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### CONGRESS ON DOMESTIC ANIMAL BREEDING GENETICS AND HUSBANDRY

### **Proceedings of the ICABGEH-21**

Virtually, UKRAINE September 27 - 30, 2021

<u>Editors</u> Dr. Hasan ONDER Dr. Ugur SEN

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# Proceedings of the ICABGEH-21

### Virtually, UKRAINE

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### PREFACE

This volume contains the papers presented at the V. International Congress on Domestic Animal Breeding Genetics and Husbandry - 2021 (ICABGEH-21) was held on September 28, 2021.

The ICABGEH-21 has been organized by the Agricultural Faculty of Ondokuz Mayıs University. ICABGEH-21 is the fifth international event of the congress series with the participation of top-rated invited speakers; Dr. Ann Van Soom (Ghent University, Belgium), Dr. Dariusz Piwczyński (Bydgoszcz University of Science and Technology, Poland), and Dr. Theodore A. Tsiligiridis (Athens University of Agriculture, Greece). 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 serious planning and preparation to ensure that the Turkish and international animal science scientific community to meet the challenges and move safely and successfully into the advanced information era. To this end, ICABGEH-2021 has been focused on recent developments and research on animal science aimed at protecting the environment and food safety. Thus, ICABGEH-2021 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,

**Congress President** 

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### TEST-TUBE EMBRYOS IN PETS AND LIVESTOCK: STATE OF THE ART

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### Abstract

During the last decades, in vitro fertilization (IVF) has become a routine technique in most domestic animals. In cattle, the first in vitro produced (IVP)-calves were born in the eighties, and the combination ovum-pick-up -IVP is now a mature technique, leading to more than a million bovine IVP embryos transferred worldwide in 2019. In small ruminants, IVP is not very popular due to the impracticalities associated with oocyte collection and the complicated transfer technique. In the horse, routine IVF is still problematic, with only two foals born in 1991, but good results have been achieved after intracytoplasmic sperm injection (ICSI), with thousands of ICSI-foals born worldwide. Recently, equine OPU-ICSI had become a commercial succes for production of foals from horses of high genetic value. In pigs, IVF is only performed for research purposes, since pigs are polytocous animals, yielding about 35 pigs per year by natural reproduction. In pet animals, research in reproduction has mainly been focused on contraception, although recently, the introduction of new drugs especially marketed for cats and dogs will probably expand fertility research in carnivores towards the previously neglected area of assisted reproduction. In the dog, the technique has been lagging behind, with a first litter born in 2015. In cats, healthy kittens have been born, but in fewer numbers than in cattle and horses. The dog in particular remains a real challenge for the reproductive biologist, due to the low meiotic capacity of canine follicular oocytes. In cats, oocyte maturation is less of a problem and embryo production rates comparable to those of cattle can be achieved. The domestic cat is a valuable model for endangered felids and it can even be used as a recipient for wild felid embryos. In this presentation, we will discuss the state-of-the-art of IVF in domestic species such as cattle, horses and pigs, and we will list some of the problems associated with the implementation of IVF in dogs and cats in relation to their reproductive characteristics.

*Key words:* Test-Tube Embryos, Assisted reproduction, Embryos transfer, Pets, Livestock, Food security, Climate change

### FORECASTING MILKING EFFICIENCY COWS MILKED IN AN AUTOMATIC MILKING SYSTEM USING DECISION TREE TECHNIQUE

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### Abstract

In an automatic milking system (AMS), the profitability of production is primarily determined by the amount of milk obtained from a cow per its time spent in the milking box, i.e. milking efficiency (ME). The study was carried out on 1823 Polish Holstein-Friesian cows milked in AMS. Data on milk performance of cows milked in AMS was derived from the T4C management and data registration system by Lely. A total of 713206 records was obtained. Different traits characterizing the milking process were investigated with the use of multivariate analysis of variance and decision tree technique. The results show that the average ME was 2.59 kg/min. Milking efficiency was affected: year of AMS operation (being the highest in the first year), number of cows per robot (the highest in robots with 61-75 cows), lactation number (highest for multiparas), season of calving (spring), age at first calving (>36 month), days in milk (in 201-305 days) and finally by rear quarter to total quarter milk yield ratio (the highest between 51 and 55). The decision tree shows that in AMS, the highest ME (3.18 kg/min) had cows that gave more than 35 kg of milk per milking, were milked for more than 150 days, in robots that had occupancy lower than 56 cows and were kept in new barns where AMS was installed at least 3 years prior. Milking efficiency may be used in a selection programme aiming at obtaining AMS-high performance cows.

Key words: Milking efficiency, Automatic milking system, Decision trees, Dairy cattle

### DEEP LEARNING: AN E-MONITORING TOOL FOR INSECT PESTS' DETECTION

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### Abstract

Insect pests are animal species causing significant damages of global crop and fruit production every year. Against this event Integrated Pest Management (IPM) monitor and control practices are always applied. Pest monitoring aims to support the decision of counteracting a given level of infestation, as well as, to select the appropriate control mechanism. However, since most insects are similar, insect pests' detection on crops and fruits is more challenging than the general case of object detection. The manual (traditional) approach is based on placing, empirically, a number of traps in a cultivation area that are checked regularly by operators, in order to visually classify and discriminate insects in cultivation fields, a process which has been proven highly labor-intensive, time-consuming, and expensive. Various feature extraction methods have been applied so far for insect classification, including wing structures, colour morphometric measurements, histogram features, as well as local and global image features. In addition of the above feature extraction methods, well known classifiers, including support vector machines (SVM), artificial neural networks (ANN), k-nearest neighbors (KNN), and ensemble methods have also been applied. However, the above methods were only recently tested in real conditions, using images taken from traps already deployed in the cultivation field for pest monitoring. Further, the methods have also extended to be applied to specific insect pest catches, provided the use of pheromone traps to attract them. Nowadays, the advent of Internet of Thinks (IoT) allows the real-time data acquisition based on electronic traps (e-traps) equipped with high resolution cameras and agro-climatic sensors for effectively monitoring the dynamics of the pest population and support the control decisions, respectively. In the semi-automatic case images of the captured flies are forwarded to experts for identification and classification analysis based on communication capabilities of the e-traps. The approach is improving the manual one in many aspects, however, there is still room for further improvements in terms of error detection made by human experts, and efficiency in terms of labor-intensive and time-consuming procedures. Various approaches for developing automatic detection and counting systems have already appeared so far, including machine and deep learning, image processing, spectroscopy, or optoacoustic techniques. Recently, deep learning algorithms have been developed to detect and count insect pests automatically and accurately that reducing to a minimum the human interventions. However, the detection problem of the various insect pests is still challenging due to their small size and similar shape, the light conditions the images were taken, and the limited data to train the learning model. We focus on summarizing the progress made on imagebased recognition approaches exploiting either machine learning, deep learning, or image processing techniques, and aiming to identify and count insect pests from images taken by a camera-equipped etraps deployed in a cultivation field. We particularly discuss the capabilities of the proposed real-time e-monitoring system, as it measures pest population dynamics on a regular basis, using a large number of e-traps following various deployment patterns. We present examples of e-monitoring of pests, based on constructing a particular e-trap devise that captures insects on a sticky panel and transfer them to the cloud for processing. Limits and benefits resulting from several pilot studies are discussed with a perspective for the future improvements and developments.

*Key words:* Internet of Thinks, Artificial Intelligence, Entomology, Integrated pest management, Electronic traps, Automated monitoring

### DETERMINATION OF MEAT QUALITY AND YIELD BY IMAGING METHODS

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#### Abstract

Muscles in living things have different functions from each other in terms of their location and function. These functional differences are also reflected in meat consumed as an important food source. Meats are divided into two as valuable meat pieces and low value meat pieces according to their nutritive value. In this respect, carcass productivity is very important in the meat industry. The factor that determines carcass productivity is the ratio of valuable meat in the carcass. The quality and quantity of these muscles can be proportional to the bones on which they are located. The purpose of this review; the aim is to provide information on the usability of imaging techniques on carcass quality and productivity, which is very important in terms of economic value.

Key words: Imaging methots, Meat quality, Muscularity index

### INTRODUCTION

In animal husbandry, meat production always takes priority. However, it is both difficult and costly to carry out yield controls related to meat yield, as it requires the slaughter of the animal. After slaughtering the carcass, butchery animals and separating the head, feet (front legs from Articulus carpi, hind legs from Articulus tarsi), skin, tail (4th tail vertebra), all internal organs (internal organs in the thorax, abdomen and pelvis cavity), they are left behind (Anar, 2012). The rest is the whole body. Estimation of carcass composition in live animals is of practical importance in terms of determining the most appropriate slaughter and marketing age of the animal, estimating the economic value of the carcass and improving the carcass characteristics (Andre et al., 2007). Carcass yield, muscle-bone and fatness ratio are the main criteria in the use of carcasses in meat production (McKiernan et al., 2007). These criteria may vary depending on race, age, gender, diet and live weight (Çetin, 2017, Sakarya et al. 2014).

Meats are divided into two as valuable meat pieces and low value meat pieces according to their nutritive value. In this respect, carcass productivity is very important in the meat industry. The factor that determines carcass productivity is the ratio of valuable meat in the carcass.

These valuable meats (Figure 1) are among the people; tenderloin (M. psoas minor, m. psoar major, m. iliacus and m. quadratus lumborum), contrafile (M. iliocostalis, mm. multifidi, part of m. longissumus dorsi extending to the gluteal muscles), ribeye (M. longissimus), egg (M. quadriceps femoris), trance (M. semimembranosus, m. adductor, m. pectineus, m. gracilis and vastus medialis of m. guadriceps femoris), nuar (M. semitendinosus), contranuar (M. gluteobiceps'in 2/3 distal part) (Kahvecioğlu et al; Yücesan ve Ergün, 2000, Bilgin S, 2018).

The quality and quantity of these muscles can be proportional to the bones on which they are located. Muscularity index can be calculated together with osteometric measurements related to meat yield characteristics and objective evaluations can be made in carcass grading (Bilgin S, 2018).



**Figure 1.** Some valuable meats. A: Nuar, B: contranuar, C: Egg, D: Trance (Bilgin S, 2018).

Evaluation of meat yield and guality can usually be done at the carcass stage and subjectively (Herring and Kemp, 2001). With the developing imaging techniques, these values can be obtained with more real results. The aim of this review is to give information about the usability of rapidly developing imaging techniques on carcass guality and productivity, which are very important in terms of economic value. With the discovery of X-rays by Wilhelm Cormack Rontgen in 1895, it was developed rapidly in the following vears and started to be used in medical fields transformed into today's advanced and technology imaging systems (Tuncel, 2007; Kaya, 2016).

The types of energy used in visual diagnosis methods are collected in two groups as electromagnetic radiations and ultrasound energy. Types of electromagnetic radiation include x-rays used in radiography and computed tomography (CT), gamma rays used in nuclear medicine (NT), and radio waves used in magnetic resonance imaging (MRI). The type of energy in the second group is ultrasound energy used in ultrasonography (USG) (Oyar, 2003; Tuncel, 2007; Kaya, 2016).

