the deterioration of reproductive indicators, including lamb rearing. For this reason, research to determine the factors contributing to their improvement is fully justified. The aim of the research was to analyze the influence of the weight and zoometric dimensions of lambs measured after parturition on the dam's course of delivery. The animal material consisted of 155 Polish Merino sheep, aged 4 to 12 years, used in a flock subject to performance assessment in the Kuyavian-Pomeranian Voivodeship (Poland). In addition, the study included 248 offspring born to them. The course of delivery of the studied mothers was divided into two categories: easy (independent or with little help from the breeder) and difficult (with complications, breeder´s help required). Both mothers and their offspring were assessed in terms of body weight and zoometric measurements: mothers - 5-6 months before delivery, lambs - immediately after birth. In the initial stage of statistical analysis, using analysis of variance, the influence of lambs´ sex and type of birth on their body weight and zoometric dimensions was examined. Next, the mothers´ birth course was statistically modeled using multiple logistic regression. When building the final model, the forward variable selection method was used. In the subsequent statistical analysis stage, Pearson correlation coefficients were calculated between the body dimensions of lambs and their mothers. Statistical processing was performed using SAS software. As a result of the analysis of variance, it was shown that the sex of the lambs had a statistical impact on their body weight at birth, oblique body length, circumference of the cannon, circumference of the chest, and circumference of the waist. Furthermore, the method of lamb birth had a statistically significant impact on both shoulder breadth and head width. A logistic regression analysis was conducted to illustrate the impact of litter size, shoulder breadth, and head width on the delivery process in mother ewes. Based on the calculated odds ratios, we can confidently state that the likelihood of a challenging birth for twin litters was 7.73 times greater compared to singletons. It was found that as the width of the lambs´ shoulders increased by 1 cm, the risk of difficult delivery increased by 7.54 times, and the risk of head width increased by as much as 20.73. All calculated correlation coefficients between the body dimensions of mother sheep and their offspring were low (<0.1) and insignificant. In summary, the research findings indicate that the delivery process of the dam's was influenced by factors such as the litter size, the shoulder width of the lambs, and the head width of the lambs. Simultaneously, no statistical relationships were observed between the dimensional characteristics of mothers and the offspring they lambed.

Key words: *Polish Merino, course of delivery, body dimensions, prediction, logistic regression*

Genetic diversity and maternal origin of Turkish Awassi Sheep based on mitochondrial genome

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Abstract

Awassi is a versatile sheep breed, selectively bred for meat, milk, and wool production, and exhibits remarkable adaptability to challenging environmental conditions. It is extensively raised across southern Türkiye, Iraq, Saudi Arabia, Palestine, Jordan, and Syria. However, factors such as prolonged droughts, diseases, climate fluctuations, intense selection, and crossbreeding have collectively led to a substantial reduction in the genetic diversity and biological richness of the Awassi sheep population in the Fertile Crescent region. As a result, it becomes crucial to identify these breeds, ascertain their phylogenetic relationships, and institute protective measures. Preserving the genetic heritage of Awassi sheep holds paramount significance given its historical and ecological importance.
In this Study, the mtDNA D-loop region of Awassi sheep reared in Türkiye, was sequenced, information about domestication processes and haplogroups were obtained and phylogenetic analyses were performed by revealing genetic similarities/differences. For this purpose, a total of 75 sheep were sampled, the control region (D-loop region) of mtDNA was amplified by PCR and then sequenced. In this study, the median-joining network analyses conducted on Awassi sheep raised in Türkiye resulted in the majority of the studied samples being grouped together with HPG-C, E.

Key words*: Awassi, Genetic diversity, Haplogroup, MtDNA D-loop, Phylogenetic analysis*

INTRODUCTION

According to archaeologists and geneticists, sheep were first domesticated around 11,000 BC in various regions of the Fertile Crescent, including the region between central Anatolia and the northern Zagros Mountains. These regions correspond to present-day Iraq, Jordan, Palestine, Syria, Iran and Türkiye (Zeder et al. 2008; Demirci et al. 2013). The sheep populations of these countries exhibit higher levels of genetic diversity (based on microsatellite, SNP, mtDNA and nuclear DNA studies) compared to sheep from other regions in Asia, Africa and Europe (Al-Atiyat et al. 2018). The high genetic diversity found is due to the fact that these countries are close to or contain some of the centers of domestication of livestock (Naderi et al. 2007).

The genetic structure of the Awassi sheep breed is important as it is a widespread breed in the Fertile Crescent and probably gave rise to most of the modern European sheep breeds (Demirci et al. 2013). It is important to determine the genetic structure of native Turkish breeds to understand their contribution to the gene pools and domestication process of modern European sheep breeds and also to understand evolutionary processes that began in the region that includes Türkiye. Determining the genetic structure of sheep breeds is also necessary to implement an effective genetic conservation program, which is needed in Türkiye as uncontrolled breeding systems have reduced the effective population size of native sheep breeds. Disorganisation in sheep breeding systems can cause both genetic pollution and genetic erosion. To protect native breeds against future unexpected circumstances and demands, the genetic structure of these breeds should be determined. mtDNA is a very useful tool for investigating genetic diversity in domestic species because it is a relatively small genome that facilities laboratory studies, contains highly conserved regions and is maternally inherited; therefore, there is no

recombination. Studies primarily utilizing mitochondrial DNA sequencing have identified five maternal lineages for domestic sheep breeds (Ovis aries) worldwide. These lineages are classified into haplogroups A, B, C, D, and E (Hiendleder et al. 2002; Meadows et al. 2011).

Haplogroup B encompasses breeds predominantly found in Northern Europe and the Eastern Mediterranean. Haplogroup A breeds are especially common in Asia and Europe. Haplogroups C, D, and E are primarily located in the Middle East and the Caucasus, and are distinguished by their fat tails, which serve as reserves to help them endure extended periods without water. These five haplogroups have been foundinTurkish sheep breeds (Meadows et al. 2011).

Türkiye is a very popular country for sheep breeding due to its multiple small ruminant habitats. In summary, the Awassi breed is widely bred in Türkiye due to its high milk yield and developmental characteristics. The main regions of distribution of Awassi sheep in Türkiye are Gaziantep, Şanlıurfa, Mardin, Hatay and Adana. Recently, Awassi sheep have penetrated into the Aegean Region and Central Anatolia and are widely used for milk production after crossbreeding with some domestic and exotic breeds. Gürsu and Aygün (2014) stated that Türkiye is a very suitable country for sheep breeding, sheep products market and climatic conditions for breeding.

MATERIALS AND METHODS

In this study, a total of 75 sheep were sampled, and Genomic DNA was extracted using the salting-out method. In addition, several reference sequences from previous studies (from haplogroup A, B, C, D and E) and wild sheep sequences (O. musimon, O. ammon, and O. vignei) were used for the analyses. The 531 bp in length of mtDNA D-loop region) was amplified by PCR using forward (5′-ACTGCTTGACCGTACATAGTAC-3′) and reverse (5′-AGTATTGAGGACGGGGTA A-3′) primers. The PCR products were sequenced.

We used FinchTV 1.5.0 (Geospiza Inc., Seattle, WA) to view the data from Sanger DNA sequencing. The sequences, 531 base pairs in length, from the mtDNA D-loop region were aligned using MEGA 7 software (Kumar et al. 2008). The FinchTV was used to view the data from Sanger DNA sequencing. The sequences, 531 base pairs in length, were aligned using MEGA 7 software (Kumar et al. 2016). To calculate the position and number of polymorphic sites, as well as corresponding haplotypes, we used DNASP software (Librado and Rozas, 2009). We constructed an unrooted neighbor-joining (NJ) tree of the sheep breeds under study using Splits Tree4 software (Huson and Bryant, 2006). Furthermore, to determine haplotypes, we generated haplotype median-joining networks using NETWORK 4.1 software based on reference sheep mtDNA sequences.

RESULTS

In total, 11 haplotypes were observed from 22 polymorpic sites for 75 Awassi sheep (Table1). The haplotype diversity was found as high and the nucleotide diversity was estimated as 0.01748. The Tajima's D value showed that the low heterozygosity and the number of haplotypes being lower than expected in Awassi sheep reared in Türkiye (Table1).

Table.1. Genetic diversity parameters of the studied populations

Population	**π**	**H_d**	**h**	**S**	**k**
Turkish Awassi	0.01748	0.933±0.048	11	22	6.450

Population	**Tajima's D**	**P**	**Fu's F_S**	**P**
Turkish Awassi	-0.110	0.503	-1.939	0.165

π: nucleotide diversity; Hd: haplotype diversity; h: number of haplotypes; S: number of polymorphic sites; k: average number of nucleotide differences., Tajima's D and Fu's F_S statistics.

After analiyzing the mtDNA D-loop, a total 11 haplotypes were identified in the Turkish Awassi population. The genetic distance between the haplotypes was visualized through heatmap and phelogenetic tree (Figre1).

Haplotipler	Polimorfik nükleotitler
H1	TTGACTTTCCATTCAAGTGTGG
H2	....T..................
H3	..A.T......CC.........
H4	....T.C................
H5	C.....CC.....TG..C..AA
H6	....TC..........A.AC..
H7	....T.......C.........
H8	..AGTC..........A.AC..
H9	...GTCC......TG..C....
H10	.C.GTCC.GGG..T.G.C....
H11	...GTC..........A.AC..

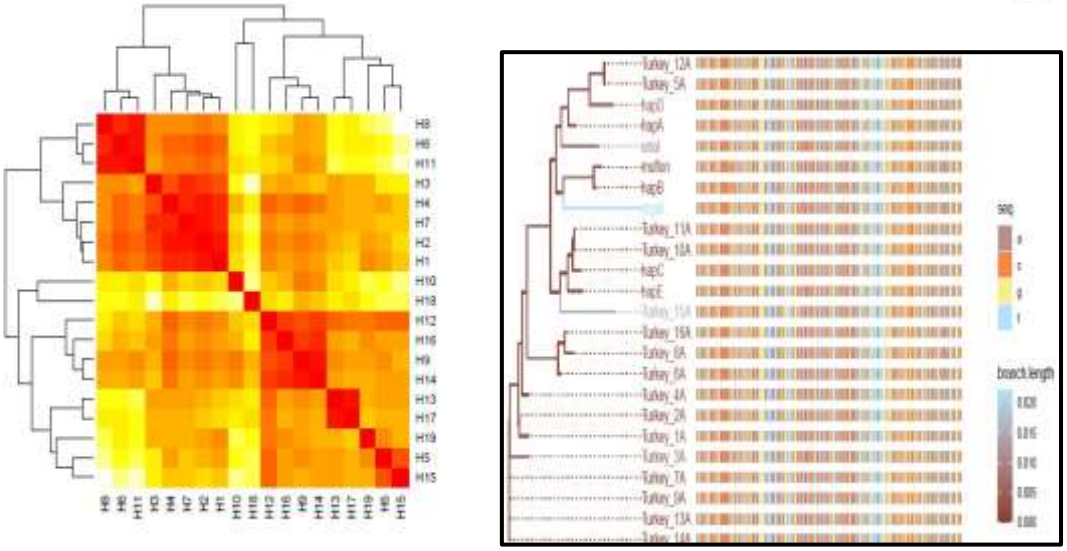

Figure 1: Haplotype sequences, Heatmap and phelogenetic tree based on mtDNA D-loop region of Turkish Awassi sheep

According to both median joining network analyses and neigbour joining phylogenetic tree results, Awassi sheep raised in Türkiye have mtDNA sequences close to the reference sheep haplogroups C and E. In contrast to the other samples, two samples (12 A and 5A) have different mtDNA sequences, but show close similarity to Urial, Muflon and Argali sheep with haplogroups D, A and B.

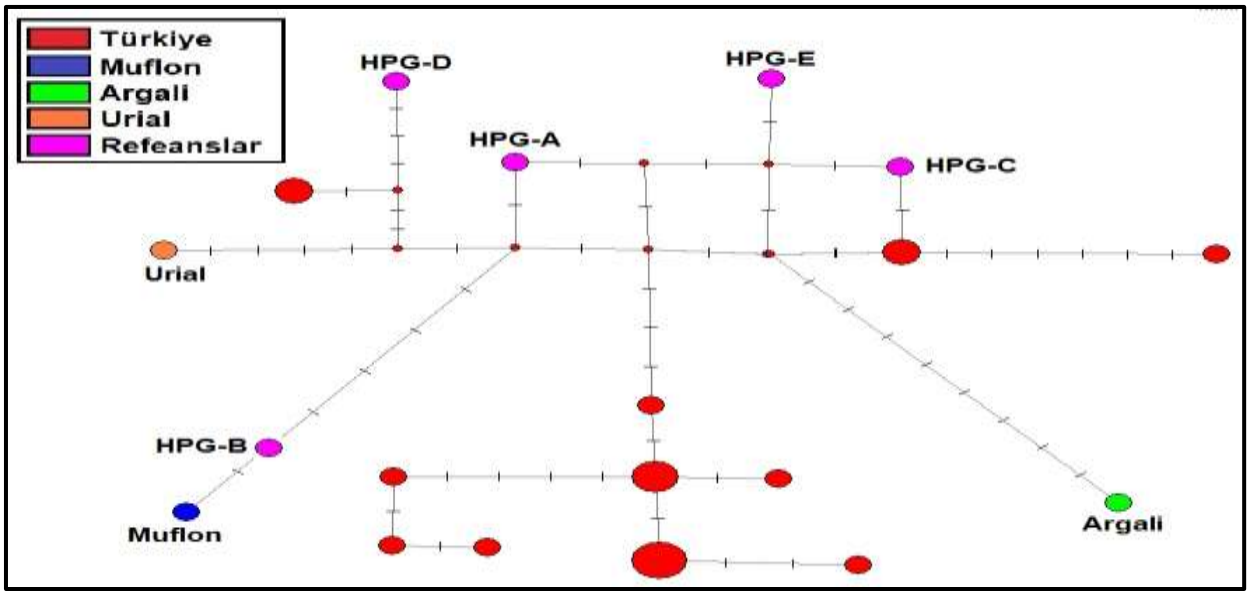

Figure 2. Median joining network analyses of the Turkish Awassi population and reference sequnces and mtDNA haplogroups.

DISCUSSION

Mitochondrial DNA, particularly the control region (mtDNA D-loop), exhibits substantial intraspecific variation, leading to a high number of haplogroups and haplotypes within species. The mtDNA D-loop is a widely utilized molecular marker for exploring the evolutionary history and phylogeny of many animals. Due to its haploid nature, mtDNA has a small effective population size and is highly susceptible to genetic drift. During migrations, certain haplotypes and haplogroups may vanish or arise, resulting in new genetic structures across different regions. This characteristic of mtDNA allows for clear visualization of geographic patterns of genetic diversity, which can be assessed in a phylogenetic context (Bruford et al. 2003).

As a result of the median joining network analyses performed on the Awassi sheep bred in Türkiye, some of the studied samples were grouped close to HPG-C and some were grouped together with HPG-D. In previous studies conducted in Türkiye, the presence of relatively high HPG-C haplogroups along with HPG-D and HPG-E (Pedrosa et al. 2005) was observed (Meadows et al. 2007). These results are similar to the results obtained in this study. In contrast to our study, they reported that HPG-B is the most common haplotype in Türkiye (Demirci 2012).

Studies to date have consistently identified haplogroup B as the most prevalent among Turkish native sheep breeds (Meadows et al. 2007; Yüncü et al. 2009; Demirci et al. 2013). The research has uncovered three primary haplotypes: haplogroup A (Asian type), B (European type), and C (Central Asian and Middle Eastern type) (Heiendelberg et al. 1998, 2002; Pedrosa et al. 2005; Meadows et al. 2007). These haplogroups are present in modern domestic sheep globally. However, our study did not detect haplogroups A and B. This discrepancy may be attributed to differences in sampling methods. One possible explanation is Türkiye's historical role as a terminus of the Silk Road—a major caravan route that traversed Türkiye, Iran, India, and China—facilitating trade in goods and livestock. Consequently, the observed weak genetic structure among Turkish indigenous sheep breeds could be the result of extensive gene flow driven by historical human migrations.

CONCLUSIONS

This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

ACKNOWLEDGEMENTS

In this section, you can acknowledge any support given which is not covered by the author's contribution or funding sections.

This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

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Advances in bioinformatics: phylogenetic analysis and data visualization using r programming language in animal biotechnology

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Abstract

Phylogenetic analyses play a critical role in population genetic studies, especially for livestock animals, providing essential insights into the historical demographic structure of populations, the genetic diversity within and between species, and the evolutionary relationships among species. Traditional methods of molecular genetic data analysis have involved the use of multiple software programs, each requiring specific input and output formats. Tools such as MEGA, DnaSP, SplitsTree, TASSEL, and Arlequin are widely employed, but necessitate users to format their data uniquely for each software, complicating the workflow.

In recent years, the field of molecular genetics has benefitted from significant advancements in computational tools and bioinformatics, streamlining the data analysis process. New-generation software, like R programming language, enables researchers to conduct various analyses and visualize results powerfully with a single data entry. R, originally developed for statistical computing and data visualization, has emerged as a valuable tool in animal biotechnology for conducting phylogenetic and statistical analyses on large molecular datasets. With the open-access environment of R, researchers can easily share and replicate computational workflows by providing command scripts and code, offering great advantages in terms of reproducibility and collaborative research. The comprehensive capabilities of R, combined with the flexibility it offers for complex analyses through language-specific commands, have made it a go-to tool in the analysis of genetic data. By leveraging these tools, animal biotechnologists can perform a wide range of genetic studies more efficiently, providing deeper insights into genetic relationships and enhancing efforts in the conservation and breeding of livestock species.

This presentation will discuss the power and application of R in molecular genetics, focusing on its utility for phylogenetic analysis, and data visualization in the context of animal biotechnology. It will highlight the advantages of using R over traditional methods and demonstrate how it simplifies the research workflow, ultimately facilitating the advancement of livestock genetic studies.

Key words: *TASSEL, DnaSP, MEGA*

POSTER PRESENTATIONS

TABLE OF CONTENT

The influence of a magnetic field on the gene expression of macrophage-like cells in vitro

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Abstract

Animal physiotherapy is currently a rapidly developing field within veterinary medicine. Among the physical treatments employed, magnetotherapy is frequently utilized to facilitate maximal patient recovery. The therapeutic efficacy of a magnetic field is influenced by its parameters, including frequency, induction, waveform, and exposure duration. This study aims to evaluate the impact of a low-frequency pulsed magnetic field (commonly applied in both human and animal physiotherapy) on the expression of specific cytokine genes. An in vitro cell culture of the HD11 line was established, with cells distributed across three six-well plates, each representing distinct experimental groups. Plates 1 and 2 were subjected to magnetic field stimulation using the BTL-5818SLM COMBI device. The stimulation protocol consisted of four 30-minute sessions. For the first group, parameters typically associated with acute-stage disease therapy were applied (frequency 5 Hz, induction 3 mT, rectangular pulse shape). The second group was exposed to parameters corresponding to chronic-stage disease therapy (frequency 50 Hz, induction 10 mT, rectangular pulse shape). Molecular analysis was performed using a two-step RT-qPCR reaction, and relative gene expression levels were calculated based on the geometric mean of threshold cycle values for reference genes beta-actin (ACTB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The cytokine gene panel included: interleukin 1 beta (IL-1β), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 17 (IL-17), and tumor necrosis factor (TNF). Statistical analysis revealed a significant upregulation ($P<0.05$) of IL-6 and IL-8 in response to both acute and chronic magnetic field parameters. IL-1β expression significantly increased only under chronic stimulation conditions. Acute-stage magnetic field parameters significantly enhanced the expression of IL-2 and TNF. Conversely, IL-17 expression was significantly downregulated compared to the control group. These findings suggest that magnetic field stimulation may induce a pro-inflammatory cellular response akin to acute inflammation, potentially promoting tissue regeneration. This in turn may stimulate the regeneration of organisms. Investigating the effects on other cell types and tissues could provide a more comprehensive understanding of the therapeutic potential and mechanisms underlying magnetotherapy. *This research was funded by a subsidy from the Polish Ministry of Science and Higher Education under the research project BN-WHIBZ-4/2022 Omics studies in the context of bird development.*

Key words: *Magnetic field, in vitro, gene expression, macrophages*

Hatchability and microclimatic conditions in duck hatching: conventional vs.

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Abstract

The hatching period is critical in the technology of artificial incubation of poultry. In mass production of chicks, hatching stress is increased by additional stressors that do not occur in natural conditions. The stress caused by the egg leaving by the chick is intensified by the hatcher´s poor environment and the provision of access to feed and water. "On-farm hatching" is one of the solutions that reduces stress and improves the welfare of the chicken around the hatching period. However, it has not been tested so far in duck production. Hatching Pekin duck eggs (Cherry Valley) were incubated in a commercial hatchery for 24 days. Next, 12 trays of eggs were candled for verification of embryo viability. The eggs of three trays (HH group, n=310) were transferred to hatch in a conventional hatcher, while the other ones (336 eggs) were transported into the experimental facility and set in 3 pens directly on litter (OH). The number of hatched ducklings and microclimatic parameters were controlled at 4-hour intervals. All unhatched eggs were break-out analyzed and hatchability established. The hatchability in the conventional and on-farm systems (mean ± SD) was 90.6 ± 2.21% and 88.1 ± 5.97% (P<0.05), respectively. Ducklings hatched in the "on-farm" system, in comparison to HH, had higher body weight by 1.7 g (P>0.05). The hatch window was between 42.8-46.8 hours. The temperature (T) and relative humidity (RH) in the experimental facility (mean ± SD) were 36.5 ± 2.25°C and 12 ± 0.1%, respectively, while in the hatcher, they were 36.6 ± 0.14°C and 61 ± 2.5%, respectively. It was found that the litter surface temperature varied greatly, e.g., 33°C observed near the heater, while 14°C near the door and the external wall. In conclusion, the results of the experiment indicate that "on-farm" hatching of Pekin ducks seems to be a fully usable solution and provides ducklings with a higher level of welfare than the conventional system. However, it currently needs further development and establishment of optimal microclimatic parameters for hatching.

Key words: *Ducklings, hatching, alternative hatch system, hatch window, welfare*

Causes and prevalence of ovine meat seizure in batna slaughterhouse (Algeria)

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Abstract

The aim of this study is to determine the main lesions and anomalies as well as the causes of seizure at the municipal slaughterhouse in Batna City. To carry out this work, an investigation was carried out over a period of three months (03) December, January and February 2024. During this investigation it was noted that the production of sheep meat in this region is important with 231175 kg and the carcasses have a average weight of 25 kg. These carcasses come from the slaughter of young males (231025 kg) while those of females are rare (150 kg) (the females admitted are at a culling age > 5 years). The results of this investigation showed that the reasons for the seizure of the organs are hydatidosis in the liver with a more or less reduced number of 24 cases which represents a prevalence of 0.24%, while we found 25 cases (0.25%) in the lungs. On the other hand, tuberculosis of the lungs and liver is 0%. Furthermore, liver infections caused by the fluke are very low (0.06%). Other conditions of various origins occupy the majority of lung seizures which are 2.07% and 1.28% of the liver. Regarding the seizure of carcasses during this period, only two cases of seizure were recorded, jaundice and septicemia. Furthermore, no cases of anomalies were recorded. From this investigation we observe that the seizure of organs and carcasses is less important (156 kg) than that recorded in other species of red meat production.

Key words: *Hydatidosis, fluke, liver, lungs, sheep meat*

Assessment of ouled-djellal lamb meat quality during frozen storage for 12 months duration

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Abstract

Freezing stands as one of the preferred and primary methods for preserving meat over the long term. Nonetheless, the product remains susceptible to physical and biochemical reactions that could undermine its long-term qualities. This study aimed to track the quality changes in vacuum-sealed Ouled-Djellal lamb meat stored in a freezer (-20°C) over four different frozen storage periods (0, 3, 6, and 12 months). Significant alterations in physical parameters were observed across various frozen storage periods, such as an increase in pH, heightened yellowness (b), and total drip loss, alongside reduced water activity, brightness (L*), and browning (a*). Regarding biochemical characteristics, there was a decline in meat protein solubility, while lipid oxidation values (TBARS) substantially rose with prolonged frozen storage duration. The frozen practice can be used to preserve Ouled-Djellal lamb meat. However, there are some changes in meat traits that deserve attention, mainly regarding lipid oxidation, meat color, and cooking losses, as these types of modifications are undesirable and can cause consumer rejection during purchase, preparation, and subsequent product consumption.*

Key words: *Ouled-Djellal lamb, freezing, quality, frozen storage periods*

Intestinal microbiota of broiler chicken influenced by dietary supplementation of the diatomite-bentonite mixture

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Abstract

Background: Diatomite is a source of biologically available silicon but in feed industry its insecticide and anti-caking proprieties have been also widely recognized. The aim of the study was to evaluate the effect of dietary diatomite-bentonite mixture (DBM) supplementation on the quantitative and qualitative composition of the bacterial microbiome of the broiler chicken gut. The trial was carried out on 960 Ross 308 broiler chickens divided into 2 experimental groups throughout the entire rearing period lasting 6 weeks. The birds were fed complete granulated diets without (group C) or with DBM (group E) in an amount of 1% from the 11 day of life. Two nutritionally balanced diets were used, tailored to the age of the broilers: a grower diet (from day 11 to 34) and a finisher diet (from day 35 to 42 of life). Diatomite used in a mixture with bentonite significantly altered the microbiome. Results: Restricting the description to species that comprise a minimum of 1% of all analyzed sequences, 36 species in group E and 30 species in group C were selected. Several bacteria species were identified in intestinal contents of chickens for the first time. Thirteen species occurred only in group E: Agathobaculum butyriciproducens, Anaerobutyricum hallii, A. soehngenii, Blautia producta ATCC 27340 = DSM 2950, Gordonibacter pamelaeae 7-10-1-b, Helicobacter pullorum NCTC 12824, Lactobacillus crispatus, L. helveticus DSM 20075 = CGMCC 1.1877, Mucispirillum schaedleri, Phascolarctobacterium faecium, Phocaeicola coprocola DSM 17136, P. massiliensis, and Ruthenibacterium lactatiformans. Conclusions: The findings highlight the intricate and potentially consequential relationship between diet, specifically diatomite supplementation, and gut microbiota composition. Furthermore, a favourable increase in some production performance indices of broiler chicken fed DBM has been demonstrated.

Key words: *Gut microbiota, sequencing technologies, diatomite, poultry*

One health approach in vector-borne disease surveillance networks

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Abstract

In recent decades, a series of changes have been taking place, including movements of people and animals, alterations in natural environments, especially climatic changes, which have influenced the possibilities of the appearance of certain diseases. This is the case of diseases transmitted by vectors, organisms whose presence, survival and multiplication are favored in habitats where they had not previously been detected. Given the zoonotic nature of some of these diseases, surveillance systems involving animals and humans are required to obtain successful results. The creation of networks based on two pillars is proposed. On the animal health side, veterinarians play a fundamental role, together with livestock farmers and environmental/forestry agents, given the intimate contact with species living in forested areas, coexisting with other wild species that are not subject to disease control. It is important to emphasize that in zoonoses, control in animals considerably limits the risk in humans, hence a great effort must be made to ensure that veterinary clinics, independent veterinarians and official bodies work in coordination with animal owners, thus constituting the first barrier of containment. On the other hand, surveillance in individuals, which is relatively simple if one has recourse to public health centers, where one can obtain very interesting information about the appearance of abnormal symptoms, which attract the attention of the physicians; another fundamental pillar is the educational centers, where one has access to the population in training, which will become the immediate future. The success of surveillance will depend entirely on the integration of all the above elements.