These imaging techniques are used to obtain results on yield characteristics (live weight, withers height, etc.) and meat quality of animals outside of medical fields (Demircioğlu, 2020, Bilgin; 2018; Yardımcı ve Özbeyaz; 1999; Stanford et al. 2001).

Bilgin (2018) reported that muscularity indices and muscle/bone ratio can be determined by bone morphometry in cattle. It gives very good results in imaging the skeletal system in imaging methods (CT and radiography) using X-rays. Thus, the efficiency of the meats in question can be revealed based on muscle bone indices. It has also been reported that CT gives good results in the determination of carcass fat distribution, lean tissue and bone ratio (Toldi, 2003). With CT and radiography, soft tissues may not give as healthy results as other methods, so meat quality and yield may not be determined directly.

MRI is superior to CT in determining carcass composition. In addition, it has advantages such as high soft tissue contrast resolution, no need for iodinated contrast material, visualization of vessels without contrast, high quality imaging of cartilage tissue anatomical structures, and evaluation of more than one anatomical and functional structure on a single modality. However, the lack of mobilization and the fact that the device is quite expensive are among its disadvantages (Ludewig at al., 2015).

USG technology is one of the most widely used methods in Veterinary Medicine. It is used in many countries in Europe (Denmark, England and Ireland, etc.) outside the medical field, especially in the evaluation of carcasses when the animal is alive. Averdunk et al. He reported that there is a very high correlation between the estimated value by USG and the actual amount of meat (as cited in Yardimci and Ozubeyaz, 1999). Due to its portability and easy accessibility, USG is more prominent than other medical imaging techniques in estimating meat yield and carcass yield in livestock. In addition to these imaging methods, carcass yield and meat quality can be determined by X-rav absorptiometry, optical probes, various impedances and video capture systems and digital image analysis. (Aktan, 2004; Stanford et al., 1998; Ince ve Ayhan, 2008).

As a result, it is necessary to develop some new technologies that will be effective in meat yield and quality in order to increase the value of meat compared to other yields and to ensure profitability. Predicting carcass composition on livestock enables early selection of young animals with desired characteristics and establishment of breeding flocks. For this purpose, cost, measurement method and speed are of great importance in the use of imaging systems.

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### THE CELL'S SELF PROTECTION METHOD; VBNC (VIABLE BUT NON CULTURABLE CELL) STATE

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### Abstract

Viable but non-culturable cell (VBNC) is a different resistance method for non-differentiating bacteria. They are living cells that have lost their ability to grow in the routine environment on which they normally grow, unlike normal cells that can be cultured in suitable environments and developed in colonies. Despite losing their ability to grow, VBNC cells are not considered dead because they have the membrane containing undamaged genetic information. Dead cells are metabolically inactive, while VBNC cells are metabolically active and perform respiration. In addition, these cells continue transcription and mRNA production. VBNC formation mechanism assumptions can be counted among the hypotheses of extreme environmental conditions leading to low quality cell formation, a loss in cell activity and becoming unculturable, cells entering the VBNC state exhibiting a survival strategy to adapt to adverse environments, and it may occur as a result of gene regulation. Detection of VBNC cells, staining procedure for direct enumeration of viable bacterial cells based on substrate absorption ability, approaches based on detecting gene expression by microscopic counting or respiratory detection method, LIVE/DEADR BacLightTM fluorescent staining method based on electron transport ability and cell membrane, (RT-PCR), qRt PCR, LAMP) and identification of cells using biological sensors. Detection of these cells is very important in preventing foodborne infections, reducing the risk of disease and determining treatments. As a result, it leads to less detection of the total viable cells present in the samples taken and causes vital risks in terms of public health. Therefore, we believe that more studies on VBNC cells are needed and fast, sensitive and cost-effective, practical methods are needed for the detection of these cells.

Key words: VBNC, Resuscitation, Public health, Bacterial pathogens

### SELECTION SIGNATURES OF CATTLE BREEDS IN PAKISTAN

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### Abstract

Selection signatures define a specific genome region, which is considered specific and important functional trait under natural or artificial selection. In this study, two different approaches to identify the selection signatures between and within ten different cattle breeds in Pakistan. The first method was used to detect haplotypes fixation on genomic regions within breeds. The second method, population differentiation index (Fst), was used to identify genomic regions having different allele frequencies between these cattle breeds. All selected breeds include Achi (18), Bhagnari (14), Cholistani (13), Dajal (10), Dhanni (10), Kankraj (12), Lohani (19), Red Sindhi (13), Sahiwal (14) and Tharparkar (13), were divided into three populations (A, B & C) based on nucleotide structural analysis. In this study, we identified fifty-three candidate genomic regions using both approaches. Seventeen genomic regions were common among three populations and forty-nine were successfully annotated with identification of some candidate genes. MCIR gene is found on BTA-18 between 14, 757, 332-14, 759, 082 bp. However, a number of putative genes linked with production traits (LAP3, CAPN3, CYP19, SAR1B and RPS6KA2), reproductive traits (PIK3CA, SPERT and IGF1R), nervous system (KIT, FGF5, ASIP and HSPB9) and immune response (IL2, IL4, SERPINA3-8 and BOLA3) are identified as being under selection. The finding of this preliminary selection signatures study in Pakistani cattle breeds provides a new insight for genomic diversity during domestication events and breed development. These results could be used to expedite the genomic assisted breeding selection in these breeds for the improvement of important economic related traits.

Key words: Cattle, Diversity, Domestication, Gene, Selection signatures, Traits

### THE RELATIONSHIPS BETWEEN SOME EGG TRAITS AND SEX IN BLUE-BREASTED QUAILS (EXCALFACTORIA CHINENSIS)

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### Abstract

Predetermination of sex in monogamous Blue-Breasted quails is particular importance. In these quails, which have recently been defined as a good model animal, time, subject of study and economic concerns are effective in sex preference. Unfortunately, the most popular sex determination methods are done after hatching with techniques that require specialist and high-tech facilities that can increase the cost of production. In addition, since Blue-Breasted quails are much smaller than other poultry, sex discrimination is determined by following the plumage and behavioral development at later ages. Therefore, in this study, some egg traits on sex were investigated in search of an easier technique. A total of 230 freshly fertilized eggs were grouped according to egg weight, eggshell colour and shape index treatment groups. Data on egg hatchability, egg weight loss during the first 15 days of incubation, hatched chicks' weight and male to female sex ratio were collected. The results showed that 22 chicks hatched from small eggs (11 males and 11 females), 71 chicks hatched from medium eggs (49 males and 22 females), 38 chicks hatched from large eggs (20 males and 18 females). In the shape index groups, 22 chicks (16 males and 6 females) were hatched from the 71-75%, 68 chicks (44 males and 24 females) were hatched from the 75-79% and 41 chicks (21 males and 20 females) were hatched from the 79-83%. Eggshell colour groups were categorized: cream (5 males and 6 females), olive (10 males: 3 females), brown (36 males and 30 females) and grevish (19 males and 12 females). According to treatment groups egg indicates hatching from both sexes cannot be a more useful method. In the study, it was concluded that other sex-related traits should also be taken into account in determining sex.

Key words: Egg weight, Egg shape index, Eggshell colour, Plumage colour

### INTRODUCTION

Chicken (Gallus gallus domesticus) and Japanese quail (*Coturnix japonica*) are traditionally known as primary model poultry. Over the last two decades, blue-breasted quail, the smallest species of the order Galliformes, has been proposed as an excellent candidate species due to characteristics including ease of handling, small body size, hardiness, high egg-laying performance, short generation interval and distinguishable gender after 4 weeks of age. (Tsudzuki, 1994; Nishibori et al., 2002; Wei et al., 2011a; Kageyama et al., 2018; Nakamura et al., 2019). This species is also called button quail in the USA, the King quail in Australia, and the Chinese painted quail in Europe. Other common names known as Indian blue quail, Asian bluebreasted quail, and blue quail. There are also two scientific names for this species, *Coturnix chinensis* and *Excalfactoria chinensis* (Tsudzuki, 1995; Shibusawa et al., 2004; Ono et al., 2005).

The earliest possible sex determination of bluebreasted quail is desirable when these birds are bred for experimental purposes. Blue breasted quail has a highlighted sexual dimorphism in plumage colour. Wild females display a brown coloration similar to that of Japanese quail, whereas wild males show a general blue pattern on its back and a red tail, and clearly delineated black and white pattern on its throat (Araguas et al., 2018). Sexual dimorphism does not become obvious until the back, breast and throat feathers emerge when the quail are 21 days of age (Wei et al., 2011b). Moreover, several plumage varieties, colour of the feathers remains identical in both sexes even after sexual maturity, e.g., the "white", "tuxedo" "splash" and albinos (Landry, 2015). In these varieties, sex determination is possible by observing the sexually active males displaying behaviors such as fidbitting, strut and, wing leg stretch with walking around the hen, as indicated in their ethograms (Schleidt et al., 1984).

These methods cause time and economic loss in studies where the experimental procedure is started at an early age and a single sex is preferred (Homma et al., 1966). Recently, sex determination in poultry is done using several methods such as examining of feet, neck, feather colour and length as well as vent sexing, laparoscopy, steroid sexing and karyotyping after hatching (Cerit and Avanus, 2007; Rathert et al., 2017) However, some of these methods are unreliable, time-consuming, and expensive while some of them could be painful and even lifethreatening for birds (Cerit and Avanus, 2007). It is also important to use easy and cheap methods in blue-breasted quails, especially because they are monogamous (Adkins-Regan, 2016) and very small.

This study was carried out to investigate whether sex discrimination can be made by evaluating egg weight, egg shape index and egg shell colour in blue-breasted quails before hatching.

### MATERIALS AND METHODS

In this study, a total of 230 fertile eggs were obtained from 36 pairs of blue-breasted quails (in two different age ranges of 65-99 and 168-192 days) reared in Isparta University of Applied Sciences, Faculty of Agriculture, Animal Breeding Laboratory and no selection criteria were applied to this population.

The birds had a wild plumage pattern and seven different plumage types: red breasted (Araguas et al., 2018), white, cinnamon (Pis and Lus'nia, 2005) or buff (Harrison, 1973), blue face (Araguas et. al., 2018) or extended brown (Kageyama et al., 2018), tuxedo (Adkins-Regan, 2016), pied and golden pearl (Landry and Rogers, 1977). Quails were fed a commercial quail breeder diet contained 17 % CP 2750 kcal of ME/kg until the end of the study (Parker and McDaniel, 2009).

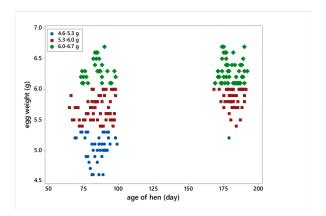


Figure 1. Egg weights by age of hen.

Fertilized eggs were collected within 12 hour of laying and stored at 15°C (Nakamura et al., 2019). Fertilized eggs were used within 10 days of collection. Before incubation egg length and egg width were measured with a caliper (0.01 mm), egg weighed (to 0.01 g) to determine initial egg weight and colour chart was used to identify and categorize eggshell colour by individually.



**Figure 2.** Image of eggshell colour goups of blue-breasted quail. Cream, olive, brown and grayish colours are shown from left to right, respectively.

They were grouped into 4.6- 5.3 g (small), 5.3-6.0 g (medium) and 6.0-6.7 g (large) egg weight, 71-75 %, 75-79 % and 79-83 % shape index (the upper full value in the groups is included in the previous group) and cream, olive, brown and greyish according to the colour of the eggshell. Fertilized eggs were incubated at 37.5°C and approximately 50 % relative humidity (Parker et al, 2017) for the first fifteen days, and at 37.1°C and approximately 60% relative humidity for the last three days. On the 15<sup>th</sup> day of incubation, turning was stopped, the eggs were weighed again and egg weight loss was found. The chicks hatched after 18 days of incubation and weighed for chick weight immediately after hatching. Egg weight loss was calculated according to the formula below (Abo-Samaha and Basha, 2021);

Egg weight loss (%) = [(egg weight at settingegg weight at  $15^{th}$  days of incubation)/ initial egg weight at setting] ×100

Hatchability was calculated according to the formula below (Bobbo et al., 2013);

Hatchability (%) = Number of eggs hatched out/Total number of fertile eggs x 100

The percentage hatchability and plumage colour were recorded. The hatched chicks were weighed and the sex determination was done according to the 21 days of age plumage colour (Wei et al., 2011b). The sex distinction of white, splash and tuxedo colours were made according to the fitbitting, strut or wing leg stretch behavior of the males about 25-35 days of ages. The sex ratio was calculated according to egg weight, shape index and eggshell colour groups, as well as the plumage colour.

The obtained values were subjected to simple descriptive statistics and examine the effect of the egg indices was modeled using binary logistic regression in the Minitab (2019) statistical package. In the model, egg weight, shape index, chick weight and egg weight loss

Table 1. Effect of egg weight on sex rat	io
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until the 15<sup>th</sup> day of hatching were taken as continuous predictors on sex. Odds ratio was used to understand the effect of the predictors. Odds ratios that are greater than 1 is how that the event is more likely to occur as the predictor increases. Odds ratios that are less than 1 indicate that the event is less likely to occur as the predictor increases (Hosmer and Lemesho, 2000).

### **RESULTS AND DISCUSSION**

The effect of egg weight on chick sex ratio is shown in Table 1. A total of 130 chicks were obtained from 230 fertile eggs incubated and hatchability was 56.97 %. These chicks included 11 females and 11 males in the small egg group, 22 females and 49 males in the medium egg group, and 18 females and 20 males in the large egg group. The male ratio of chicks was found to be 61.07%, and the male percentage is higher as Homma et al. (1966) reported. Hatchability value was highest (67.62 %) in medium egg group followed by 52.38 % and 45.78 % recorded in small and large groups, respectively. The number of male chicks in the medium group was about two times higher than the females. Male and female chick weights were found to be similar in egg weight groups.

Table 2 shows the effect of index on sex ratio. Considering the index groups, hatchability value was highest (78.57 %) in 71-75 % index group followed by 75-79 % (54.84 %) and 79-83 % index groups (52.56 %).

Trait	Eg			
	4.6-5.3 g	5.3-6.0 g	6.0-6.7 g	Total
	Small	Medium	Large	
Number of fertilized eggs	42	105	83	230*
Number of hatched chicks	22	71	38	131 (56.95)
Number of hatched female chicks	11	22	18	51 (22.17)
Number of hatched male chicks	11	49	20	80 (34.78)
Hatchability (%)	52.38	67.62	45.78	
Male to female ratio of hatched chicks	1:1	49:22	10:9	
Egg weight (g)	5.04±0.03	5.75±0.01	6.28±0.02	
Hatched egg weight (g)	5.04±0.04	5.75±0.02	6.24±0.02	
Female chicks weight (g)	3.63±0.09	4.19±0.07	4.56±0.06	
Male chicks weight (g)	3.80±0.06	4.23±0.04	4.59±0.07	
Hatched chicks weight (g)	3.71±0.05	4.22±0.04	4.57±0.05	

\*= Values in the parentheses is the corresponding percentage. Values are presented as mean±standart error.

In the index groups, the male to female chicks' ratio value was determined to be 8:3 (71-75 %), 11:6 (75-79 %) and 20:21 (79-83 %). There were

more male chicks hatched in the 75-79% and 71-75 % index groups and in remain group the male to female ratio was close to one to one.

Trait	Shape index groups				
	71-75 %	75-79 %	79-83 %		
Number of fertilized eggs	28	124	78		
Number of hatched chicks	22	68	41		
Number of hatched female chicks	6	24	21		
Number of hatched male chicks	16	44	20		
Hatchability (%)	78.57	54.84	52.56		
Male to female ratio of hatched chicks	8:3	11:6	20:21		
Shape index (%)	73.71±0.18	77.18±0.10	$80.50 \pm 0.14$		
Shape index of hatched eggs (%)	73.65±0.20	77.15±0.14	80.30±0.15		
Shape index of female chicks' eggs (%)	73.59±0.39	77.22±0.25	80.15±0.21		
Shape index of male chicks' eggs (%)	73.67±0.24	77.11±0.18	80.45±0.22		

Table 2. Effect of shape index on sex ratio

Values are presented as mean±standart error.

As seen in Table 3, the hatchability of cream, olive, brown and grayish eggshell groups found as 57.89 %, 52.00 %, 58.93% and 54.41 %, respectively. In additions to this, the effect of eggshell colour on sex ratio is given in Table 3. The male to female ratios in the cream, olive, brown and grayish eggshell colour groups were found to be 5:6, 10:3, 13:10 and 29:12,

respectively. Among the eggshell colour groups, only the number of females was found to be higher than the males in the cream eggshell colour. This may be due to the fact that some tuxedo plumage colours are only found in females. In addition to this, the other group with a higher number of females was brown plumage.

Table 3. Effect of eggshell colour on sex ratio

Trait	Eggshell color groups					
	Cream	Olive	Brown	Greyish		
Number of fertilized eggs	19	25	112	74		
Number of hatched chicks	11	13	66	41		
Number of hatched female chicks	6	3	30	12		
Number of hatched male chicks	5	10	36	29		
Hatchability (%)	57.89	52.00	58.93	54.41		
Male to female ratio of hatching chicks	5:6	10:3	13:10	29:12		
Number of plumage colour of hatched						
chicks						
White	-	-	1 (0:1)	1 (1:0)		
Brown (Cinnamon)	2 (1:1)	-	13 (5:8)	-		
Red-Breasted	4 (1:1)	5 (3:2)	4 (1:3)	10 (4:1)		
Golden Pearl	-	-	17 (13:4)	14 (5:2)		
Dark Brown (Blue Face)	-	-	13 (7:6)	2 (2:0)		
Tuxedo	1 (0:1)	-	6 (1:2)	3 (2:1)		
Pied	-	-	-	1 (1:0)		
Splash	-	-	-	1 (1:0)		
Wild (basic colour)	4 (1:1)	8 (7:1)	12 (2:1)	9 (4:5)		

\*The male to female ratio is shown in parentheses.

According to the white, brown, red-breasted, golden pearl, dark brown, tuxedo, pied, splash and wild plumage colours, the male to female

ratio values were found as 1:1, 2:3, 14:9, 23:8, 3:2, 2:3, 1:0, 1:0 and 7:4, respectively.

Source	DF	Wald Test	Odds Ratio	95 % CI	P-Value
		Chi-Square			
Regression	4	12.90			0.012
Egg weight	1	2.96	0.878	0.745; 1.019	0.085
Shape index	1	1.65	0.340	0.066; 1.759	0.199
Egg weight loss	1	10.57	0.752	0.633; 0.893	0.001
Hatching weight	1	0.81	1.916	0.463; 7.936	0.369

	<b>D</b> .				C .1	· · ·	
Table 4.	Binary	logictic reg	ression a	inalysis (	of the ego	j indicates c	on chick sex

\*Egg weight loss during the first 15 days of incubation, CI= Confidence interval

Table 4 provides the binary logistic regression analysis of the egg indicates on the chick sex. As seen in the table 4, 1 gram increase in egg weight reduces the probability of being male by 0.878 times, a 1% increase in shape index reduces the probability of being male by 0.340 times, and 1 gram increase in hatching weight increase the probability of being male 1.916 times, but not statistically significant (P= 0.085, 0.199 and 0.369, respectively). On the other hand, even if the egg weight loss is significant (P=0.001), the P value is ignored because the confidence interval of the odds ratio contains 1 or less than 1 value (OR 0.752

(0.633; 0.893)). Therefore, egg weight loss also does not affect the probability of being a male. Egg weight, shape and shell colour may not be effective in determining chick sex (Idahor et al., 2015).

### CONCLUSIONS

The results obviously showed that egg weight, shape index, eggshell colour, egg weight loss and chick weight did not affect to sex. Thus, egg and chick traits may most likely not be suitable factors on blue-breasted quail sex. As a result, a more comprehensive study that includes a larger sample size and evaluates more traits may be useful in determining the effects on sex.

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### GENETIC VARIATION OF B-LACTOGLOBULIN AND KAPPA CASEIN GENES AND THEIR EFFECT ON MILK COMPOSITION IN LOCAL AND IMPROVED AWASSI SHEEP REARED IN PALESTINE

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### Abstract

The purpose of this study was to look into the genetic polymorphism and association of milk protein genes and yield in Palestinian Awassi sheep strains. On a DNA level, the polymorphism of the beta lactoglobulin (8 -LG) and Kappa casein (k -CN) genes of two Awassi sheep reared in Palestine, known as indigenous (local) and improved Awassi sheep, was evaluated. The DNA of 54 sheep (23 native Awassi and 31 improved Awassi) was extracted and genotyped for  $\beta$ -LG and  $\kappa$  -CN using by DNA sequencing. The amplified products for the  $\beta$ -LG and  $\kappa$  -CN genes were 471 bp and 670 bp in size, respectively. Three genotypes were discovered in the B-LG gene (AA, AB, and BB). In both local and improved Awassi sheep, the  $\beta$ -LG sequence of exon 2 showed a point mutation (C>T) in the (X12817.1:c.112 T>C) locus. The existence of three different patterns, CC, TC, and TT, was discovered via κ -CN sequencing analysis. Furthermore, a single base pair mutation (C T) in exon 3 of codon 56 was discovered. Significant impacts of 6 -LG genotypes on fat content and density of milk were observed in both sheep, while a specific  $\kappa$  -CN genotype had effects on solid non-fat milk content exclusively in local Awassi. For both sheep, significant (P>0.05) differences in all measured parameters were observed between local and improved Awassi in the early season of milk, and between fat and lactose towards the end of the milk season. It is recommended to breed local Awassi sheep for outdoor rearing or extensive system in order to get more content of milk components.

Key words: Genetic polymorphism, β -lactoglobulin, κ -casein, DNA sequencing, Awassi

### USAGE OF BICLUSTERING METHODS IN ANIMAL SCIENCE

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### Abstract

Cluster analysis, which has developed especially with the widespread use of computers, has had a wide application area in different disciplines. Today; Applications are seen in data mining, banking, marketing, medicine, agriculture, sociology and economy. Cluster analysis is a multivariate method that enables to classify the units examined in a study by gathering them into certain groups according to their similarities, to reveal the common features of the units and to make general definitions about these classes. The aim of this study is to explain biclustering methods that bring a different perspective to clustering analysis and to give information about their use in animal science.