Key words: *vector, surveillance, network, One health, zoonoses*

INTRODUCTION

Some VBDs (vector-borne diseases) have (re)emerged in Europe and its surrounding areas, partly due to climate variation and other anthropogenic changes, as described for mosquito-borne diseases like malaria, chikungunya, dengue, Zika and West Nile virus infection (Wint et al., 2023). By other hand, certain tick-borne diseases (Lyme, encephalitis), as well as sandfly-borne diseases (leishmaniosis) and biting-midge-borne diseases (Schmallenberg virus disease, bluetongue) in livestock (Trájer et al., 2013; Lievaart-Peterson et al., 2015; Jacquot et al., 2017).

Because of the VBDs progress in the last years and specially in new areas, appropriate surveillance appears essential to ascertain the risk for animals and human populations (Guillot et al., 2021).

According to the World Health Organization, surveillance is defined as *the ongoing systematic collection, collation, analysis and interpretation of data and the dissemination of information to those who need, to know in order for action to be taken* (World Health Organization 2001). In this way, it can be stated that disease surveillance would be addressed to identify changes in the infection and/or health status

of animal and human populations (Salman, 2003).

Two main points have been described, the surveillance of the spatial distribution of competent vectors, and the surveillance of proper sentinels (Guillot et al., 2021). The information about the spatial distribution of competent vectors contributes to determine the risk of the diseases they might transmit, as vector presence is a prerequisite for their transmission. The importance of sentinels relies on early detection and identification of outbreaks can be possible, which looks vital to the success of control and prevention efforts (Kahn, 2006) and to reduce the magnitude of subsequent outbreaks (Ferguson et al., 2005).

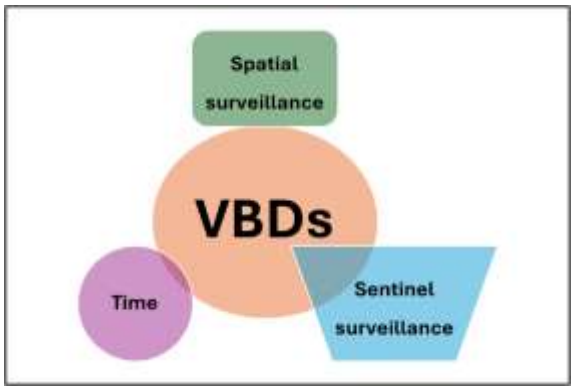

Figure 1. Surveillance of vector-borne diseases requires adequate observation in a specific area, definition of appropriate sentinel species, and sufficient time to obtain the expected information.

The first objective of this study was to design a surveillance network for animal and/or human disease vectors in an area where abundant information had not been collected so far, due to the lack of appropriate climatic conditions to facilitate the survival and growth of certain species of diptera and ticks that act as disease vectors, an aspect that has been modified in the last decade. The second objective was to indicate which could be the most suitable sentinel species to evaluate the presence of some diseases. Special attention was given to several zoonoses, i.e. diseases shared by animals and humans.

SURVEILLANCE OF THE SPATIAL DISTRIBUTION OF COMPETENT VECTORS

When establishing surveillance of the geographic distribution of competent vectors, certain information is needed. First, the disease or pathogen of interest, which defines the vector to look for. One possibility is to try to identify the presence or absence of the disease, or also the etiological agent, and accordingly, the areas where it should be investigated its presence. or capturing early warnings of disease emergence. In this case, it would be necessary to know the suitable environments in which the vector maintains, since if surveillance is carried out in ecologically unsuitable habitats, its absence could erroneously indicate a low risk of BDV in the entire area of examination (Guillot et al., 2022). Conversely, if the objective is to determine the size of populations, monitoring should be conducted on a random basis.

The surveillance of sentinels is counseled for the purpose of improving the possibilities of focusing on specific sites, which will provide information regarding risk in larger areas, reducing thus the resources required by limiting sampling units and effort (McCluskey, 2003; Botella et al., 2021).

VBDs AND HUMAN PATIENTS

The importance of vector-borne diseases is reflected on circa 17% of all deaths attributed to infectious diseases seem caused by VBDs, which means more than 700000 yearly deaths are related to the participation of mosquitoes and/or ticks (The World Health Organization, 2017). The interactions between pathogens (viruses, bacteria, protozoa), vectors (diversity, density), hosts (humans, animal reservoirs), and environment (climate, geographical location) are complex. In relation to this, changes in climate and environment are directly involved in the risk of appearance of both vectors and VBDs into new areas, as demonstrates the recent detection of dengue and chikungunya outbreaks in Southern Europe, or malaria, Zika or yellow fever outbreaks in China (De Silva et al., 2012).

An interesting point related to certain VBDs affecting humans relies on the habitats

where people are living. From a rather generalist point of view, there is a tendency to associate the VBDs to a dichotomous classification involving rural or urban settlements (Onstwedder et al., 2022). As results, the risk of Lyme disease and CCHF is more possibly frequent in rural areas, while others such as malaria, Chagas disease, and sleeping sickness do it to urban zones (Symmons et al., 2012; De Silva et al., 2012), because of unplanned and extensive urbanization, invasion by different vectors belonging to the genus *Aedes* (Bracks et al., 2011). Besides this, new opportunities for VBDs to flourish and spread originates in the cities of the developing world, compromising the well-being of populations.

In addition to all explained here, it is needed to take into account the information indicated in previous sections regarding the importance of changes in environment and climate, because people lifestyle might profoundly affect, improving they can enjoy of walking across natural spaces in forests, increasing the time spent on agricultural work such as planting vegetables, rising the time destinated to visiting zones suitable for survival and distribution of vectors, etc.

SENTINELS SURVEILLANCE: ANIMALS

This is a widely used term referring frequently the idea of standing guard or keeping watch. Since the second half of the 20th century, it has been recognized that animals can act as important sentinels for a wide range of environmental health threats (CAMEH, 1991). There is a widespread view that a sentinel is a naive animal that is also intentionally placed in an environment of possible infection, being monitored at time intervals to detect infection (Hoinville, 2013). If the sentinel is placed near human settlements, it should react to the infectious agent without becoming infected, thus providing early warning of risks to human health in the environment (George et al., 2020). Besides this, sentinels can go from individual animals to herds or larger populations, from animals of the same species to different, more susceptible, or more accessible species. In terms of geographic location, sentinels can be deliberately placed or inserted into existing sentinels in a given location.

In recent decades, passive surveillance has been gaining relevance, especially when supported by the existence of certain animal species that do not become infected after exposure to a pathogen, but are able to develop a response against it, which can be advantageously used for detection (Aguilar-Vargas et al., 2022). An example is the Crimean-Congo Hemorrhagic Fever (CCHF), a VBD caused by a virus transmitted by *Hyalomma* infected ticks biting the hosts, or by direct contact with blood or tissues of infected ticks, people and livestock. More than 80% of cases are asymptomatic or mild, with any symptoms appearing within 2-7 days of infection (ECDC, 2024).

Finally, the sentinel concept can also refer to a physical location, such as a farm, slaughterhouse, veterinary practice or laboratory, a so-called *sentinel unit*, which is selected to monitor a specific disease (Neo & Tan, 2017).

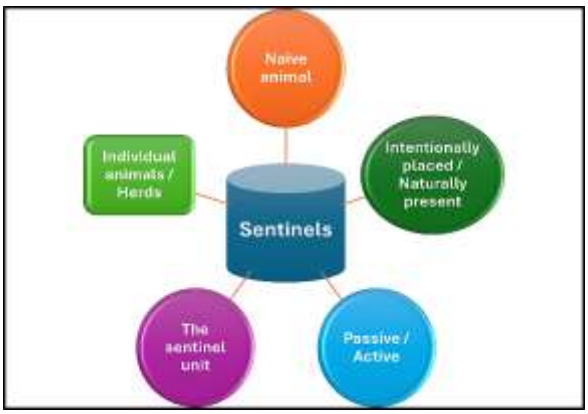

Figure 2. Surveillance of vector-borne diseases requires adequate observation in a specific area, definition of appropriate sentinel species, and sufficient time to obtain the expected information.

VBDs SURVEILLANCE NETWORK PROPOSAL

Capture of vectors

Figure 3 shows a proposal which includes the main points to consider in the designing of vector sampling procedures. For the purpose to integrate them, the designing includes surveillance on two main blocks, environment and sentinels.

All the tick species which are involved in disease transmission develop a phase in the soil, from the egg to the larval stage, then surveillance should include sampling of questing ticks by means of the blanket *dragging/flagging* method (Newman et al., 2019) (Figure 4), comprising that a white cotton flannel cover (1 × 1 m) is dragged/flagged for 10 -25 min, taking care to maintain full contact with the litter throughout the sampling and stopping every 2-3 min to remove all attached ticks.
Concerning the sampling places, it must be performed in different localizations including several habitats surrounding farms, buildings, gardens, roadsides… It should be emphasized that the *Hyalomma* species are difficult to catch with this strategy (Valcárcel et al., 2020).
At the same time, dipteran trapping should be carried out using different procedures advised by the Centers for Disease Control and Prevention (CDC), which (ideally) should be placed at or near points with water, organic matter (manure) and animals that serve as blood donors (Figure 5).

With the aim to gain information on the vectors encompassed in the transmission of diseases, certain animal species and people are both very useful and then could be monitored (Figure 3). Surveillance of people seems especially helpful and productive because of the possibility of picking ticks off the skin of patients while when attend the primary health care office (Benzoni, 2024). Other helpful strategy involves population participation by using social media (mainly networks), with the aim to get their collaboration.

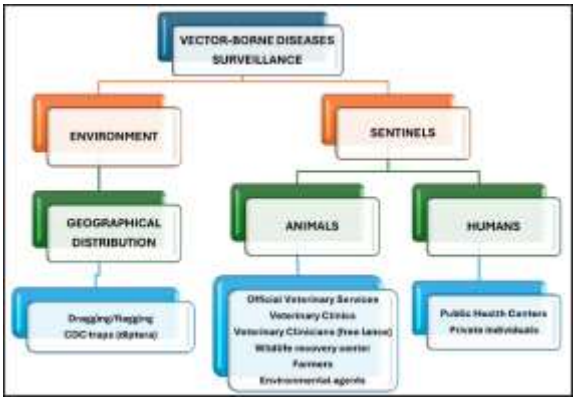

Figure 3. A proposal of surveillance of vector-borne diseases in a specific area should be supported on the surveillance of proper vectors together with animal species and finally humans.

Figure 4. Surveillance of questing ticks is done by the dragging/flagging (left) method, which allows to catch ticks as adult *Dermacentor* specimens (right).

As for animal surveillance, it seems more realistic among domestic species, but would probably restrict the vector species captured to those present in specific habitats with proximity to human settlements, infesting mainly domestic animal species.

Figure 5. Capture of dipera can be conducted by placing CDC light traps (left) or BG Sentinel traps (upper right Side).

A possible solution, especially for ticks surveillance, could include sampling of wild species that live mainly in forests and open areas, for example game species, or those received in wildlife recovery centers (Matei et al., 2023). Another choice could encompass monitoring free-roaming animal species (cattle, horses) but this could present some disadvantages related to their proper immobilization or seasonality. Diptera surveillance could also present similar troubles.

Detection of pathogens circulation

Surveillance of vector-borne diseases needs not only monitoring vector species and studying their phenology but also to establish the possibilities that they are transmitting diseases. Two appear the most practical options, a) analysis of vector specimens for identifying the pathogens directly or their nucleic acids (Plowright et al., 2017).

b) serological surveys which might offer the possibility to give information on the exposure to a pathogen by monitoring immunological responses in the blood of animals or humans (Carrasquilla et al., 2023).

CONCLUSIONS

Surveillance of VBDs is a complex task entailing different points as designing protocols for the capture of competent vector species, identification of targeted mammals and checking for the active infection or exposure to certain pathogens. This underlines the importance of integrating different areas of work, i.e. environmental, animal and human health. All the three are fundamental to achieve the expected results, and remark also the essence of One Health approach which should enhance the efforts to be performed with this objective.

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Sustainable control of pig helminths by using filamentous saprophytic fungi

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Abstract

Nowadays, due to the increasing difficulty in controlling certain helminths in pig farms, together with the economic losses and the reduced growth of piglets, it is necessary to search for an alternative method of control and prevention to drugs. A hopeful option could be found in biological control, using some saprophytic fungi, which would also solve the problem of resistance to the different families of antiparasitic drugs. In the present study, the effect of fungi such as Clonostachys rosea *and* Trichoderma atrobrunneum *on the eggs of the nematode was analyzed. For this purpose, 19500 eggs of the nematode Ascaris suum were exposed to fungal for 45 days. To check the viability and performance of the experiment during the experiment, five observations were made (days 0, 12, 25, 35 and 43 of exposure), assessing the morphology and degree of development of the eggs. Thus, it was established that the fungus* C. rosea *had an ovistatic effect during this interval, which consisted of inhibiting the development of the eggs, which remained viable but did not embryonate. On the other hand,* T. atrobrunneum *showed a reversible ovistatic effect until day 35 of the experiment, and from that moment on, the percentage of viable eggs developing increased. These results indicate that the use of these saprophytic fungi could be a good alternative for the prevention of helminths in pig farms.*

Key words: *parasites, swine, biological control, prevention, ovistatic, ovicidal*

INTRODUCTION

Helminths are described as among the most common parasites in all pig farms worldwide, but they generally receive less attention than in other species, possibly because they rarely present with clinical signs noticeable to the farmer (Roepstorff et al., 2011).