Key words: Animal science, Biclustering, Cluster analysis

### COMPARISON OF BICLUSTERING ALGORITHMS TO REDUCE DURING GESTATION PROBLEMS IN SHEEP BREEDING

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### Abstract

The aim of this study is to compare the clustering performance of biclustering methods in order to evaluate the solutions developed to reduce during gestation problems in sheep breeding and to evaluate the benefits brought by these solutions. For this purpose, the data set of the Sheepnet platform containing the solutions and benefits developed to reduce during gestation problems and lamb deaths in sheep breeding was used. Bimax, Xmotif and Bayes biclustering algorithms were used in the study. The Jaccard index was used to evaluate the clustering performance of the algorithms. As a result of the study, it was determined that the jaccard index value obtained from the Bimax algorithm and the Bayes algorithm is lower than the Xmotif algorithm and the clustering performances are higher.

Key words: Biclustering, Gestation, Lamb, Sheep breeding

### DROUGHT AND ITS EFFECTS ON NOMADIC AND RURAL LIVESTOCK BREEDERS IN IRAN

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### Abstract

Decreasing amount of rainfall in the past autumn and winter caused deficiency in pastures and shortage and unavailability and very high prices of fodder and forage in most regions of Iran. This unfavorable phenomenon forced many nomadic and rural people that breeding livestock and especially goat and sheep herds to selling their animals even reproductive livestock under normal prices of them in market to middlemen and before maturing them completely and with low weights and killing them in livestock slaughterhouses in Iran. This imposed many economic damages to them. Slaughtering reproductive livestock will cause reducing in milk and dairy production and increasing their various products prices in future. Drought is a spreading phenomenon, and it has caused a lot of damages in different parts of the country in recent years. The driest conditions in 53 years have brought chronic mismanagement of water resources in Iran. According to Iran's meterological agency, the country's average temperature has increased by 2C since the 1960s, rainfall has decreased 20% in the last 20 years and October 2020 to June 2021 was the driest period in 53 years. Environmental problems in general and water problems in Iran in particular are multi-faceted issues. Iran's water resources have been depleted by a lack of rain, the building of hydro-electric dams and farming of water-intensive products like rice, wheat and sugar cane. Farmers hit by water shortages are fleeing their villages to live in precarious settlements on the outskirts of cities. Some villagers just do not have water to drink. They have to go and get water from small puddles on the ground. Type of doing this research is mainly qualitative that observations, experiences and field visits of author are main sources for gathering information. In these regards, author in article state various aspects of drought and its effects on nomadic and rural people that breeding livestock and especially goat and sheep herds in Iran. In the end of article present solutions and approaches for confronting to drought and its unfavorable effects on these nomadic and rural people in Iran.

Key words: Drough, Nomadic, Rural, Breeding, Livestock, Iran

### INTRODUCTION

Iran has been under a prolonged drying condition since the early twenty-first century, revealing itself by vanishing lakes and wetlands along with excessive water stress across the country (Ashraf et al., 2021). Drought is a common natural hazard in arid and semi-arid regions. It is a prolonged period of abnormally low rainfall that negatively affects land managers, ranching enterprises, and pastoral systems.1 Drought may be the most complex but least understood of all natural hazards, and it directly affects more people globally than any other natural hazard (Moghaddas Farimani et al., 2017).

Drought is a spreading phenomenon, and it has caused a lot of damages in different parts of the country in recent years (Sobhani et al., 2020). Drought can be defined as a temporary climatic anomaly with no rain, especially during the planting and growing season. It is one of the most complex and least-understood of all natural events and affects more people than any other hazard. In contrast to the effects of floods, earthquakes and hurricanes, the damage created by drought is usually nonstructural, but is spread over a larger geographical area. Its effect accumulates slowly over time and lingers for years. Since the onset and duration of drought is difficult to determine; people generally are not fully aware that they are in the midst of such an event (ANSARI AMOLI et al., 2007).

Iran is a country with significant cross-sectional variations in amenities and climate. While the country has been hit hard by drought, provinces of Iran are not equally affected by drought. Moreover, the provinces have different climates and are unlike Malaysia or Finland where regional areas have almost similar climates (Farzanegan et al., 2019).

In Iran, geographically distribution of drought showed that southern and southeastern of the country are more sensitive to drought both in intensity and frequency (Shokri et al., 2016).

Rainfall had dropped by nearly 50% in South Khorasan province this year compared to the long-term average and by as much as 80% in southeastern Sistan and Baluchistan province *(Gebeily,* 2021).

Iran enjoys a diverse topography and climate variability. Temperature can vary between -20 and +50 °C while precipitation varies from less than 50 mm to more than 1,000 mm per year. Iran's average annual precipitation is 250 mm (less than one-third of the global average) with most of the country receiving less than 100 mm of rain per year. Iranians have been successful in coping with this natural limitation, establishing one of the world's oldest civilizations and sustaining life for thousands of years in a mostly arid to semi-arid region with limited water availability. This was done through the invention of ingenious water harvesting techniques, which made farming and food production feasible in a water-scarce region of the world in ancient times (Madani et al., 2016).

For example, the pastoral nomads in Kerman province of Iran breed goats, sheep, cattle and camels in different combinations, but the main livestock are sheep and goat (Shokri et al., 2016).

### EFFECTS OF DROUGHT ON RANGELAND USERS (RUs)

In recent decades, the frequency of drought in arid and semi-arid regions such as West Asia, North Africa, Eastern Australia, and Southwestern United States has been increasing. This climatic phenomenon has negatively affected agriculture (e.g., crop and livestock production) and natural resources (e.g., rangelands and surface waters).

Rangeland users (RUs) in arid and semi-arid regions consider drought to be a significant problem because it can lead to forage production losses between 30% and 100%. Rangelands within advanced economies are not immune to this hazard. For example, from 2014 to 2015, drought caused an 80% forage production loss in San Luis Obispo County, California. The widespread droughts of the early 1990s in particular had a major detrimental impact on rangelands and livestock production. A drought usually entails a number of different and interconnected social, economic, and environmental consequences. For example, a drought will significantly affect rangeland activities, and in developing countries in arid and semi-arid regions, it can be a primary cause of poverty and emigration (Moghaddas Farimani et al., 2017).

Turning the focus to Iran specifically, in the past 40 years, the country has experienced 27 droughts. Drought is clearly not an unusual climatic hazard in Iran, but it nonetheless remains a phenomenon that has not been fully considered in the country, despite the clear challenges that drought presents for RUs' livelihoods and environmental management policy making. Droughts in Iran affect large numbers of people, causing tremendous economic losses and social hardships as well as severe environmental damage (Moghaddas Farimani et al., 2017).

RUs may employ a variety of drought risk management options to minimize drought's effects. More options (e.g., moderate use of rangelands, reduced stocking levels, deferred grazing, and so on) allow for greater flexibility to reduce damage to a rangeland's soils and vegetation, the health of livestock, and the RUs' livelihoods. Although there is no "cookbook" approach to drought risk management, many of these points are principles of range management that can be relevant to all RUs.

Drought affects rangeland ecosystems in many ways. For rangeland managers, the most concerning impact is lowered soil moisture levels that inhibit plant growth and thereby reduce forage yields (Moghaddas Farimani et al., 2017).



**Figure 1.** A small herd of goats that grazing in the top of a mountain near a village with 15 km distance to Birjand, east of Iran. Main reason for grazing goats in the top of a mountain was unavailability of herbs and shrubs in the fields because of facing country with worst drought in last 50 years (By author, Sep 9, 2021).

### KNOWLEDGE AND SKILLS ON DROUGHT MANAGEMENT:

For effective operation management, drought combat activities the knowledge regarding comprehensive watershed management shall develop and there should be coordination at national and provincial levels among relevant organizations in different levels. Proper drought management methods in agriculture shall reinforce empowerment of farmers and ranchers so that they can adopt proper technology to follow sustainable agriculture principles. Success or failure of strategies and plans towards promotion of water and land use systems such as water distribution, flood water management and constructions of artificial feeding pools and methods of mitigating evaporation and sustainable agriculture in farmlands of each province or area is stressed. There is need for proper technologies, management measures and technical expertise within national strategy framework of drought preparedness. Applying local competence to support sustainable farmland use systems shall be promoted by involvement of the government in drought preparedness strategies. Also, ganat water network system is a proper method to access underground water resources for irrigation and supply of potable water (Garshasbi, 2016).



**Figure 2.** Grazing of a small sheep and goat herd in a poor rangeland because of drought, near a village of Darmian city, 220 km distance to Birjand, center of South Khorasan province, east of Iran (By author, summer 2021).



**Figures 3.** Slaughtering of sheep and goat herds that purchasing under normal prices of them in market by middlemen, because of drought and unavailability and very high prices of forage and fodder, before maturing them completely and with low weights in a livestock slaughterhouse near Birjand, center of South Khorasan province, east of Iran (By author, summer 2021).

### **MATERIALS AND METHODS**

Type of doing this research is mainly qualitative that observations, experiences and field visits of author are main sources for gathering information. In these regards, author in article state various aspects of drought and its effects on nomadic and rural people that breedinglivestock and especially goat and sheep herds in Iran. The study was conducted on villages and rangelands of Nehbandan, Darmian, Sarbisheh, <u>khoosf</u> and Birjand cities, in South Khorasan province, east of Iran at spring and summer 2020 and 2021.

### **RESULTS AND DISCUSSION**

Economic effects of drought are massive and multifaceted and in subsectors such as change of land use, rain fed, livestock, and range and forest management and at primary and developing levels it surpasses processing and complementary industries. Lack of occupational opportunities and income, sales of land and livestock, high production costs, low food supply and tax income and high governmental costs are examples of drought effects.

Economic effects of drought are massive and multifaceted and in subsectors such as change of land use, rain fed, livestock, and range and forest management and at primary and developing surpasses processing levels it and complementary industries. Lack of occupational opportunities and income, sales of land and livestock, high production costs, low food supply and tax income and high governmental costs are examples of the effects of drought on economy. One of the most important measures is to establish effective communication among public and non-governmental organizations with the aim of timely response during emergencies. Establishment of technical crisis committees in the fields of emergency food and water supply and damage control is essential (Garshasbi, 2016).

Poor rainfall in Iran this year highlights challenges in providing enough water and energy, adapting to a hotter climate and preventing mass migration, and following:

- Drought leaves many Iranians without water, power
- Climate change and poor policies behind shortages
- Lack of action could drive migration from rural areas (*Gebeily*, 2021).



**Figure 4.** Qanat repairing and reconstructing in Binabad village, 70 km distance to Birjand, south east of Iran. Qanat has major role for sustainable exploiting of groundwater resources in Iranian rural people Indigenous Knowledge (IK) (By author, spring 2021).

### CONCLUSIONS

"May Ahura Mazda protect this land, this nation, from rancor, from foes, from falsehood and from Dariush the Great (550 B.C.). King drought" of the Iranian (Persian) Achaemenid Empire. Decreasing amount of rainfall in the past autumn and winter caused deficiency in pastures and shortage and unavailability and very high prices of fodder and forage in most regions of Iran. This unfavorable phenomenon forced many nomadic and rural people that breeding livestock and especially goat and sheep herds to selling their animals even reproductive livestock under normal prices of them in market to middlemen and before maturing them completely and with low weights and slaughtering them in livestock slaughterhouses in Iran. This imposed many economic damages to them. Slaughtering reproductive livestock will cause reducing in milk and dairy production and increasing their various products prices plus increasing meat price in future (Ministry of Agriculture Jihad, 2021).