The genus *Ascaris* spp. belongs to the group of soil-transmitted helminths. The species found in swine farms is *Ascaris summ*, a nematode with a direct biological cycle, without free-living larval stages. Adult females release eggs with the feces into the environment (soil), where larvae 1 and 2 (L1 and L2) develop. Swine become infected by ingesting eggs with L2 that contaminate the soil or are attached to the skin of the progenitors. Inside the stomach or small intestine, L2 hatch and penetrate the mucosa of the cecum and colon and migrate through the portal vein to the liver (4th day pi) (Masure et al., 2013), where they cause inflammatory lesions commonly known as "milk spots", leading to the seizure of that viscera (Dold & Holland, 2011). Then, after moving through the caudal vena cava to the lungs, the larvae ascend the bronchial tree, reaching the pharynx and being swallowed and passing back to the digestive tract (10-15 days pi). Once in the small intestine, they mature into adults, where the females, after copulation, eliminate the eggs that are

released into the environment with the feces (Bowman, 2022).

Eggs of *A. summ* are highly resistant to environmental factors, which allows them to remain viable and infective in the soil for 6 years (Roepstorff et al., 2011).

Despite its subclinical course, this parasite has been shown to cause a decrease in average daily gain (ADG) and an increase in feed conversion ratio (FCR) in animals (Vandekerckhove et al., 2019), an aspect that is related to the fact that farms with a high number of seized livers also show an increase in FCR (Martínez-Pérez et al., 2017).

There are different treatments for the control of ascarids. Fenbendazole is administered in drinking water for two days in the finishing period in intensive swine farms (Lassen et al., 2017). Other drugs are amines (piperazine salts), imidazothiazoles (levamisole) and macrocyclic lactones (ivermectin) (Urquhart et al., 2001). An increasing problem is their diminishing effect and resistance to antiparasitics (Lassen et al., 2017).

The resistance and longevity of eggs are a drawback for the control of ascariosis, especially in outdoor production systems, because of most drugs are not ovicidal, and common disinfectants are not effective against *A. summ* eggs (Oh et al., 2016). In addition, there is an increasing demand for organically produced meat and dairy products (Szewc et al., 2021).

The use of different fungi for the treatment of parasitic forms, especially helminths, is a promising alternative that is being increasingly tested (Canhão-Dias et al., 2020; Pérez-Anzúrez et al., 2022; Lozano et al., 2024; Salmo et al., 2024a). Among the best known parasiticidal fungal species are:

- *Duddingtonia flagrans* which has been shown to have high efficacy against larvae of the most important species of gastrointestinal nematodes. Its efficacy has been altered if combined with other fungal species, being higher when combined with *Monacrosporium* spp. and *Pochonia chlamydosporia* (Szewc et al., 2021).
- *Mucor circinelloides* is a filamentous saprophytic fungus with action against different eggs to which it attaches to its surface and penetrates inside, feeding on their contents. It has been shown that high ovicidal and larvicidal activity can be achieved by culturing *M. circinelloides* together with *D. flagrans* (Arias et al., 2013; Voinot et al., 2020).
- *Pochonia chlamydosporia* attacks helminth eggs through specialized structures that allow its adhesion to the egg surface and penetration by mechanical and enzymatic action (Araújo et al., 2021).

The present study aimed to determine the antagonistic activity of the fungi *Clonostachys rosea* and *Trichoderma atrobrunneum* on eggs of the gastrointestinal nematode *A. suum*, two species utilized in the control of parasites affecting plants (Fanelli et al., 2018). For this purpose, eggs of the nematode *A. suum* were directly faced with the spores of the two fungi in aqueous solution.

MATERIALS AND METHODS

Fungal spores

Spores of two saprophytic filamentous fungi, isolated by the COPAR Research Group (GI-2120; USC) from samples of animal feces and soil from agricultural farms, were used in this work. Once identified as *Clonostachys rosea f. catenulata* (CECT21110) and *Trichoderma atrobrunneum* (CECT20999), they were deposited in the Spanish Type Culture Collection (Valencia, Spain).

The two fungi were cultured in the submerged medium COPFr (Arias et al., 2013) until a concentration around 10^6 spores / mL was attained.

Collection of A. suum *eggs*

Samples were collected from two pens at the farm of the PRODEME Foundation (Monforte de Lemos, Lugo, Spain), a non-profit institution dedicated to the care of adults with functional diversity. The pens housed two groups of 12 piglets of 5 months of age, and 4 samples were taken directly from the floor in each one, for a total of 8 samples of approximately 100 g. Once in the laboratory, each was analyzed by the quantitative McMaster or saline flotation technique to visualize eggs or oocysts. Samples were processed within 24 hours to avoid deterioration of possible parasitic formations. With the aim to get elevated amounts, the eggs of *A. suum* were

washed in water and then centrifuged at 3500 rpm for 5 min.

Experimental design

Thirty-six 2-mL plastic tubes were added 600 eggs of *A. suum* in 30 µL water (day 0), then divided into three lots of 12 tubes each. One group was added 200 µL medium containing $5x10^6$ *C. rosea* spores, other group 200 µL medium containing $5x10^6$ *T. atrobrunneum* spores, and the control received 200 µL distilled water as controls.

All tubes were maintained at RT and 12 h light-dark cycles for 48 days.

Antagonistic effect on A. suum *eggs*

Evaluation of the parasiticide activity encompassed the examination of three tubes per group on days 12, 24, 36 and 48. Analysis consisted of determining the egg-viability to establish the ovicidal role of the fungal species (Hernández et al., 2018). Then viable eggs were sorted into undeveloped (VU) or developing (VD), based on the presence of cellular division or larva inside (Figure 1), to check if the fungal species influence the egg development rates (ovistasis) (Ciarmela et al., 2018).

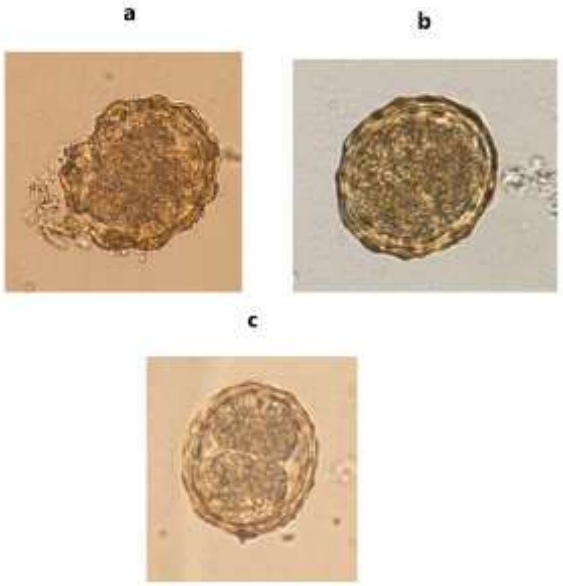

Figure 1. Eggs of *A. suum* observed under 20X. a) Non-viable (NV); b) Viable undeveloped (VU); c) Viable developing (VD).

The procedure consisted of centrifuging each tube for 20 seconds to 6000 rpm, then collecting a 25 µl aliquot of the sediment and placing it on a slide covered with a glass coverslip and evaluating it under the Leica DM2500 optical microscope at 20x magnification, which took 15-20 min per aliquot. Sometimes it was necessary to use more volume to count at least 150 eggs, so it was necessary to take more than one aliquot per tube.

The percentage of reduction of the eggs was estimated by using equation 1:

$$\% \text{ Red} = (EPG_{day0} - EPG_{day48}) / EPG_{day0}) \times 100 \quad (1)$$

Statistical analysis

Because of the Kolmogorov-Smirnov test showed the data collected were normally distributed (Z= 1.202, *P*= 0.111), and the Kolmogorov-Smirnov that variances were homogeneous (F= 0.010, *P*= 0.922), the results were analyzed by One-way ANOVA with repeated measures, followed by the Bonferroni test for the post-hoc evaluation.

Significant differences were considered when *P*< 0.05. All tests were done using SPSS for Windows (20.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Viability of A. suum *eggs*

As can be seen in Figure 2, the viable eggs remained constant in the controls throughout the study. Slight reductions (~6%) were also recorded in the presence of *C. rosea*, which increased with *T. atrobrunneum*, with a 16% diminution at the end of the trial. No statistically significant differences were obtained among the three groups (F= 0.618, *P*= 0.555).

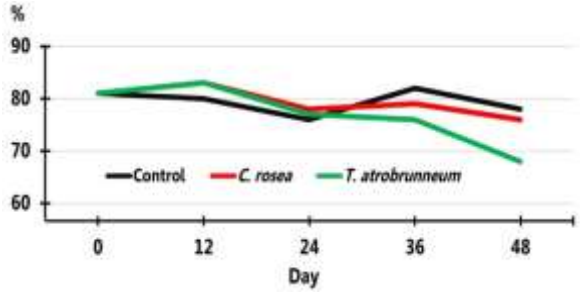

Figure 2. Evolution of viable eggs of *A. suum* exposed to saprophytic filamentous fungi.

Development of A. suum *eggs*

The percentages of VU eggs in the controls decreased along the study (20.4%; Figure 3). When exposed to *C. rosea*, the lowest percentages of VU were obtained until day 36, then increased in 12.2% to the end, and less than half of the eggs did not develop.

Regarding the exposure to *T. atrobrunneum*, the pattern was reversed, with percentages of 50-60% until day 36 post-exposure, and then decreasing to 35%, which means a 26.5% reduction. The differences according to the group were not statistically significant (F= 1.339, *P*= 0.299).

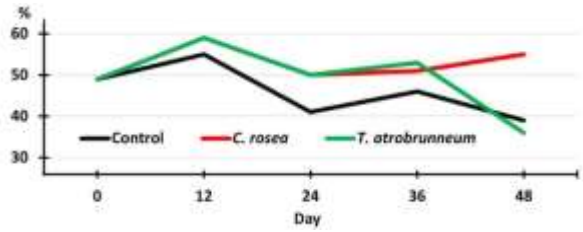

Figure 3. Evolution of viable undeveloped eggs of *A. suum* exposed to saprophytic filamentous fungi.

As drawn in Figure 4, the percentages of VD eggs increased significantly in the controls towards the day 48, when the maximal values (39%) were attained.

After 48 days of exposure to *C. rosea* the percentages of VD reduced to 21%, which means a 34.4% reduction in respect to day 0 (Figure 4). In the presence of *T. atrobrunneum*, the VD percentages diminished by 28% until day 36, then returned to the initial values.

No infective eggs (with L2 larvae inside) were observed in none of the groups.

Significant differences were recorded for the VD values (F= 6.120, *P*= 0.015), and with the Bonferroni test the differences were attributed to the controls and the eggs exposed to *C. rosea* (*P*= 0.023) and to *T. atrobrunneum* (*P*= 0.046).

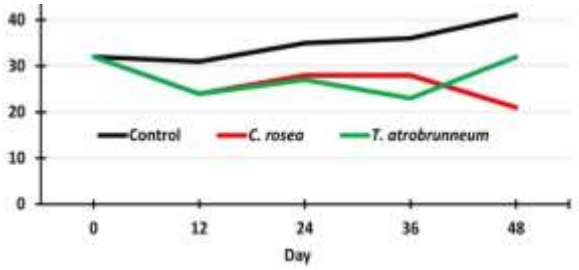

Figure 4. Evolution of viable developing eggs of *A. suum* exposed to saprophytic filamentous fungi.

DISCUSSION

Exposure of porcine gastrointestinal nematode *A. suum* eggs to two saprophytic filamentous fungi, *Clonostachys rosea* and *Trichoderma atrobrunneum* revealed a weak to moderate effect, respectively, on their viability, and the best results (16% reduction compared to controls) were achieved 48 days after being faced with *T. atrobrunneum*. Prior studies reported a 57.3% reduction on viability of eggs of *A. suum* in pigs given orally *Pochonia chlamydosporia* spores, and a 60% reduction by providing piglets mash feed-added *M. circinelloides* spores (Cortiñas et al., 2015). It is noteworthy to take into consideration that the viability of *A. suum* eggs dropped by one half and two thirds, respectively, when spores of *C. rosea* and *T. atrobrunneum* were directly sprayed onto the feces of pigs naturally infected (Viñas et al., 2020).

For the purpose of getting information about the two fungi might exert any kind of activity on the development of the eggs, the percentages of undeveloped and developing eggs were estimated. Thus, it was concluded that *C. rosea* has an ovistatic activity, influencing the development of more than half of the eggs until preventing them from evolving during a period of 48 days, and avoiding them from reaching the infective stage, thus eliminating the risk of infection in swine. The observation of the percentage of viable and developing eggs maintained between 20-30% for 48 days confirms the ovistatic effect previously reported in other studies conducted on eggs of *Parascaris equorum*, roundworm affecting equids (Hernández et al., 2017). By other hand, a reversible ovistatic effect

during the first 36 days seems to occur in the presence of *T. atrobrunneum*, which reverses from this time. This phenomenon of ovistasis is of great importance, since the ingestion of undeveloped eggs (without a L2 inside) does not entail any risk, thus reducing the chances of infection in swine along this period (Hernández et al., 2018). Even in the case of reversible ovistasis, this delay in development could favor its destruction with abiotic agents (solar radiation, dryness) or biotic agents (fungi, viruses and bacteria) (Blaszkowska et al., 2014).

One helpful procedure appears to be to apply other methods such as the combination with other fungi, pharmacological treatments or rotation of animals between boxes (Viña et al., 2020; Araújo et al., 2021).

In recent years, progress has been made in the isolation, characterization and use of fungi as biological control tools (Canhão-Dias et al., 2020; Paz-Silva et al., 2023; Salmo et al., 2024b), but even so there are still many characteristics to be known, as their mechanisms of action, or adequate conditions to reach the highest antagonistic activity (Cortés-Sánchez & Mosqueda-Olivares, 2013). The finding of a lower ovicidal effect in the present investigation than in previous studies could be attributed to differences in the substrate containing the eggs (Viña et al., 2020). Consequently, it could be thought that aqueous media do not provide suitable conditions for the fungi to develop and exert their parasiticidal effect, but further research is required to confirm this.

CONCLUSIONS

The analysis of the results obtained lead to state the ovistatic activity the filamentous fungus *Clonostachys rosea* and *Trichoderma atrobrunneum* exert on the eggs of *Ascaris suum*. In this way, their use provides a very useful sustainable tool to reduce the infectivity of *A. suum*, and even could increase the effect of conventional treatments in limiting their survival in the soil.

FUNDING

Study partially financed by FEADER Project 2022/017B (Operational Groups AEI 2022, Xunta de Galicia), and by Project PID2020-120208RB-I00 (Ministry of Science and Innovation, Spain; European Regional Development Fund).

ACKNOWLEDGEMENTS

We would like to thank to the Head of the "Granja Gayoso Castro" (Deputación Provincial de Lugo, Spain) for the valuable cooperation in producing fungal spores.

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First biochip based screening of antibiotic residues in livestock products produced in Kazakhstan

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Abstract

Meat, milk, and poultry products are the main food products in the domestic consumer market of Kazakhstan. Antibiotics and hormonal drugs are used in veterinary medicine and livestock farming to treat various diseases of animals and stimulate their growth. Our study aimed to test whether antibiotic residues were present in the different types of meat. In this study, for the first time in Kazakhstan, screening of antibiotic residues in animal products such as beef, horse meat, and chicken was carried out based on the Antimicrobial I Ultra Array kit and Antimicrobial I Array Plus kit biochip systems (Randox Food Diagnostics, UK). Beef and chicken samples were positive for antibiotics. The study was conducted in the frame of the scientific and technical program BR21882327 for 2023-2025. Screening of 123 beef samples showed that 20% were positive for sulphamonomethoxine, 17% dapsone, 17% streptomycin, 23.5% tylosin, 23.5% tetracyclines, and 23.5% thiamphenicol. Testing of 63 chicken meat samples revealed 15% dapsone, 17% streptomycin, 5% tylosin, 5% tetracyclines, and 10% thiamphenicol. No residues of the above antibiotics were found in horse meat. In Kazakhstan, the presence of antimicrobial and growth-promoting drugs in animal products is regulated by Technical Regulations of the Customs Union TR CU 021/2011 2 "On the safety of food products" and TR CU 034/2013 "On the safety of meat and meat products," where specified are their maximum permissible levels in meat. In general, the obtained indicators did not exceed the standards regulated in the documents TR CU 021/2011 and TR CU 034/2013. Our findings indicate the need for more in-depth research on screening antibiotics in livestock products and increased attention to monitoring and regulating the use of antibiotics in livestock production. Uncontrolled use of antibiotics in livestock will cause the development of antibiotic resistance and increase health-related threats to the population.

Key words: *Livestock products, Meat, Antibiotics, Antibiotic resistance, Veterinary medicine*

Evaluation of β- and κ-casein variants in 16,000 Polish Dairy Heifers

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Abstract

In 2023, the "A2A2: A New Path in Dairy Products" program genotyped 16,000 heifers to investigate the potential for creating A2A2 and A1A2 subpopulations, while also establishing a foundation for analyzing other functional traits, such as κ-casein. Casein proteins, which make up about 80% of the proteins in cow´s milk, include β-casein and κ-casein—both crucial for dairy production. The A2 variant of β-casein is considered easier to digest and is targeted at people with milk allergies. κ-Casein, encoded by the CSN3 gene, plays a critical role in stabilizing micelle formation in milk, preventing casein precipitation, which is essential for cheese-making. The BB variant of κ-casein is particularly advantageous, as it enhances milk's coagulation properties, leading to higher cheese yields and superior texture compared to the AA or AB variants. This makes the BB variant highly desirable for optimizing both the efficiency and quality of cheese production. The analyses focused on presenting the percentage occurrence of various β-casein and κ-casein variants, highlighting the distribution of the "best genotypes" in different regions of Poland. The study involved 16,000 heifers from the "A2A2: A New Path in Dairy Products - Utilizing Genomic Selection for Reduced Allergenicity - 2023 Edition" project. Ear tissue samples were collected by ear tag, and DNA was isolated using MagnifiQ™ kits. Single Nucleotide Polymorphisms (SNPs) were analysed using Illumina BeadChip XT, a DNA microarray technology that enables large-scale SNP genotyping across the genome. Data were processed with GenomeStudio 2.0 and analyzed in Microsoft Excel. Sample distribution varied significantly across regions, with the highest counts in Podlaskie (5,106), Warmian-Masurian (1,723), Opole (1,468), Łódź (1,269), and Silesian (1,267). The A2A2 variant was present in 51.20% of samples, while the BB variant was found in 20.73%. The kappa casein variant was undetermined in 0.04% of samples. The combination of A2A2 and BB genotypes was found in 11.63% of the population. These results suggest the potential for developing A2A2 and reserve A1A2 subpopulations in Poland. The BB variant may benefit dairy producers, though further research and market analysis are needed to evaluate the economic potential and optimize marketing and selection strategies for A2A2 milk.

Key words: *SNP, cattle, beta-casein, kappa-casein*

Influence of prebiotic Immunobeta and the combination Immunobeta + Zoovit probiotic on blood biochemical parameters in Ile-de-France lambs

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Abstract

The aim of the present study was to determine the effect of the addition of prebiotic Immunobeta and probiotic Zoovit on the biochemical parameters of blood in Ile-de-France lambs. The research was carried out at the Agricultural Institute - Stara Zagora. It includes a total of 45 Ile de France lambs, divided into three groups of 15 - one control and two experimental. The groups were formed by the method of analogues, equalized by live weight at the beginning of the experiment, type of birth and sex. The animals of the I experimental group received 8 g of the prebiotic Immunobeta individually once a day, and those of the II experimental group the same amount of prebiotic with the addition of 4 g of the Zoovit probiotic. Blood for the study of 8 animals from each group was taken at the beginning and at the end of the experiment. In the indicators of albumin, urea, glucose, choleterol, creatinine and bilirubin, significant differences were reported after the addition of the prebiotic Immunobeta compared to those at the beginning of the trial. A significant decrease in albumin (P<0.05), glucose (P<0.001), cholesterol (P=0.001), bilirubin (P<0.001) and increase in urea and creatinine levels (P<0.01) were found after administration of prebiotic Immunobeta. The addition of prebiotic Immunobeta + prebiotic Zoovit to the ration of lambs leads to a significant decrease in the values of albumin (P<0.05), glucose (P=0.012), cholesterol (P<0.001), bilirubin (P<0.001), alanine aminotransferase (P<0.05), increase in urea (P<0.001), urea/creatinine ratio (P<0.05) and tendency to increase in creatinine (P=0.082) after taking the combination prebiotic Immunoneta + probiotic Zoovit. A trend toward lower cholesterol was reported in the control group and the group supplemented with the prebiotic Immunobeta, as well as between the synbiotic group and the group supplemented with Immunobeta. An unproven trend towards an increase in the level of alkaline phosphatase was found in lambs receiving Immunobeta + Zoovit compared to the control group.

Key words: *Ile-de-France, lambs, prebiotics, probiotics, blood, cholesterol*

INTRODUCTION

The use of subtherapeutic doses of antibiotics in farm animals, as growth promoters and therapeutic doses for the control and treatment of infectious diseases, is the reason for the development of antimicrobial resistant microorganisms (Abreu et al., 2023; Caneschi et al., 2023). One of the possibilities to limit AML is preventive strategies with the use of probiotics, prebiotics and other biologically active substances. Probiotic therapies are at this stage an alternative to address the problems associated with increasing resistance due to the ability of probiotics to modulate the gut microbiota and the host's immune system, as well as their ability to act as a growth factor. Regardless of numerous studies to clarify the effect of probiotics on strengthening the immune system, improving the intestinal microflora and the health status of animals (Al-Shaar et al., 2023; Fenta et al., 2023; Mao et al., 2023; Santana et al., 2023; Zhang et al., 2023; Zhou et al., 2023; Abdullah et al., 2022; Kholif et al., 2022; Reuben et al., 2022; Elaref et al., 2020; Chashnidel et al., 2020; Mousa et al., 2019; Vosooghi-Poostindoz et al., 2014) new multidisciplinary studies are needed to determine which therapies are effective in a given animal species and how to apply them to overcome AMR.

Administration of probiotics helps improve the overall health status of the body by accelerating protein and carbohydrate-fat metabolism with an increase in the concentration of serum total protein, albumin and globulin (Devyatkin et al., 2021).

Hussein, (2018), El-Katcha et al. (2016) and Moarrab et al. (2016) reported no significant effect on blood plasma parameters except for cholesterol, which was lower in animals receiving probiotic and synbiotic compared to the control group. It is likely that the reduction in the level of cholesterol in the blood caused by the action of probiotics and synbiotics is the result of inhibition of its synthesis through assimilation processes. The decrease in cholesterol concentration is due to the hypocholesterolemic effect of inulin and the deconjugation of bile fatty acids, in the process of which lactic acid bacteria take part. This leads to reduced absorption of fatty acids in the intestine and, accordingly, to their lower concentration in the blood (Yoo and Kim, 2016; Moarrab et al., 2016).

In an experiment with dairy goats receiving a probiotic in the ration Mohammed et al. (2013) found an unreliable increase in the blood serum levels of urea, creatinine and cholesterol and a decrease in the transaminases acetaminotransferase, alanine aminotransferase and glucose. Milewski et al. (2009), conducting an experiment with sheep supplemented with a probiotic with the participation of Saccharomyces cerevisiae found a reliable increase in the concentration of glucose, Na+ and Cl- ions, a decrease in the level of creatinine and a change in the acid-base balance. The authors indicate that changes in blood biochemical indicators suggest that the probiotic supplement has a stimulating effect on energy metabolism, as well as a protective effect on kidney function, but also helps prevent acidosis in experimental animals.

Despite a number of data, the beneficial effect of probiotics in animals is not consistent, necessitating the development of probiotic therapies for the relevant species and categories of animals, as prevention, which can delay and influence the development of resistance

The aim of the present study is to determine the effect of the addition of prebiotic Immunobeta and a combination of prebiotic Immunobeta + probiotic Zoovit on blood biochemical parameters in Ile-de-France lambs.

MATERIALS AND METHODS

The experiment was conducted in the experimental base at the Agricultural Institute - Stara Zagora. The experiment involved 45 lambs of the Ile de France breed (ILF), divided into three groups - control and two experimental - 15 in each. The groups were formed by the method of analogues, equalized by live weight at the beginning of the experiment, type of birth and sex. The lambs were raised in groups in pens, equipped with feeders for hay and concentrated feed and with drinkers with constant access to clean drinking water, according to the requirements of Ordinance No. 40, for the conditions for raising farm animals, taking into account their physiological and ethological characteristics. The animals were fed ad libitum (+ 5 to 10% residue) in a ratio corresponding to their age and meeting the requirements for nutrients and biologically active substances. The ration included concentrate and alfalfa hay (Table 1 and Table 2).

Table 1. Composition of the combined fodder for feeding
IlF lambs

Component	% of investment
Soy meal	4.00
Chalk	3.00
Salt	0.50
Wheat	42.00
Premix-16-97-K	0.20
Sunflower meal	20.00
Corn	3030

Table 2. Nutritional composition of the combined feed

Component	% of investment
Component	15.90
Protein	2.40
Fats	5.43
Fibers	11.40
Moisture	1.20
Approx	0.50
P	0.570

Table 3. Chemical composition of Zoovit probiotic

Component	% of investment
Protein	29.29
Lactose	52.14
Fats	0.95
Dry substance	94
Lactic acid	2.75
Propionic acid	3.10
Mineral substances	8.76
Number of active cells	no less from 2,5 x 10^7 cfu/g
Coliforms	are not established
Molds and yeasts	are not established
Salmonella in 25 g	are not established
Coagulase-positive staphylococci in 1 g	are not established

Preparation

The combined feed contains 1.12 feed units, 2778.25 Kcal/kg and TDN 0.174.

The animals of the I experimental group received 8 g of the prebiotic Immunobeta individually once a day, and those of the II experimental group the same amount of prebiotic with the addition of 4 g of the Zoovit probiotic.

The probiotic preparation Zoovit includes four strains of lactic acid bacteria: Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, Lactobacillus acidophilus, Lactobacillus lactis and one strain of Propinibacterium.

Immunobeta is a prebiotic preparation with a pronounced immunostimulating effect, obtained from certain strains of Saccharomyces cerevisiae yeast through a process of enzymatic autolysis. Its composition includes 30% β-glucans, 25% mannanoligosaccharides and 7% nucleotides.

Blood for the study of 8 animals from each group (total 24) was taken in the morning after a 12-hour fast from the external jugular vein (v. jugularis interna) at the beginning and at the end of the experiment. After collection, the blood samples were analyzed on a MN Chip biochemical analyzer.