Drought is a spreading phenomenon, and it has caused a lot of damages in different parts of the country in recent years. The driest conditions in 53 years have brought chronic mismanagement of water resources in Iran. Drought is a normal, recurrent feature of climate that may occur anywhere, even if its characteristics and impacts vary significantly from one region to the next (Moghaddas Farimani et al., 2017).

Iran has been experiencing long cycles of drought for the last 50 years (Farzanegan et al., 2019). Vulnerability factors such as an underdeveloped infrastructure, lack of facilities, absence of authority, lack of communication between political and economic systems, ineffective markets, lack of social benefits, and inadequate institutional support contribute to the negative effects of drought on a countrywide scale. High population growth, urban development, expansion of deserts and deforestation are additional factors that intensify drought. The effects of drought can occur months or years after the drought has ended. These effects are largely nonstructural and are spread over a larger geographical area than those from other natural disasters. The nonstructural characteristic of drought hinders the development of accurate, reliable, and timely estimates of severity (ANSARI AMOLI et al., 2007). The Iranian climate is mostly arid or semi-arid and it is heavily affected by depleting water resources, as a result of rising demand, salinization, groundwaters overexploitation, and increasing drought frequency. The drought disaster is placing an extreme strain on water resources, drinking water supply systems, livestock and agriculture. It is the cause of great hardship and human suffering, impacting vulnerable groups, particularly in rural areas, who have no alternative source of income and are still feeling the heavy losses they incurred last year. The drought has adversely affected nearly all drinking water supply systems in both rural and urban areas.

RUs in some areas of arid and semi-arid regions (such as in southwest Iran) have developed and applied coping strategies to manage drought effects (Moghaddas Farimani et al., 2017). Because drought is one of the most existential threats facing pastoralists in arid and semi-arid regions, they can use similar strategies to cope with drought in their own local contexts. Such strategies may prevent or reduce the effects of drought and grant RUs access to more rangeland resources. Such strategies also increase forage production during drought, reduce the impacts of drought on livestock and the range, and hasten recovery once the drought subsides. Among these strategies, those that are needed in the short term to improve rangelands (e.g., the gradual reduction of inefficient, elderly, and sick livestock) are more important from the RUs' point of view. Accordingly, it is necessary to reconsider and develop short-term strategies during drought. Moreover, because the damage that results from drought may take a long time ameliorate, long-term strategies to (protection/exclosure) along with the short-term ones should be considered as complementary tools. These strategies, especially long-term ones such as range protection/exclosure, are socially and economically acceptable and implementable among all of the RUs and what alternative livelihoods may be practical for RUs while their rangelands are under exclosure.

In the last two decades, recurring drought becomes a challenge for Iran's economy, which is located in a drought-prone area, and it has been expected that drought will become more severe and frequent in the future (Shahpari et al., 2021).

In countries such as Iran, the nonexistence or deficit of inter-ministerial cooperation is a challenge to drought management. It is clear that effective drought management requires intensive cooperation and accurate programming in different divisions. Drought management requires cooperation between ministries and between organizations (ANSARI AMOLI et al., 2007). Farmers do not have enough water to grow their crops and that the over-use of aquifers has led to subsidence and sinkholes. "If you don't have water, you have to leave and in the last few decades, millions of villagers have left their homes behind and migrated to slums and shanty towns (UN Mission Report. August. 2000). According to Iran's meterological agency, the country's average temperature has increased by 2C since the 1960s, rainfall has decreased 20% in the last 20 years and October 2020 to June 2021 was the driest period in 53 years.

A recent Nature study found that this trend is set to continue. Compared to 1980-2004, 2025-2049 is going to have more frequent and severe heatwaves, droughts and floods. Over 90% of Iran's water is used for agriculture. Due to international sanctions, the government wants the country to be self-sufficient and has encouraged farmers to grow water-intensive crops like wheat, rice and sugar cane. "Iranian officials have acknowledged that outdated agricultural and irrigation systems, as well as poor water management policies in the past three decades, have contributed to nationwide water shortages.

It is suggested that addressing the following issues:

National and regional coordination networks to reduce the effects of drought and to exchange information and know how and establish links for regional drought response as well as develop regional plans and mitigation activities.

While political boundaries are important in planning and responding to droughts, improving the geographical/regional effects of drought need to become better-integrated into future drought preparedness and response.

Cross-border livestock migration is a regular feature of rangeland use in all of the countries in the region. Rangeland management (as well as issues of disease transfer) is an important issue for all concerned.

In the end, following solutions can recommend for improving conditions and solving problems of nomadic and rural people that breeding livestock and especially goat and sheep herds in present drought crisis in Iran:

- Guaranteed purchase of livestock and especially goat and sheep herds from nomadic and rural people by government.

- Security of supply imported forage and fodder to livestock breeders with low and subsidized prices by government.

- Preparing approaches for preventing nomadic and rural people that breeding livestock and especially goat and sheep herds from selling and slaughtering their reproductive livestock.

- Providing balance between number of goat and sheep herds and capacity of rangelands in all of the regions of country. With considering this important point that according to last statistics from Ministry of Agriculture Jihad in 2021, there are more than 75 million head of light trap and small livestock (goat and sheep) in country whereas capacity of rangelands only can feed 60 million of them. This most consider as an important strategy in government policies of Iran.

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### EVALUATION OF THE RENGGONG HORSE'S BODY CONFORMATION IN CONGGEANG AND UJUNG JAYA SUB-DISTRICTS SUMEDANG REGENCY

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### Abstract

Conformation is a description if the general appearance of an animal, which is then associated with performance and health. The research "Evaluation of the Renggong Horse's Body Conformation in Conggeang and Ujung Jaya Subdistricts, Sumedang Regency" was carried out on March 15, 2021-March 20, 2021 in Conggeang and Ujung Jaya subdistricts. The purpose of this study is to determine the size of the actual conformation and its suitability to the ideal conformation based on the ideal conformation according Mc Bane-Helen. The method used in this research is descriptive method by taking data from the entire population. The fit between the actual and ideal conformations was tested using the Chi Square test. Based on the results of the Chi Square statistical test, the body measurements of the Renggong horse in Conggeang and Ujung Jaya Districts are in accordance with the ideal body measurements according to Mc Bane-Helen.

Key words: Actual body measurement, Mc Bane- Helen body conformation, Renggong horse

### INTRODUCTION

Renggong Horse is one of the performing arts originating from Sumedang Regency, West Java which has been designated as a National Intangible Cultural Heritage by the Minister of Education and Culture on May 15, 2015. The word Renggong comes from the word "ronggeng" which means dance movements that follow the rhythm (Gustianingrum and Affandi, 2016). Renggong horse art is performed by horses which have been trained, so they have the skills to move the horse's legs, head and body following the rhythm of the accompanying music. Conggeang and Ujung Jaya sub-districts are one of the sub-districts in Sumedang Regency that have a lot of Renggong horses, with a total population of 49 Renggong horses in the two sub-districts.

Based on the narrative of the horse breeder, the horse used in the Renggong horse art is a horse that comes from a cross between a Sandel horse and a Race horse. This type of horse is widely used by Renggong horse owners because of its ideal height to be ridden during Renggong horse performances. Generally, horses begin to be used for Renggong horse performances at the age of 3.5 years, depending on the horse's ability to perform leg, head and body movements during a Renggong horse show. The age of rejection of the Renggong horse is uncertain, depending on the performance of the horse itself at the time of the show. During the show, Renggong horses work for 6-7 hours in one day of the show. Renggong horses to show the skills of leg, head and body movements to the accompaniment of music, must have good posture.

Conformation relates to the appearance of the horse's body proportions, refers to the defined structure of an animal from its body parts, and plays an important role in the horse's ability to perform. Conformation is important selection criterion in Renggong horses. Evaluation of the ideal horse's body conformation is generally done with one of the formulas of the ideal horse's conformation formula, such as the Mc Bane-Helen formula.

The ideal horse according to Mc Bane and Helen is a horse that has an equal distance between the throat, the highest part of the shoulders, hips, back of the elbows, and the fetlock of 40% of the total length of the throat to the base of the tail. Meanwhile, the length of the hip distance and the base of the tail is half the length of the other distance or 20% of the total length of the throat to the base of the tail (Mc Bane and Helen, 1990). Based on this description, the author is interested in knowing the body conformation of the Renggong horse in Conggeng and Ujung Jaya sub-districts based on the ideal body conformation according to the Mc Bane-Helen formula and the magnitude of its suitability to the actual conformation.

## MATERIALS AND METHODS

The method used in this research is descriptive quantitative method by taking data from the entire population. Quantitative properties are properties that can be measured or properties in the form of numbers (Sudjana, 2005). The object of the research used was 49 adult stallions aged at least 3.5 years who had been trained as Renggong horses in Conggeang and Ujung Jaya. The fit between the actual and ideal conformations was tested using the Chi Square test. Based on the results of the Chi Square statistical test, the body measurements of the Renggong horse in Conggeang and Ujung Jaya Districts are in accordance with the ideal body measurements according to Mc Bane-Helen.

This research was conducted by taking pictures of the horse side view. The shooting distance has been standardized so that the measurement data from the image is then converted to a predetermined scale. The measurements of the body parts studied were the length from the throat lutch to the highest part of the withers, the length from the withers to the hip, the length from the hip to the dock. the length of the distance from the withers to the back of the elbow, and the length of the distance from the back of the elbow to the fetlock (proximal sesamoid).

## **RESULTS AND DISCUSSION**

Horses, like other domestic animals, are formed from the results of selection and breeding so as to produce a body skeleton morphology in accordance with the desired purpose (Brooks, et al., 2010). The representation of horse visual morphological characters, such as withers height, chest circumference, and cannon bone circumference were used as estimates of standard body measurements in horses (Onar et al., 2015).

must have Renggong horses an ideal conformation to be able to carry loads and ensure continuity of motion. Conformation is a and correlates aenetic trait with qait characteristics in horses, so conformation can be taken into consideration in horse breeding. The best athletic ability of a horse can be estimated by looking at proportions such as height at the withers and at the highest point of the hip provided that height (croup), must be proportional to body length (Sánchez, et al., 2013).

Horse measurements use an estimation system based on horse images that have standardized the shooting distance using a template. According to Kainz et al. (2015), measurement of objects based on images is almost always accurate, with an object dimensional error rate of 10%, the height of the object when it appears in the image display area depends on the distance of the object from the camera. The actual object distance from the camera consists of the distance between the object area and the viewing area, and the distance not captured by the camera, with the same shooting distance, the object's height will be the same in each shot. The assumption is that when two images with the same distance, one with an object and one without an object, each pixel of the two images has the same number and position so that the two images can be said to be identical.

The ideal horse according to Mc Bane-Helen is a horse that has an equal distance between the throat, the highest part of the shoulders, hips, back of the elbow, and fetlock of 40% of the total length of the throat to the base of the tail. Meanwhile, the length of the hip distance and the base of the tail is half of the length of the other distance or 20% of the total length of the throat to the base of the tail (Mc. Bane and Helen, 1990). Then the length (a) the distance from the throat lutch to the withers, (b) the distance from the withers to the hip, (d) the distance between the withers to the back of the elbow and (e) the distance from the back of the elbow to the fetlock (proximal sesamoid) is 40% of a+b+c, and the length (c) the distance from the hip to the base of the dock is 20% of a+b+c. Based on the actual value and the calculation results of the ideal value calculated from all parts a, b, c, d, and e which are then analyzed using the Chi Square Test, the results of the Horse Renggong calculation can be seen in Table 1.

Table	1.	Calculation	Results	Using	the	Chi
Square	Tes	st of Renggo	ng Horse	es in Co	ngge	eang
and Ujung Jaya Sub-Districts						

	Average Actual	Average Ideal	
	Body	Body	Chi
	Conformation	Conformation	Square
	(Centimeter)	(Centimeter)	
а	65,49	67,78	
b	70,17	67,78	
с	34,54	33,89	0,19
d	66,74	67,78	
e	67,43	67,78	

Significant difference (P<0.05). a = The distance from the throat lutch to the highest part of the withers, b = The distance from the withers to the hip, c = The distance from the hip to the dock, d = The distance between the withers and the back of the elbow, e = Length of distance from the back of the elbow to the fetlock (Proximal sesamoid).

The matches obtained from averages parts a, b, c, d, and e have results that are close to ideal, this indicates that based on body measurements according to Mc Bane-Helen, the Renggong horse in Conggeang and Ujung Jaya Sub-Districts is close to the ideal value. To see the proportion of ideal body size as a whole, the Chi Square Test statistical proportion test was used.

Based on Table 1. the calculated Chi Square value is 0.19 with the table Chi Square value of 9.49, which shows that the calculated Chi Square value is smaller than the table Chi Square value. This shows that based on the Chi Square statistical test, the body sizes of the Renggong Horse in Conggeang and Ujung Jaya Sub-Districts, Sumedang Regency are in accordance with the ideal horse body sizes according to Mc Bane-Helen.

## CONCLUSIONS

In conclusion, the results of the present study imply that comparison between the actual body size measurement results and the ideal body size according to Mc Bane-Helen, has a match. The Mc Bane-Helen method is a formula used to assess ideal body sizes in Race Horses and can also be used to assess ideal body sizes in Renggong Horses.

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## EXAMINATION OF THE RELATIONSHIP BETWEEN FEEDING TYPE AND CALF BEHAVIOR

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#### Abstract

This study was aimed to investigate diet selection and animal behavior to Holstein calves different feeding methods. For this aim, 3 treatment group were used as TMR, choice feeding and pellet. As for calf behavior, feeding, resting, litter eating, rumination, water consumption and other behaviors were examined with these 3 treatment groups. The  $\chi^2$  test was used to test the dependence of all behavioral traits on the feeding type. When the dependence of the calves behavior on the feeding type was examined, it was statistically determined that it was not dependent.

Key words: Holstein calf, Animal behaviour, Feeding type, Nutrition, Chi-square test

## AN OVERVIEW OF REPRODUCTION AND ARTIFICIAL INSEMINATION IN HONEY BEES: TURKISH EXAMPLE

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#### Abstract

High productivity in agriculture depends on ensuring inter-plant pollination (fertilization). Bee health and production have been critical in the production of honey and other bee products, which have provided thousands of years of food and income since ancient Egypt when beeswax was widely used. According to physicist A. Einstein (1949), who wanted the bees to be protected in the ecosystem; "If the bees disappear, human beings can only live for four years. Without bees, there would be no plant, no animal, no human, just as there would be no fertilization". Comprehensive research on beekeeping is very limited in Veterinary Medicine. In the sector, there is an urgent need for the protection of resistant and abundant honey-producing/pollen-collecting native breeds through reproduction (features of male and female gametes) and artificial insemination, as well as ensuring animal welfare, preventing high temperature, humidity and stress within the hive. Turkey's vegetation is very rich with thousands of numbers and diversity. Our region has plants that allow the original production of specific honey types (e.g. thyme, eucalyptus, and red pine). Turkey, as located on a peninsula between three continents, with very rich vegetation and thousands of years of civilization, is one of the leading countries in the world in beekeeping; first in the world in the total hive (7 million; OMU-TR, 2021), second in the world in honey production (104 thousand tons; FAO-2014). In beekeeping, as normally depending on water and rich flora (nectar and pollen), honey is produced more intensively in Muğla, Adana and Ordu provinces of coastal regions (Aegean, Mediterranean and the Black Sea, resp.). Especially Muğla province, located in the Southern Aegean, is covered with 68% forested (mostly red pine, Pinus brutia) scrub and wetlands. In addition to olive growing, citrus production and tourism, our province is the leader in gulf fishing (bream and sea bass) and has a voice in the world with its honey production (total 3.5 million hives) (90% of our country in pine honey, 75-80% in Muğla). Due to its climate and geographical structure, seasonal transitions are not very clear herein, so it is the main haunt of mobile beekeepers coming from outside the province. However, most producers continue traditional beekeeping without expert assistance. Accordingly, reproduction in bees is mostly done by traditional/classical methods (via the queen's mating flight). Due to the presence of migratory beekeepers going to different regions for wintering purposes, the rate of cross-breeds (hybrids) has gradually increased. There are wrong practices related to ignorance and inexperience in beekeeping. Incorrect hive management, environmental/agricultural pollutants and various bee diseases (Varroa, American or European foulbrood, Nosema, Calcification, hive worm) adversely affect honey production as well as colony existence and health in bees. As a result, massive bee deaths, colony losses or low yields are frequently seen. Not only bees but also the flora are adversely affected by increasing fires, extreme temperatures, drought and rising temperatures with the north wind. Inevitably, the presence of nectar and pollen, having a critical role in the development of bees, will gradually decrease under not only these unfavourable factors but also improper feeding and insufficient parasitic control (namely for Varroa). Although there are different (Anatolian, Thrace, Aegean, Caucasian, Syrian, Iranian and Cyprus) types among the main honey bee (Apis mallifera) breeds found in our country, the most common one is the "Anatolian bee" (Apis mallifera anatolica). Aegean ecotype of the Anatolian bee (Apis mallifera anatolica ecotype) is common in the Muğla region. Global warming, fires and climate

change are gradually increasing in the world. Years without precipitation, the late arrival and short duration of the winter seasons, as well as the extreme heat in the dry summer months, prevail. In addition, since the seasonal transitions became rapid and sudden, the bees access to green plants and water resources for their survival and production may be severely limited. This situation leads to a decrease in the hive accommodation distance between the beekeepers and to the fact that many colonies are in a congested condition in the same narrow area. The "Muğla bee", which prefers the presence of the "Basra insect" (Marchalina hellenica), frequently seen on red pine trees and contains fructose in its faecal secretion, should be obtained and protected as a pure breed. In a practical solution, a successful beekeeper in the field has to give the bee in the hive the daily "work to be done". Scientifically, it is also imperative to improve bee welfare and medicine (including reproduction and artificial insemination). In summer (active) hive, there are three types of bees (a specially fed, egglaying long queen with 2n chromosomes, some/2-3 thousand large males/n and mostly/70-80 thousand small worker-females/2n). There can be 'parthenogenetic' (asexual) reproduction in bees (infertile-mitotic eggs bear male/n while fertile-meiotic eggs bear female/2n). The queen (over 170 mg, 5-6 days old) is fertilized by drones (8-10 male) during her natural mating flight (1-3 times). In artificial insemination performed under stereo-microscope, fresh semen (8 mm3) collected (15-20 males) hygienically is pipetted via abdominal and back hooks under the special cuticle on the back (sperm storage place) of the female after stunning (CO2). Semen samples can be stored (using antibiotic saline/Ringer, 1:5, semen ratio) by aluminium foil wrapping at +16oC up to 15 days or in straws (preferably using TES/TRIS- "Kyiv" extender and DMSO cryoprotectant) by freezing in a stepwise manner (-3oC/min rate to -40oC, then plunged into the LN2) for a long time (thawing at 35-40oC for 20 sec). However, it is recommended to use fresh semen (with high motility-viability) for high brood yield (over 50%, worker bees). In the literature, few studies on the protective efficacy of honey as a source of carbohydrates (sugars, 76%) and its by-products like propolis having antimicrobial and antioxidative effects in mammalian reproduction (sperm-egg quality, storage, fertility) are important. Therefore, in the future, beekeeping products (namely propolis) should be considered widely as cellprotective agents for semen handling in livestock breeding. Undoubtedly, scientific studies on beekeeping will contribute to the sustainability of our country's high level in the world in terms of our national colony population and honey production, and to the goal of increasing our international market share. But, in sustainable production towards bee health and welfare, it is essential for the beekeepers to encounter experts in the field and adapt the science thereby.

Key words: Honey bee, Reproduction, Artificial insemination, Turkey

## UPDATE ON REPRODUCTION AND ARTIFICIAL INSEMINATION IN FOWL AND TURKEY

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#### Abstract

Reviewing the literature, an update on breeding and artificial insemination (AI) in poultry (fowl, turkey, goose, and duck) will be given herein. High commercial circulation of fowl and high market value of turkey are apparent. Historically, in Europe, Turkey is the undisputed exports leader of the intensive industrial sector for chicken production. Chicken meat/egg at affordable prices can largely meet the individual daily animal protein requirement. Additionally, the meat of turkey with its large body is popular at a reasonable price especially in the Western world and in our big cities. Its industrial production is limited, but semi-intensive culture is common nationwide. The famous taste of goose meet and its breeding culture (grazing, swimming) is typical in North-eastern Anatolia. Finally, duck production is carried out in semi-intensive and water-accessible enterprises while its meat is in demand especially in big cities and tourist areas. In poultry, the reproductive cycle has a 'photoperiodic' nature and, the breeding season (under 'heat- light- feeding' effect) starts in spring in the Northern hemisphere including Turkey. However, this cycle may be altered by excessive/continuous lightening, as a popular protocol for intensive breeding. Additionally, unlike cage breeding with ready feed and limited moveable area, semi-intensive care and feeding culture (roaming chicken) is becoming increasingly popular especially in commercial enterprises. There exist variabilities in reproduction in females, such that; pronounced oestrus lacks but just sudden sitting seen, oviposition periods differ between species, progesterone -instead of oestrogen- causes LH release for ovulation, and in fowl, the next ovulation (laying) occurs one day after fertilization and the incubation period of eggs takes about 3 weeks. Histo-anatomically, females have a very long threesegmented oviduct (infundibulum, magnum, and isthmus), fertilization is formed in the infundibulum area, and no polyspermy block forms. Following a single mating/AI weekly, the sperm deposition sufficient for a week or so is facilitated by sperm collecting tubules within the utero-vaginal area of females. Males (turkey, rooster) do not have a classic penile organ, whereas waterfowls (goose and duck) have a protrusion (phallus) or large birds (ostrich) have a big penis. Male accessory reproductive glands and urethra are absent and the ductus deferens is opened directly into the cloaca (turkey and rooster). The use of AI in fowls is striking mainly for breeding purposes. As a large source of consumable animal protein (chicken meat/egg) in the world, AI is used in poultry due to the increasing size of broiler breeding males. Likewise, in turkeys with lymphatic/large bodies, the AI is necessary since the heavy (three-fold) male harms the female during prolonged mating. The poultry AI sector continuously develops, but it requires to care for hygienic semen collection/AI and foot diseases, and also avoid inbreeding. Al prevalence in goose and duck is limited due mainly to difficult semen collection. Poultry semen is collected on alternate days by a routine abdominal massage under hygienic conditions. Due to the lack of male accessory glands, semen is very dense and its volume is rather low, so it should be diluted with non-toxic diluents at low rates. Sperm with elongated morphology (head) has a high rate of unsaturated fatty acids in chemical composition. Due to vulnerability to oxidation as commonly observed during processing, poultry semen requires the presence of antioxidants in the diluent. Semen cryopreservation procedures are not as practical as in the bull. However, it is possible to inseminate turkeys and hens at weekly intervals by keeping fresh-