Data were statistically processed by one-way analysis of variance ANOVA using IBM SPSS software (ver. 19) and Student's t-test for dependent samples was performed to compare the values of hematological indicators.

A check was made for homogeneity of variances and for normal distribution of individual variables. When rejecting the null hypothesis, a non-parametric analogue of the one-factor analysis of variance - the Kruskal-Wallis test - was applied, and for the non-parametric alternative of the t-test for related samples - the Wilcoxon test.

One-way parametric analysis of variance (ANOVA) was performed on variables with equal variances and a normal distribution.

In cases of established statistical significance of the F-statistic ($P \leq 0.05$), an additional post hoc LSD test for multiple comparisons was performed.

RESULTS

Table 4 presents the results of the biochemical analysis of blood serum in ILF lambs.

The albumin values in the three groups of lambs were close: 30.45g/L, 29.89g/L and 29.69g/L for the control, I and II experimental groups, respectively. The values for the globulin are close in the I and II experimental groups - 25.58 and 26.40g/L, respectively, and the differences are unreliable.

The lowest levels of total protein were found in the serum of animals from experimental group I - 55.46 g/L, followed by lambs from experimental group II - 56.09 g/L and the control group - 58.79 g/L. No mathematical differences were found.

The concentration of glucose recorded the highest value in the II experimental group - 5.36 mmol/L, followed by the I group with 5.18 mmol/L and the control group with 4.79 mmol/L, the differences being mathematically unreliable.

The highest cholesterol levels of 2.22 mmol/L were recorded in the animals of the control group, followed by the II experimental group with 2.18 mmol/L and 1.73 mmol/L in those of the I experimental group ($P=0.062$). There was a tendency to decrease the levels of the indicator between the control and the I experimental group, as well as between the II experimental group and the I experimental group.

Table 4. Biochemical indicators of blood in Ile de France lambs in control group, I experimental group with the participation of prebiotic Immunobeta and II experimental group with prebiotic Immunobeta + probiotic Zoovit

Indicators	Groups of animals			
	Control group (n=8)	I experimental group (n=8)	II experimental group (n=8)	p-value
	x̄±SD	x̄±SD	x̄±SD	
Albumin (G/L)	30.45±1.94	29.89±2.73	29.69±2.09	0.788
Total protein (g/L)	58.79±2.45	55.46±5.54	56.09±2.76	0.182
Globulin (g/L)	28.34±1.89	25.58±3.16	26.40±2.49	0.098
Ratio albumin/globulin	1.08±0.10	1.19±0.10	1.16±0.15	0.166
Glucose (mmol/L)	4.79±0.70	5.18±0.64	5.36±0.60	0.214
Urea (mmol/L)	9.65±1.20	9.29±1.74	9.13±1.98	0.820
Phosphorus (mmol/L)	2.14±0.44	2.43±0.74	2.54±0.19	0.146
Cholesterol (mmol/L)	2.22±0.39 **a**	1.73±0.27 **b**	2.18±0.58 **a**	0.062
Alanine aminotransferase (U/L)	14.25±8.56	31.00±48.45	15.25±7.59	0.893
Bilirubin (umol/L)	5.12±2.53	3.32±0.94	3.98±1.22	0.129
Alkaline phosphatase (U/L)	35.13±25.5 **b**	52.00±27.00 **ab**	68.13±26.27 **a**	0.051
Creatinine (umol/L)	79.00±6.97	76.38±9.09	76.00±5.78	0.682
Ratio urea/creatinine	30.50±3.42	30.13±4.82	30.13±7.43	0.988

a; b – when using the LSD test, different letters in a row mark a trend for statistically significant differences

Regarding the level of urea, the highest values are the control group - 9.65 mmol/L, followed by the animals of the I experimental group - 9.29 mmol/L and those of the II - 9.13 mmol/L, as the differences are statistical unproven. In the lambs of I and II experimental groups, which received as supplements, the serum concentration of urea was lower compared to the control group. No statistical significance between groups was reported.

Alkaline phosphatase in the blood serum had the highest values in the animals of the II experimental group - 68.13 U/L, followed by the animals of the I experimental group with 52.00 U/L, and the animals of the control group had the lowest values. group - respectively 35.13 U/L Between the control and II experimental groups, an unproven tendency to increase the levels of the indicator was established (P=0.051).

The results of the analysis of the biochemical indicators in the 1st experimental group at the beginning and end of the experiment are presented in table 5. The data show a decrease in the albumin values from the beginning of the experiment - 33.52 G/L to 29.89 G/L at its end, with the differences were proven at P<0.05.

A similar trend of lowering the serum concentration of total protein - 59.76 g/L at the beginning to 55.46 g/L at the end of the experimental period, the differences being unreliable.

A significant reliable decrease was found in the glucose values - from 8.55 mmol/L at the beginning to 5.18 mmol/L at the end of the experiment (P<0.001), as expressed in percentage this decrease is 39.42%.

For the urea indicator, we found higher values (9.28 mmol/L) at the end of the experiment compared to the beginning of the experimental period (6.48 mmol/L), and these differences were proven at P<0.01.

The serum cholesterol level decreased significantly from 4.67 mmol/L at the beginning to 1.73 mmol/L at the end, the difference being significant at P=0.001.

Alanine aminotransferase is an indicator of normal liver function. It should be noted that the serum alanine aminotransferase level decreased in an unproven trend (P=0.074) from 25.43 U/L at the beginning to 14.14 U/L at the end.

Serum bilirubin values showed a highly significant decrease at the end of the experiment (3.32 umol/L) compared to the beginning (8.66 umol/L) at P<0.001.

Creatinine showed an increase at the end of the experimental period (76.38 umol/L) compared to the beginning (58.50) at P<0.01.

Table 5. Biochemical indicators of blood in Ile de France lambs with the participation of prebiotic Immunobeta in the I experimental group at the beginning and at the end of the experiment

Indicators	I experimental group		
	In the beginning (n=8)	At the end (n=8)	p-value
	$\bar{x}$±SD	$\bar{x}$±SD	
Albumin (G/L)	33.52±2.35	29.89±2.73	0.027*
Total protein (g/L)	59.76±2.55	55.46±5.54	0.093
Globulin (g/L)	26.24±3.16	25.58±3.16	0.712
Ratio albumin/globulin	1.30±0.24	1.19±0.10	0.288
Glucose (mmol/L)	8.55±1.53	5.18±0.64	0.000***
Urea (mmol/L)	6.48±1.33	9.28±1.74	0.004**
Phosphorus (mmol/L)	2.33±1.03	2.43±0.74	0.889
Cholesterol (mmol/L)	4.67±1.56	1.73±0.27	0.001***
Alanine aminotransferase (U/L)	25.43±16.89	14.14±9.32	0.074
Bilirubin (umol/L)	8.66±2.09	3.32±0.94	0.000***
Alkaline phosphatase (U/L)	48.63±33.11	52.00±27.00	0.851
Creatinine (umol/L)	58.50±10.65	76.38±9.09	0.007**
Ratio urea/creatinine	27.75±5.15	30.13±4.82	0.470

* - P≤0.05 ** - P≤0.01, *** - P≤0.001

Table 6. Biochemical indicators of blood in Ile de France lambs with the participation of prebiotic Immunobeta + probiotic Zoovit in the II experimental group at the beginning and at the end of the experiment

Indicators	II experimental group		
	In the beginning (n=8)	At the end (n=8)	p-value
	$\bar{x}$±SD	$\bar{x}$±SD	
Albumin (G/L)	32.05±2.33	29.69±2.09	0.029*
Total protein (g/L)	58.16±4.39	56.09±2.76	0.195
Globulin (g/L)	26.11±3.99	26.40±2.49	0.855
Ratio albumin/globulin	1.24±0.21	1.16±0.15	0.378
Glucose (mmol/L)	8.74±1.45	5.36±0.60	0.012**
Urea (mmol/L)	5.01±0.92	9.13±1.98	0.000***
Phosphorus (mmol/L)	2.71±0.20	2.54±0.19	0.196
Cholesterol (mmol/L)	5.04±0.96	2.18±0.58	0.000***
Alanine aminotransferase (U/L)	32.13±19.95	15.25±7.59	0.035*
Bilirubin (umol/L)	10.40±2.30	3.98±1.22	0.000***
Alkaline phosphatase (U/L)	46.38±24.08	68.13±26.27	0.114
Creatinine (umol/L)	63.50±18.98	76.00±5.78	0.082
Ratio urea/creatinine	21.50±8.93	30.13±7.43	0.035*

* - P≤0.05 ** - P≤0.01, *** - P≤0.001

Table 6 presents the results of the biochemical analysis of the blood serum of the animals of the II experimental group at the beginning and at the end of the experiment. The obtained results show similar trends as in the 1st experimental group.

When adding prebiotic + probiotic, analogously to the addition of only the prebiotic supplement in the feed of the lambs, the albumin values decreased reliably at the end of the experiment compared to its beginning - from 32.05 g/L to 29.69 g/L at P<0.05.

Total protein values also showed a decrease from 58.16 g/L at the beginning of the trial to 56.09 at the end, the differences being non-significant.

Regarding glucose in the blood serum, a significant decrease (P=0.012) was reported from 8.74 mmol/L at the beginning to 5.36 at the end.

The concentration of urea in the serum increased significantly from 5.01 mmol/L at the beginning to 9.13 at the end of the experiment (P<0.001).

There was a significant decrease in cholesterol level from 5.04 mmol/L at the beginning to 2.18 mmol/L at the end of the experimental period at P<0.001.

We find a significant decrease (P<0.05) in the level of alanine aminotransferase from 25.43 U/L at the beginning of the experimental period to 14.14 U/L at its end.

Serum levels of the bile pigment bilirubin decreased significantly (P<0.001) from 10.40 umol/L at baseline to 3.98 umol/L at the end

of the trial. Expressed as a percentage, the reduction is 61.73%.

We found a difference in the urea/creatinine ratio between the beginning and the end of the experiment at P<0.05.

DISCUSSION

In view of the increasing antimicrobial resistance in humans and animals, a thorough search is needed for new alternative sources of biological basis such as prebiotics, probiotics and synbiotics, with a pronounced cumulative effect on the complex physiological, microbiological and biochemical processes in animals and humans.

The results of the effect of probiotic substances on hematological and biochemical parameters in small ruminants are relatively few and contradictory. According to Ayala-Monter et al. 2019 the effect of similar substances depends on the selected microbial strain or combination of strains, dose, time and frequency of intake, diet, breed of animal, physiological stage, production system.

Elliethy et al. (2022) cited significant differences in serum levels of urea, creatinine and minor differences in cholesterol and triglycerides in lambs fed diets supplemented with prebiotics and probiotics.

Albumin is the most abundant protein in the body. It accounts for half of the total plasma protein content in healthy humans and animals.

In the conducted research, we found close values of albumin in all three studied groups, and the differences are unreliable. Similar to our results for albumin and globulin in probiotic-supplemented lambs were reported by Chen et al. (2021). No differences were found between the control group and the synbiotic-supplemented group. Similar results cited by Sheikh et al. (2019) in an experiment with Corydell lambs.

Dar et al. (2022) found an increase in total protein and globulin concentration when consuming a synbiotic in calves. Increased levels of total protein following probiotic intake in lambs were reported by Abed et al. (2018), of albumin and globulin Abdel-Salam et al. (2014), Hussein, (2014), Ismaeel et al. (2010), and in goats Abu El-Ella and Kommonna (2013) and Singer et al. (2023).

Serum glucose concentration is affected by many factors, including nutrition, stress, age. We found the highest content of the metabolite in the synbiotic group compared to the other groups (P>0.05). Chen et al. (2021) found a decrease in serum glucose concentration in lambs supplemented with probiotic feed. Tabagde and others. (2020) reported a decrease in serum glucose concentration in lambs fed synbiotics.

Cholesterol plays an important role in the composition of the animal organism and some hormones.

We found the highest cholesterol levels in animals from the control group and the lowest in lambs from the group receiving the prebiotic Immunobeta.

Research on cholesterol content should continue in this direction.

A definite reduction in serum cholesterol concentration in lambs fed synbiotic supplements was reported by Moarrab et al. (2016). Chen et al. (2021) also reported lower cholesterol levels by 2.32 mmol\L in the probiotic-supplemented group compared to the control - 2.49 mmol\L

Urea and creatinine are interrelated. Creatinine is a waste product of muscle activity that is formed in the liver. The serum urea test provides important information about kidney and liver function, helping to diagnose various kidney diseases. Its importance is great in connection with the protein nutrition of animals.

The highest urea values were found in the control group, and the lowest in the lambs that received the prebiotic + probiotic combination. Different ones are statistically unproven. Higher levels of urea in lambs receiving a probiotic were reported by Chen et al. (2021). Dimova et al. (2013) reported an increase in magnesium (P<0.05) and a decrease in urea and calcium in lambs supplemented with the probiotic Zoovit.

Alkaline phosphatase is an enzyme that occurs in several forms in the animal body. It is formed in the liver, bones and kidneys, as well as in the placenta of pregnant animals.

In our study, we found increased levels of alkaline phosphatase with no proven trend between the control and II experimental groups (P=0.051). An increased serum

alkaline phosphatase concentration in sheep receiving a synbiotic supplement was reported by Fenta et al. (2023).

Sahib et al., 2023 in an experiment with sheep receiving probiotic supplementation did not find significant and reliable variations in this parameter.

We found a tendency of lowering the total serum protein at the beginning of the experiment and at its end, in the group that took the prebiotic Immunobeta.

Hussein (2018) cited a significant increase in total protein, glucose, urea nitrogen and aspartate aminotransferase levels compared to the control group.