diluted semen (with low dilution) kept at the refrigerator (around +4oC) for up to one week without losing its fertility potential. Using fresh (diluted) semen as collected by standard abdominal massage, intravaginal insemination (into the utero-vaginal area) is quite simple and hence successful hatching rates are achievable. Fertility rates with frozen semen are generally poor, but the results of recent semi-programmable freezing seem promising. Reasonable AI results with frozen semen are achievable by using an increased (four-fold) inseminate dose. Apparently, the financial gain from the poultry AI sector is expected to increase by the accumulation of innovative knowledge and experience in terms of bird's spermatology, semen handling and freeze-thawing. As a leading country of the sector, the sustainability of our regional high position depends surely on specialization, scientific research, funding and publication activities, especially in fowls and turkeys. Depending on the size of breeding males (broiler roosters and turkeys) in the field, weekly AI with fresh-diluted semen usually gives encouraging results along with sufficient experience and hygienic precautions. Furthermore, sectoral awareness seems to be critical, especially in fowl AI, as one of the most common sources of 'animal protein' (meat and eqq). Finally, critical attempts expected in reproductive biotechnologies including the assisted breeding would also allow for the genetic conservation of hundreds of wild bird species (bustard, bald ibis, etc.) under a heavy threat of extinction.

Key words: Poultry, Reproduction, Artificial insemination

## PASTURE PLANTS AND THEIR BIOLOGICAL FEATURES FOR SUSTAINABLE LIVESTOCK DEVELOPMENT

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#### Abstract

For sustainable livestock breeding and its further development, the role of pasture lands is steadily increasing. Pastures vary greatly in the degree and intensity of management, ranging from extensively managed pasturelands, where the main management variables are the density and mortality regimes of livestock in the pasture, to intensively managed (with soil fertilization, irrigation, shifts of vegetation types) long-term pastures and grasslands. The specific gravity of annual grasses increases on heavily overburdened pastures. Grazing affects the vegetation and, through it, the soil. Remaining animal feces and urine on pasture also affect the soil and plants. The role of grazing in seeding of pastures is significant, creating more favorable conditions for the germination of seeds when the sod fails and they are trampled into the soil. When pastures are overloaded or improperly grazed with a change in the plant composition, their economic value deteriorates. Excessive grazing of animals can intensify the processes of water and wind erosion. When using hayfields, first of all, the tall forbs Libanotis intermedia, Heracleum sibiricum, Thalictrum angustifolium, annual and biennial late sowing grasses disappear from the grass stand; the fast-growing herbs Bromus inermis, Festuca pratensis, Phleum pratense, Trifolium pratense, Medicago sativa last longer. Low cereals and legumes are found in the lower tier. Early mowing periods lead to a decrease in early maturing grasses in the herbage. Animals, on average, digest 60-80% of the dry mass of eaten plants. The digestibility coefficient of nutrients decreases with the age of the grasses, the highest digestibility is in the tillering phase. Early grazing of livestock, especially immediately after the snow melts, sharply disrupts the normal course of accumulation and consumption of reserve nutrients in plants, which entails deterioration in the botanical composition of the herbage, and subsequently - a decrease in the yield of grasses. The reason is that developing green shoots in the spring consume a lot of reserve nutrients, and they begin to accumulate again only 10-15 days after grazing. However, one should not be late with grazing, because the fodder value of the grass stand deteriorates, the grasses become coarser, the consumption of the grass by animals' decreases, and the productivity of the pasture decreases. Therefore, for the sustainable development of animal husbandry, a reasonable approach to the use of pastures is required.

Key words: Haymaking, Pasture, Livestock, Use intensit

## THE RELATIONSHIP BETWEEN THE BREEDING VALUE OF BULLS AND MILK YIELDS OF THEIR DAUGHTERS MILKED IN AUTOMATIC AND CONVENTIONAL MILKING SYSTEMS

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## Abstract

The inclusion of information on the level of milk yield (MY) and characteristics related to milking parameters into the breeding practice should be crucial when selecting parents in dairy herds. Currently, in the assessment of the breeding value (BV) too little attention is paid to the features related to the milking parameters, which may help farmers with improving selection of bulls for herds equipped with an automatic milking system. Statistical analysis included information on 2,533 Polish Holstein-Friesian black-and-white bulls and their daughters (a total of 25,761 females and their 65,275 305-day lactations). The data on daughters were gathered form a total of 24 herds equipped with automatic (AMS) and 15 with conventional milking system (CMS). The results show a higher positive correlation between the BV of bulls and MY of their daughters milked in AMS (0.313), than in herds equipped with CMS (0.224). It also showed that although the average results of the assessment of the BV of the group of bulls whose semen was used in the herds equipped with CMS was high, the correlations between the bulls' BV and the production level of their daughters were at a lower level compared to the herds equipped with AMS. The fact that bulls in herds that use AMS had lower BV than herds with CMS may suggest that breeders paid more attention to bulls' BV in CMS than AMS herds. Therefore, in order to improve efficiency of milking in AMS farmers should pay more attention to selecting bulls with higher BV for breeding. It seems necessary to refine the breeding value assessment for cows milked with different milking systems (AMS and CMS).

Key words: Bull, Breeding value, Milk yield, Milking systems

## POLYMORPHISM IN MITF GENE AND MILK PERFORMANCE OF POLISH HOLSTEIN-FRIESIAN COWS

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#### Abstract

The aim of the study was to determine polymorphism within melanocyte inducing transcription factor (MITF) gene in a population of 897 Polish Holstein-Friesian cows. Test-day phenotypic records were analysed. Genetic data were collected in the process of routine genetic estimation of breeding value (GEBV) that is performed in Poland with the use of EuroGenomics arrays. The relationship between SNPs in MITF gene with the milk yield in full lactation and chemical composition of milk (percentage of fat, protein, lactose and dry matter) was statistically examined with the use of one-way analysis of variance. One SNP, intronic variant that is characterised by G to A transition (g.31723626G>A, rs41642495), was proven to be polymorphic. Out of all tested animals 45.04% were AA homozygotes, 43.92% heterozygotes and 11.04% GG homozygotes. Hardy-Weinberg equilibrium test was performed and results (X2 0.04, p-value 0.84) suggest that observed and expected frequencies did not vary significantly and the population was in Hardy-Weinberg equilibrium. Animals with AA genotype in the full lactation had higher milk yield (10,793.18 kg in full lactation) compared to heterozygotes (by 3.77%) and GG homozygotes (by 1.16%). However, these difference were not proven to be statistically significant. The analysis revealed statistically significant ( $p \le 0.05$ ) differences between fat, protein, lactose and dry matter percentage in milk. Cows with GG genotype were characterised by the highest fat (3.75%), protein (3.35%) and dry matter contents (12.75%) compared to other animals, while AA homozygotes had the highest lactose content (4.87%). Even though the MITF gene is generally associated with the coat colour this study shows that SNPs that are added to microarrays may be also used in the research on associations with other traits.

Key words: Genotypes, Milk performance, MITF, Polymorphisms

#### EFFECTS OF SLAUGHTER WEIGHT ON SOME CARCASS CHARACTERISTICS AND QUALITY TRAITS IN ANGUS BULLS RAISED IN TURKEY CONDITIONS

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#### Abstract

This study was performed to evaluate the effects of slaughter weight on marbling, fatness score, backfat thickness, meat color score, and the ultimate pH in Angus bulls (n= 104). According to the slaughter weights (SW), bulls were allocated into three groups including SW1= 505-550 kg; SW2= 551-600 kg; and SW3= 601-659 kg. The statistical analysis was carried out using the least square methods of the GLM procedure and Pearson's correlation analysis. Results showed that group SW3 was characterized by the higher marbling, fatness score, and backfat thickness (P<0.001). Moreover, significant correlations were found between marbling, backfat thickness, and fatness score (P<0.001). Meat color and pH values were not differentiated within the groups.

Key words: Marbling, Meat quality, Carcass traits, Slaughter weight, Angus

## INTRODUCTION

Meat quality traits are becoming increasingly important to meat processors and consumers. It is also crucial for upscale restaurants. Although meat prices in Turkey are higher than in many developed countries, the demand for highquality meat products is also increasing. Many important factors remarkably influence carcass characteristics and meat quality, including breed, sex, slaughter age, stress conditions, etc (Ardicli, 2018). It is important to note that significant differences have also been identified depending on the individual genotypic background (Ardicli et al., 2017). Considering meat quality characteristics, there are significant differences between countries and even between different regions of the same countries. In general, highly marbled beef is considered to be high-quality by meat distributors and consumers (Park et al., 2002). Marbling can be defined as the amount and spatial distribution of the visible fat in the muscle. This fat appears as fine flecks and it gives an appearance similar to marble (Munoz et al., 2015). The degree of marbling can be determined according to the presence of white particles or flecks of intramuscular fat (IMF) between muscle bundles (Hocquette et al., 2010; Beak et al., 2021). In addition to the degree of marbling, one of the most important criterions consumers use to judge high-guality meat for

the price and acceptability is meat color (Horcada et al., 2013). Low color properties, especially remarkably light red or extremely dark colors, are perceived as the signs of low quality and unhealthy meat by consumers. This situation causes significant economic losses in the market. Although there are many traits related to the color of meat, ultimate pH plays a pivotal role in the formation of the desired color properties (Ardicli, 2018). Optimum pH values should be at the range of 5.4–5.8, 24 h after slaughter. High ultimate pH leads to unfavorable changes in meat color scores and causes dark color at the surface. This type of meat can be defined as dark, firm, and dry (DFD) (Weglarz, 2010). On the other hand, fatness traits and backfat thickness are important indicators when evaluating carcass and meat quality (Nogalski et al., 2014).

It is well known that slaughter weight is one factor affecting meat quality and carcass value (Park et al., 2002). In Turkey, the importation of beef cattle is becoming increasingly frequent to regulate meat supply and demand. In this sense, one of the most common breeds is Angus. This is an excellent beef cattle breed and it is usually slaughtered at high live weights. However, there is limited information on the effect of slaughter weight on carcass characteristics and quality traits in Angus raised in Turkey conditions. Therefore, this study aimed to evaluate the effects of slaughter weight on marbling score, fatness score, backfat thickness, meat color score, and the pH in Angus bulls.