Alanine aminotransferase and choleterol levels can be determined as indicators of normal liver function. In our study, the serum alanine aminotransferase level decreased with an unproven trend after taking the prebiotic Immunobeta. A decrease in serum alanine aminotransferase concentration in goats supplemented with prebiotics was reported by Yuan et al. (2023).

Total protein values in the group receiving the prebiotic + probiotic combination showed a decrease from 58.16 g/L at the beginning of the trial to 56.09 at the end, the differences being insignificant. According to Wang, et al. (2022) administration of probiotics improves immunity and increases serum total protein levels in dairy calves.

Yasmin et al. (2021) and El-Sayed and Mousa (2019) found that after administration of probiotics serum glucose levels in cattle and lambs decreased, and Toghdory et al. (2022), found a reliable decrease in serum glucose in lambs receiving probiotic + prebiotic. Mansilla et al. (2024) also cited a reduction in serum glucose, lipid profile and C-reactive protein values in calves receiving a probiotic based on lactic acid bacteria.

We note an increase in the concentration of urea in the serum, which increased significantly from 5.01 mmol/L at the beginning to 9.13 at the end of the experiment ($P<0.001$) after taking the synbiotic. A trend of increased urea levels after probiotic intake was cited by Azzaz et al. (2015) in goats.

We report a decrease in the level of cholesterol after taking the synbiotic, the differences being significant at $P<0.001$. Other authors also found a reduction in cholesterol values after administration of probiotics in cattle and lambs (Yasmin et al., 2021; Saleem et al., 2017). Abdel-Salam et al. (2014) also cited the lowering of cholesterol values in lambs after synbiotic supplementation.

CONCLUSIONS

1. A significant decrease in albumin ($P<0.05$), glucose ($P<0.001$), cholesterol ($P=0.001$), bilirubin ($P<0.001$) and increase in urea and creatinine levels ($P<0.01$) after taking the prebiotic Immunobeta.
2. The addition of prebiotic Immunobeta + prebiotic Zoovit to the ration of lambs leads to a significant decrease in the values of albumin ($P<0.05$), glucose ($P=0.012$), cholesterol ($P<0.001$), bilirubin ($P<0.001$), alanine aminotransferase ($P<0.05$), increase in urea ($P<0.001$), urea/creatinine ratio ($P<0.05$) and tendency to increase in creatinine ($P=0.082$) after taking the combination prebiotic Immunoneta + probiotic Zoovit.
3. A trend toward lower cholesterol was reported in the control group and the group supplemented with the prebiotic Immunobeta, as well as between the synbiotic group and the group supplemented with Immunobeta.
4. An unproven trend towards an increase in the level of alkaline phosphatase was found in lambs receiving Immunobeta + Zoovit compared to the control group.

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Blood serum electrolytes of heritage turkey hens and toms administered aqueous *Moringa Olerifera* leaves and seeds extracts

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Abstract

The serum electrolyte levels are essential in the regulation of nerve and muscle function, hydrates the body, balance blood acidity and pressure as well as help build damaged tissues. Hence the need to evaluate the impact of aqueous Moringa olerifera leaves and seeds extracts on heritage turkey toms. The study was carried out with 72 heritage turkeys for both hens and toms of about 8 to 9 weeks old. There were nine turkeys per treatment groups, grouped as; T_1 (control) T_2 (1% seed w/v) T_3 (0.5% leaf and 0.5% seed w/v) and T_4 (1% leaf w/v). Each group was replicated three times with three turkeys per replicate. The study latest for 154 days with all routine management practices duly observed. Data was collected for serum electrolyte analysis through the wing vein using sterile needles and syringes and were analyzed within 2 hours of collection. Three (3mls) of blood samples were drawn from three turkeys in each treatment. This was done on the last day of the trial. The blood serum electrolytes which are very essential in the maintenance of the body homeostatic function differed significantly (P<0.05) among the treatment evaluated for the toms (Potassium: T_1 $3.70^{\pm 0.10^{a}}$, T_2 $3.1^{\pm 0.07^{c}}$, T_3 $3.7^{\pm 0.13^{ab}}$ and T_4 $4.13^{\pm 0.00^{a}}$) with the exception of calcium. The calcium level though not significant was higher among the Moringa administered treatments than the control. Potassium levels differed significantly (P<0.05) among the treatment with treatment T_4 (4.13± 0.00) being the highest and T_2 (3.19±0.01) lowest which suggests that Moringa seed extract may have reduced the potassium levels in the turkey toms. The heritage turkey hens' serum electrolyte levels differed significantly (P<0.05) only in the calcium levels (T_1 $8.40+0.16^{a}$ T_2 8.86 ± 0.88^{a}, T_3 7.78±0.23b and T_4 7.89±0.03b). The sodium and potassium levels did not differ significantly (P<0.05) among the female turkeys. Cases of high potassium leading to paralysis and heart problem or low potassium resulting or leading to dehydration and excessive sweating was not witnessed in this study. The sodium level among the male turkeys differed significantly (P<0.05) among the treatment. The observed levels were lowest in T_2 (119.043 ±0.234) thus suggesting that Moringa seed extracts may have the potential to balance the body electrolyte level and reduce the risk of high blood pressure as envisaged in this study. This was lower than the results from leaf extracts. This confirms the anti-hypertensive qualities of Moringa. It may be that the Moringa seed has these thiazide or loop diuretics properties that enabled the male turkeys on T_2 to have reduced levels of sodium and potassium while laying affected the calcium levels in the hens.

Key words: *Serum, turkey, extracts*

INTRODUCTION

The blood serum electrolyte levels are essential in the regulation of nerve and muscle function, hydrates the body, balance blood acidity and pressure as well as help build damaged tissues The electrolytes found in the blood plasma include calcium, chloride, magnesium, phosphorus, potassium and sodium. These electrolytes are essential in the homeostatic function of the body system. They are part of the body metabolic system. Electrolyte imbalance can result from imbalances of regulatory hormones such as aldosterone and antidiuretic hormone (Miller-Keane, 2015). Electrolyte values are important in the diagnosis of clinical signs and symptoms when affected by diseases (Suchint *et al.,* 2005). Abnormal electrolyte levels were associated with abnormal functioning of the nerves and muscles of birds (Akinola and Egwuanumku, 2017). Low potassium levels

may cause dehydration and excessive sweating, while high potassium levels may lead to paralysis and heart problems (Ruotsulo and Tant, 2004). High calcium levels in the blood indicates the presence of diseases associated with the thyroid gland (Akinola and Egwuanumku, 2017). Low levels of sodium in the blood of broiler birds show that treatment has digestive effect on the birds (Chevallier, 1996). The potassium levels of 3.63 to 5.89mmol/l, calcium level of 5.06 77.46, and sodium 37.00-137.00mmol/l have been reported by Akinola and Egwuanumku (2017) among broiler birds fed red pepper as feed additives. The normal potassium level of chicken of 4.6-6.5mmol/l, sodium level of 148-163mmol/l and 158-165mmol/l was reported by Yakoub *et al* (2011). Etuk *et al.* (2012) reported potassium level of 4.50-5.70mmol/l, sodium range of 115.00-120.00mmol/l among turkey poults fed two varieties of sorghum in place of maize based diet.

MATERIALS AND METHODS:

Experimental site

It was approved and supervised by the Michael Okpara University of Agriculture Umudike (College of Animal Science and Animal Production CASAP) Research and Experiment committee and was carried out at the Poultry unit of Chirex Agro Services Limited in Abakuru, Ohaji-Egbema Local Government Area of Imo State Nigeria. It is on latitude $N5^{0}20^{I}$ 27^{II} and longitude E60 55^{1} 44^{11} and lies on the estimated terrain of 54 meters above sea level.

EXPERIMENTAL ANIMALS

A total of 36 heritage turkey toms and 36 hens of about 8- 9 weeks' old were used for the study. There were nine turkeys per treatment groups, grouped as; T_1 (control) T_2 (1% seed w/v) T_3 (0.5% leaf and 0.5% seed w/v) and T_4 (1% leaf w/v). Each group was replicated three times with three turkeys per replicate. The study latest for 154 days with all routine management practices duly observed. Blood for serum biochemistry was collected through the wing vein of the male and female local turkeys using sterile needles and syringes and were analyzed within 2hours of collection.

STATISTICAL ANALYSIS

Data collected were subjected to analysis of variance for the main effect of treatment using the GLM procedure of SAS (version 94: SAS Institute Inc., Cary, NC, USA). Means were tested using Duncan multiply range test.

RESULTS AND DISCUSSION

The blood serum electrolytes differed significantly ($P<0.05$) among the heritage turkey toms' treatment evaluated with the exception of calcium. The calcium level though not significant was higher among the *Moringa* administered treatments than the control. Potassium levels differed significantly ($P<2005$) among the turkey toms' treatment with treatment T_4 ($4.13^{\pm}$ 0.00^{a}) being the highest and T_2 ($3.19^{\pm}0.01^{d}$) lowest which suggests that *Moringa* seed extract reduced the potassium levels in the turkeys. However, the results are all within the stipulated level of 2.4-4.6mmol/l in avian. Cases of high potassium leading to paralysis and heart problem or low potassium resulting or leading to dehydration and excessive sweating as was reported by Ruotsalo and Tant (2004) was not witnessed in this study. The sodium levels among the male turkeys differed significantly ($P<0.005$) among the treatment. The observed levels were in line with report of Grey (2014) on avian except T_2 (119.043 ± 0.234) but all the treatments were lower than 141.6 to 152.6mml/l reported by Martin *et al.* (2010). This suggests that *Moringa* seed extracts may have the potential to balance the body electrolyte level and reduce the risk of high blood pressure as envisaged in this study. This was better than the results from leaf extracts. This confirms the anti-hypertensive qualities of *Moringa* (Dahot, 1988, Ogbu, 2019). It may be that the *Moringa* seed has these thiazide or loop diuretics properties that enabled the male turkeys on T_2 to have reduced levels of sodium and potassium.

There was no significant difference ($P<0.05$) on the sodium and potassium levels measured among the heritage turkey hens. There was significant variation ($P<0.05$) on the calcium level of female turkeys administered *Moringa oleifera* extract.

The calcium levels were significantly higher ($P<0.05$) in the control (T_1) and seed extract treatments (T_2). Although the serum calcium level did not differ in males, the lower levels of blood calcium in females on leaves extract treatment could be attributed to no chelating effect of the leaves of *Moringa* extract or due to the physiological statues of the turkey hens at the point of sample collection. Blood calcium level in our study was still within the range of 7.5 – 11.5mg/dl reported by Grey (2014).

The blood serum electrolyte levels are essential in the regulation of nerve and muscle function, hydrates the body, balance blood acidity and pressure as well as help build damaged tissues. Most supplements boost the functionality of these in the body. Thus from the study, it is safe to conclude that *Moringa* leaves and seed extracts did not negatively affect the heritage turkey toms and hens blood serum electrolyte.

ACKNOWLEDGMENTS

Tertiary Education Funding Federal Polytechnic Oko chapter for their support.

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The molecular mechanism of heat tolerance in goats

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Abstract

Climate impacts are related to changes in precipitation, temperature, atmospheric greenhouse gas concentrations and temperature levels that affect each biological system. These factors contribute to changes in biodiversity, oceans warming, natural reproductive cycles, feeding behavior, heat stress, animal agitation, water-related stress and other consequences such as increased parasitic infections, reduced reproductive performance, declining trends in food and forage resources for animals and other species. In recent years, the number of publications on the effect of temperature on goats have increased. Especially, publications investigating the expression levels of heat shock protein genes are quite popular for measuring of heat stress in different goat breeds. Heat shock factor 1 (HSF1), heat shock protein 60 (HSP60), HSP70, HSP90 and ubiquitin have been associated with the capacity of small ruminants to withstand heat stress challenges. Among these heat tolerance genes, HSP70 has been identified as the ideal genetic marker for heat tolerance in small ruminants. Identification of cellular and molecular markers and use of marker-assisted breeding programs may pave the way for the development of goat breeds that are resistant to changing temperature conditions due to global warming.

Key words: *Climate changes, Heat tolerance, Heat shock proteins, Goat*

Research trends in small ruminant genetics: a bibliometric analysis using the wos database

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Abstract

A bibliometric analysis was performed on 1790 research articles retrieved from the Web of Science (WoS) database, spanning the period from 2000 to 2024. This study aimed to provide a thorough understanding of the key trends, influential journals, and the most investigated topics in the field of genetic studies, particularly concerning sheep and goats. Bibliometric analysis plays a crucial role in mapping the intellectual landscape of a research field, as it allows for the systematic evaluation of scholarly output, identification of emerging trends, and the determination of the most influential sources and themes. Researchers can uncover gaps in the literature and potential areas for future inquiry, thus facilitating more impactful research efforts. The analysis revealed that Journal of Animal Science, Small Ruminant Research and Animal Genetics were the leading journals in terms of h-index, demonstrating their significant influence and impact on the field. Among these, Small Ruminant Research was identified as the most productive journal, contributing the highest number of publications. In addition to identifying leading journals, the study also examined the prevalence of certain key terms within the research articles. Analysis of keywords highlighted that terms such as expression, growth, and association were among the most frequently used and were important in genetic research on sheep and goats. These findings reflect the growing interest in exploring the genetic basis of economically valuable traits in small ruminants, particularly those related to growth and development. Thus, this bibliometric analysis not only provides insight into the prominent publications and research topics over the past two decades but also offers valuable guidance for researchers in animal breeding programs. Specifically, the results can inform studies aimed at improving economically important traits in livestock, such as growth rate, carcass quality, and reproductive efficiency. Moreover, by highlighting the most productive areas and journals, this study underscores the potential for future research to build upon these findings and drive advancements in animal genetics.

Key words: *Goat, Sheep, Bibliometrics, Reproductive, Growth traits*

Application of thermography in health and productivity assessment of cows

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Abstract

Measuring the heat emitted from the surface of the body (thermography) is now becoming a recognized diagnostic method in human and veterinary medicine. Thermography is particularly widely used in the prevention and diagnosis of musculoskeletal disorders in horses. However, there is little information in the literature on the use of this method in cattle. The aim of the study was to evaluate the usefulness of thermography in monitoring the health of cows, taking into account the factors that influence or interfere with the results of these measurements. An InfraCAM SD thermal imaging camera (FLIR system) was used for imaging. The thermograms were analyzed using the FLIR-QUICK-REPORT program. The studies were carried out in two stages on three cattle farms. In the first stage, thermograms (n=350) of 50 cows and calves (from 2 days to 5 years old) were taken to analyze the distribution of heat emission from the skin surface and its disturbances, and to detect cold and hot spots. In the second stage, thermograms (n=364) were taken of the surface of the udders of cows (HF breed) before and after milking. The effect of the stage of lactation, number of milkings, and milk yield (productivity) on udder temperature was studied by two-way ANOVA followed by a post hoc Tukey test. It was found that the heat emission through the skin of cows depends on age and breed, but mainly on hair length and density. At the same time, spotted short-haired animals showed differences in heat loss at the site of the spots. In addition, soiling of the skin surface is a factor that often leads to misinterpretation of thermograms. Disease lesions were found in a few animals. In the case of a pressure ulcer on the hind limbs (heel and ankle), the temperature of its surface was more than 5°C higher than the surrounding tissue. However, damage to the sphincter or one of the quadrants of the udder did not change its temperature. The udder surface temperature changed during the following stages of lactation ($P \leq 0.05$), while it did not depend on the age of the cow ($P > 0.05$) and did not differ between the beginning and the end of milking ($P > 0.05$). A decrease in mammary gland temperature was observed from the seventh month of lactation. This is probably related to the natural decrease in productivity of cows at this stage. In conclusion, thermography can become a non-invasive method for assessing productivity and a tool for the early detection of lesions in cattle. However, the correct interpretation of thermographic measurements should take into account factors such as breed, age, coat color and length, and filth.

Keywords: Cows, cattle, mammary glad, sin temparature, thermography

First molecular identification of Demodex sp. Infection in dog in Iran

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Abstract

Canine demodicosis is a prevalent skin disease of dogs in most countries and has been found worldwide. It causes localized and generalized lesions that can be life-threatening for dogs. The current cause of canine demodecosis is Demodex canis. Although the incidence of D. canis has been discovered in different regions of Iran, no molecular epidemiological and characterization investigation on D. canis has been conducted. The current study used the PCR technique to assess the infestation rate of dogs with D. canis in the northwestern region of Iran and finally characterize the amplified product by analyzing sequence.50 female and male stray and household dogs of various ages were sampled from skin lesions. DNA samples were extracted from skin samples and were subjected to PCR for molecular identification. The 16srRNA gene was amplified by PCR using amplify a 166 bp fragment of the sequence from D. canis primers and compared with D.canis sequences in Genbank using BLAST analysis.62% of the samples were positive for D. canis, and it is noteworthy that all positive D. canis samples were identified among stray dogs kept in shelters. The difference in infestation rates between domestic and stray dogs was an exciting finding in our study. The absence of infection in domestic dogs is due mainly to superior living conditions for these animals in terms of diet, cleanliness of the surroundings, and timely treatment of their illnesses. There was no relationship discovered between sex or age and parasitism. The sequencing results of positive samples and their blast and alignment of them with other Demodex sequences in GenBank (NCBI) demonstrated that two positive samples had 98% identity to each other and had 92-97% identity with other sequences of Demodex species in GenBank with most identity to Demodex canis. The phylogenetic analysis grouped these Demodex species TBZ isolates in a separate clade and shared most genetic similarity with Demodex canis isolate Wayanad 4 (MN161403) in dog in India.

Key words: *Demodex canis, Dog, PCR, Phylogenetic tree, Sequencing, Iran*

Usefulness of in Situ and in vitro methods to estimate the protein rumen degradation of heat-treated rapeseed cake

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Abstract

In the diets of high-producing dairy cows, protein characterized by a high content of exogenous amino acids, especially methionine, and reduced susceptibility to rumen degradation should be fed. These requirements can be met by rapeseed products, which contain protein of high biological value and, under certain conditions, allow achieving higher milk yield and milk protein content than when using soybean meal. However, it has not yet been unequivocally demonstrated whether and to what extent additional thermal processing of rapeseed products is necessary to reduce the rate of protein degradation in the rumen. Due to limited possibilities for conducting such research under production conditions, the aim of the present studies was to demonstrate the possibility of using in vitro techniques (CNCPS, including rumen undegraded protein UDP) and studies on cows equipped with rumen (in situ - rumen degradation protein RDP) and duodenum (intestinal digestibility ID) cannulas to assess the effectiveness of laboratory and industrial thermal processing methods of rapeseed cake protein (CP). In laboratory studies (LS), rapeseed cake was heated in a forced-air dryer at temperatures ranging from 90 to 150°C for 30 or 60 minutes. In industrial studies (IS), temperature and pressure were applied using specialized feed processing equipment. Laboratory tests showed that applying a temperature of 140°C for 60 minutes reduced RDP CP from 71.6 to 56.8% ($P < 0.05$), while simultaneously increasing ID CP from 32.2 to 50.9% ($P < 0.05$). As a result, protein digestion was shifted from the rumen to the intestines by approximately 100 g of protein per kg of feed. In samples used in IS, RDP CP was reduced to approximately 60%, with ID values exceeding 70%. In summary, it was found that the results of samples obtained after thermal processing under laboratory conditions are of limited usefulness in optimizing industrial feed processing processes. Additionally, no statistically confirmed relationship ($P > 0.05$) was found between the RDP CP results of the tested samples (in situ) and their UDP results ($r = -0.65$) or protein fraction content (highest $r = 0.69$ for fraction B3) obtained in in vitro studies. Due to the specificity of the structures and properties of rapeseed proteins - napins (2S albumin; 20% CP) and cruciferins (12S globulin; 60% CP), the usefulness of the results obtained using the methods used in the current studies may be limited in practical dairy cow nutrition. The authors believe that moderate thermal processing of rapeseed products is advisable, due to feed hygienization, reduction of anti-nutritional factors, and improvement of nutrient utilization by ruminants

Key words: *Rapeseed cake, ruminants, rumen degradation, protein, in vitro, methods, nutritive value*

Prevalence of Toxoplasmosis and Trypanosomiasis among Camels in Southern Algeria

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Abstract

Trypanosomiasis (Trypanosoma evansi, T. brucei) and toxoplasmosis (Toxoplasma gondii), are two parasitic diseases affecting camels throughout the world. In addition to their medical and economic impacts, they constitute an obstacle hindering the progress and development of the dromedary, a major source of animal protein for the local population (southern Algeria). Although the contamination mechanisms and specific symptoms may vary, these two protozooses can be associated with reproductive losses in camelids, particularly abortions. Without forgetting the fatal outcome of animals affected by trypanosomiasis following prolonged recumbent position. Without forgetting the fatal outcome of animals affected by trypanosomiasis following prolonged recumbency. In Algeria, an epidemiological investigation on camel trypanosomiasis was carried out between November 2005 and March 2006 in the wilayas of EL Bayadh, Ouargla, Béchar, Tamanrasset, Tindouf and Adrar on 1074 camels and revealed a strong infection for the wilaya of Béchar : 12.27% for parasitological prevalence against 68.71% for seroprevalence. A cross-sectional study was conducted from October 2015 to August 2018 to assess the seroprevalence of Toxoplasma gondii infection in 320 camels in south-eastern Algeria using the ELISA technique. The prevalence of infection was found to be 15% in camels.

Key words: *Algeria, trypanosomosis, toxoplasmosis, abortion, prevalence.*

Comparison of results coming from automatic milking system in selected countries in Europe and United States in 2021-2022

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Abstract

Milking cows using milking robots is an increasingly popular phenomenon, which results from the lack of qualified labor on the market, but also from the desire of the breeder himself to change the work model. The purpose of the study was to compare selected parameters recorded by the automatic milking system Lely Company in selected European countries and the United States in 2021-2022. The analysis assumed numerical material obtained from the data recording system by Lely concerning: the average number of robots per herd (n), the number of cows per robot (n), the daily milk yield per robot (kg), the daily milking frequency (n), the daily number of refusals (n), the milking speed (kg/min.), the daily milk yield per cow (kg), the fat and protein content (%) and the consumption of concentrated fodder per 100 kg of milk (kg). The accumulated data were recorded in the Czech Republic France, Germany, Italy, Latvia, Lithuania, the Netherlands, Poland and the United States in 2021-2022. The statistical analysis of the numerical material collected was carried out, applying the two-factor variance analysis, using the SAS v. 9.4 software. The following effects were taken into account in the linear model describing the variability of milking parameters: the country, the milking year and the country × milking year interaction. In the study, it was demonstrated that the country in which cows were milked had a statistically significant impact on all evaluated milking parameters. The highest average number of robots per farm was found in the United States (3.78), while the lowest was in Germany (1.26), with an average of 2.13 robots per farm across the studied countries. On average, there were 55.37 cows per robot in the compared countries, with the highest density in Germany (59.03) and the lowest in Lithuania (51.06). The average number of milkings per day was 2.75, with the highest in Latvia (2.87) and the lowest in France (2.53). The average milk yield per robot per day was 1693.21 kg, with the highest in the United States (2021.93 kg) and the lowest in the Netherlands (1535.69 kg). The average free time for a robot per day was 22.87%, with the shortest in Poland (18.35%) and the longest in Italy (26.71%). The proven, significant differences between the levels of milking parameters in the studied countries can probably result from the differentiated genetic potential of the milked cows and the diversity of the fodder base.

Key words: *Automatic milking system, milking parameters, dairy cattle, European Union, United States*

The influence of taurine administered

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Abstract

Cadmium (Cd) is currently considered to be one of the most important environmental pollutants and hazardous to living organisms, causing, among other things, damage to mitochondria or DNA, destabilisation of lysosomal membranes, and disturbance of the redox balance and metabolism. Therefore, the search is on for substances that can neutralise the toxic effects of heavy metals. One such substance is taurine, a naturally occurring aminosulphonic acid that is widely distributed in animal tissues. The aim of the study was to estimate the potential of taurine to counteract cadmium poisoning using an in ovo model. Chicken embryos (ROSS 308, Aviagen EPI) on day 17 of incubation (E17) were administered in ovo a solution (100 µL) containing: 0.7% NaCl (K), cadmium (6 µg/egg; Cd), taurine (5 or 25 mg/egg; T5/T25) or a mixture of these substances (T5Cd/T25Cd). Blood and liver samples were collected from randomly selected embryos (n = 10/group/phase) at phase: 24 h after injection (E18), external pipping (EP) and after hatching (D1). Total Antioxidant Status (TAS) in liver homogenate was determined colorimetrically (No. 709001, Cayman Chemical Company, USA), histological preparations were stained with haematoxylin and eosin (histomorphometry) and Sirius red in picric acid (collagen content). The effect of group (substance) and embryonic developmental stage on TAS was tested by two-way ANOVA, and on morphometric parameters by the Kruskal-Wallis test. Differences between groups were determined by the Tukey test (SigmaStat 3.5; Systat Software, Inc., USA). An effect of taurine and/or Cd and embryonic stage ($P \leq 0.001$) and an interaction between these factors on TAS in liver homogenate were found ($P \leq 0.001$). Taurine and Cd decreased liver collagen content ($P \leq 0.05$). The highest value ($P \leq 0.05$) of the circumference and area of hepatocyte nuclei was found in the T25Cd group (20.33 ± 0.26 µm and 18. 5 ± 0.38 µm2) and T25 (21.1 ± 0.23 µm and 19.6 ± 0.34 µm2). This regularity also applied to the Feret diameter. The circularity coefficient was lowest in the T05Cd group (0.53 ± 0.006; $P \leq 0.05$). The highest number of hepatocytes was found in the K group (7913.1 ± 150.95 cells/mm2) and T05Cd (8396.4 ± 338.93 cells/mm2) ($P > 0.05$). The Cd (7774.0 ± 244.01) and T25Cd (7812.6 ± 177.86) groups were characterised by the lowest number of nuclei per mm2. The total number of mononuclear hepatocytes decreased significantly ($P \leq 0.05$) in the Cd group (6444.2 ± 180.99 cells/mm2) and T25Cd (6451.89 ± 119.18 cells/mm2), and a tendency towards polymorphonuclear hepatocytes was observed. In conclusion, it appears that taurine may have a regulatory effect on the total antioxidant potential and the regenerative function of the liver in newly hatched chicks.

Key words: *Chick embryo, hepatocites, collagen, embryotoxicity, total antioxidant status*

Author Index

Nationality of Presenters

Country	n	%
Algeria	14	11,02
Bulgaria	2	1,57
Chile	2	1,57
China	1	0,79
Denmark	1	0,79
Egypt	4	3,15
Germany	1	0,79
Hungary	1	0,79
India	1	0,79
Indonesia	1	0,79
Iran	1	0,79
Kazakhstan	5	3,94
Mexico	1	0,79
Nigeria	4	3,15
Pakistan	5	3,94
Palestine	1	0,79
Poland	13	10,24
Portugal	1	0,79
Russia	1	0,79
South Africa	5	3,94
Spain	2	1,57
Tunisia	1	0,79
Türkiye	58	45,67
Ukraine	1	0,79
TOTAL	127	100