#### **MATERIALS AND METHODS**

A total of 104 purebred Aberdeen Angus bulls were used. They were raised in the same commercial farm located in the South Marmara region of Turkey with the same feeding and management practices. Slaughter weights were determined immediately before slaughter. According to the slaughter weights (SW), bulls were allocated into three groups as follows: SW1= 505-550 kg; SW2= 551-600 kg; and SW3= 601-659 kg. Animals were slaughtered according to the standard procedures and hot carcass weights were recorded. After a 24h chill in a ventilated room, chilled carcass weights were measured, and thus chilling loss was determined. Moreover, the ultimate pH was measured in the m. longissimus thoracis (between the 12th and 13<sup>th</sup> thoracic vertebra) 24 h post-mortem using a digital pH meter (Testo 205, Lenzkirch, Germany). Fatness score was evaluated according to the degree of carcass fatness by using a scale indicating 1: trace and 5: very abundant. Backfat thickness was evaluated in terms of thickness of fat over the longissimus muscle measured perpendicular to the outside surface (12<sup>th</sup> rib level). The degree of marbling (1-10) was evaluated according to Zhao et al. (2004) and the meat color score was determined using a scale (1-5) by visual attributes.

Concerning the statistical analysis, initially, all the data were tested for normality by the Ryan-Joiner normality test ( $\alpha$ =0.05). Afterward, a general linear model (GLM) procedure of Minitab (MINITAB®, Pennsylvania, USA, v17.1.0) according to the following statistical model:

 $Y_{ijkl} = \mu + SW_i + SS_j + \beta SA_k + e_{ijkl}$ where:

 $Y_{ijkl}$  = the studied traits;

 $\mu$  = the overall mean;

SW<sub>i</sub> = the fixed effect of slaughter weight groups (i: SW1, SW2, SW3)

SS<sub>j</sub> = the fixed effect of slaughter season (j: spring, summer, winter);

 $\theta$  SA<sub>k</sub> = the regression effect of slaughter age;

 $e_{ijkl}$  = the random residual effect.

Tukey's test was used as a post hoc comparison. Correlation coefficients were generated using the Pearson's correlation coefficient (PCC) option, indicating low correlation if PCC is < 0.30, intermediate correlation if PCC is between 0.30 - 0.70, and high correlation if PCC is >0.70 (Buyukozturk, 2002).

Heatmap was generated using Morpheus matrix visualization software (https://software.broadinstitute.org/morpheus/) based on the r values of the Pearson correlation.

## **RESULTS AND DISCUSSION**

In recent years, the trend in beef production has gradually changed from quantity to quality. Many factors, including genetic and non-genetic, influence carcass and quality traits. Although it is well known that the meat quality of beef cattle is remarkably higher than dual-purpose or milktype breeds, there is still a need for detailed studies on carcass characteristics and quality traits in beef cattle, especially in Turkey conditions. This study was performed to evaluate the selected carcass characteristics and quality traits in three slaughter weight groups of Angus cattle. Descriptive statistics of slaughter weight and some carcass characteristics and quality traits in Angus cattle are presented in Table 1.

**Table 1.** Means, standard errors, and minimum and maximum values for slaughter weight and some carcass characteristics and quality traits in Angus cattle (n = 104).

Trait	Mean	SE	Min	Max
SW	577.93	3.91	505.00	659.00
HCW	328.91	2.22	289.00	386.70
CCCW	323.44	2.23	283.00	380.60
CL	5.48	0.06	4.11	6.92
Ma	5.98	0.09	4	8
FS <sup>b</sup>	3.46	0.05	2	4
BFT	17.14	0.17	14.00	21.00
MCS <sup>c</sup>	3.37	0.06	2	5
$pH_{24h}$	5.56	0.01	5.36	6.00

SW = Slaughter weight (kg); HCW = Hot carcass weight (kg); CCW = Chilled carcass weight (kg); CL = Chilling loss (kg); M = Marbling; F = Fatness score; BFT = Back fat thickness (mm); MCS = Meat color score.

<sup>a</sup>1 = devoid; 2 = practically devoid; 3 = traces, 4 = slight; 5 = small; 6 = modest; 7 = moderate; 8 = slightly abundant; 9 = moderately abundant; 10 = abundant.

 $^{b}1 =$ trace, 5 = very abundant.

 $^{c}$ 1 = very light cherry red, 5 = very dark red.

In the present study, ANOVA results showed that the SW groups have significant differences

regarding marbling, fatness score, and backfat thickness (Table 2). In this context, the SW3 group has significantly higher values than the SW1 SW2 groups (*P*<0.001). and Not surprisingly, the increase in fat-related traits can be associated with the higher live weights before slaughter. These results indicated that bulls with >600 kg SW were characterized by distinctively higher marbling, fatness score, and backfat thickness. Fatness is known to be significantly related to the ultimate eating quality of beef (Jeremiah, 1996). It is important to note that consumer preference can widely vary concerning different levels of beef fat in different countries. Moreover, the choices may be changeable among the different regions of Turkey. On the other hand, trends that low red meat fat consumption is more favorable for human health may lead to increased production and sales of low-fat meat and processed meat products and removal of fat from non-retail meat pieces. It is important for the consumer whether the fat chunks are large or small for steaks, roasts, and veal cubes; and the fat content of ground beef is high or low (Resurreccion, 2004).

**Table 2.** Carcass characteristics and quality traitsmeans  $(\pm SE)$  within slaughter weight (SW)groups of Angus cattle.

		SW Groups	
Trait	SW1	SW2	SW3
	(n=31)	(n=35)	(n=38)
М	5.26±0.11 <sup>b</sup>	5.47±0.12 <sup>b</sup>	$7.05 \pm 0.10^{a}$
FS	3.03±0.08 <sup>c</sup>	$3.31 \pm 0.09^{b}$	3.94±0.07ª
BFT	16.26±0.29 <sup>b</sup>	16.71±0.32 <sup>b</sup>	18.56±0.28 <sup>a</sup>
MCS	3.40±0.12	3.62±0.14	3.50±0.12
рН	5.54±0.03	$5.57 \pm 0.03$	5.57±0.02

M = Marbling; F = Fatness score; BFT = Back fat thickness (mm); MCS = Meat color score.

<sup>a,b,c</sup>Means with different superscripts within a row are different (P < 0.001).

Apart from fat-related traits, one of the most important indicators that significantly affect consumer choices is meat color. The evaluation of color properties is a complex one. It consists of pre-and post-slaughter determinants and genotypic structure (Mancini and Hunt, 2005). In this study, there was no significant difference among the SW groups (P>0.05). One possible explanation for this situation may be the method used for the determination of meat color. Because a subjective technique was performed by evaluating the visual color properties (1= very light cherry-red to 5= very dark red). Instrumental color evaluation methods (CIE L\* a\* b\*, CIE L\* u\* v\* or Hunter Lab systems) can provide more reliable and explanatory results (Ardicli, 2018). Another decisive parameter in meat quality assessment is pH and it is also known as the main factor in optimum meat color characteristics (Pipek et al., 2003). Carcasses with ultimate pH between 5.40 and 5.80 are evaluated as acceptable (Wêglarz, 2010). In the present study, the vast majority of carcasses were in this range. However, there were five carcasses with the  $pH_{24} \ge 6.00$  (Figure 1). There was no significant association between the SW groups and pH (Table 2).

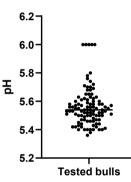
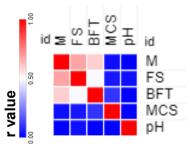


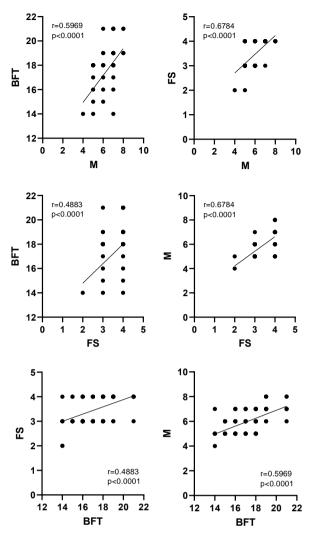
Figure 1: The pH values observed in this study.

Pearson correlation analysis showed significant relations between the traits analyzed, except for pH and meat color score (Figure 2).



**Figure 2:** Heatmap of the Pearson correlation analysis concerning carcass characteristics and quality traits.

M = Marbling; F = Fatness score; BFT = Back fat thickness (mm); MCS = Meat color score.



**Figure 3:** Graphics of the significant Pearson correlations observed in this study.

M = Marbling; F = Fatness score; BFT = Back fat thickness (mm).

All of the significant correlations were determined to be an intermediate correlation (PCC= 0.30 - 0.70) (Figure 3).

#### CONCLUSIONS

This study focused on the slaughter weight groups and their effects on some carcass characteristics and quality traits in Angus bulls raised in Turkey. Bulls with SW<601 kg were characterized by the higher marbling, fatness score, and backfat thickness. In addition, significant correlations were found between marbling, backfat thickness, and fatness score.

#### ACKNOWLEDGEMENTS

This work does not contain any invasive procedures in animals performed by any of the authors. The data were collected in a slaughterhouse during routine standard slaughtering.

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(Korean native cattle) carcasses. Journal of	Resurreccion AVA. 2004. Sensory aspects of
Animal Science, 80: 129-136.	consumer choices for meat and meat products.
Pipek P, Haberl A, Jeleniková J. 2003. Influence of	Meat Science, 66: 11-20.
slaughterhouse handling on the quality of beef	Wêglarz A. 2010. Meat quality defined based on
carcasses. Czech Journal of Animal Science, 48:	pH and colour depending on cattle category
371-378.	and slaughter season. Czech Journal of Animal
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## SCREENING FOR GALWAY MUTATION (FECXG) IN KIVIRCIK BREED

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#### Abstract

High litter size or twinning is an economically important trait that enhances sheep productivity. The FecX<sup>G</sup> has been influentially associated with the ovulation rate in various sheep breeds. However, there is limited information about this locus in the Kivircik sheep breed. Therefore, the aim of this study was to evaluate the presence of the Galway (FecX<sup>G</sup>) mutation in Kivircik sheep (n=91). Genomic DNA was isolated from whole blood using the phenol-chloroform extraction method. The genotyping was performed by the PCR-RFLP method. Results revealed that all ewes were the Galway mutation (FecX<sup>GG</sup>) carriers and the corresponding genotype was fixed in the studied population. The present analysis showed that the Galway mutation which is a nucleotide alteration (cytosine to thymine) at position 718 bp of the BMP15 (also known as FecX) gene may be considered in enhancing twinning in the Kivircik breed. However, further analyses with larger populations are needed to confirm the present results and to provide more detailed information before focusing on this genomic region in breeding programs for purebred Kivircik sheep.

Key words: Sheep, Kivircik, FecXG, mutation, PCR-RFLP

## INTRODUCTION

The transforming growth factor- $\beta$  (*TGF-B*) gene superfamily encodes multifunctional proteins that play fundamental roles in cell growth and differentiation (Kumar et al., 2008). These proteins have also important effects on embryogenesis in mammals. TGF-B consists of fecundity genes in sheep, including bone morphogenetic protein receptor IB also known as Booroola fecundity gene (*FecB*), growth differentiation factor 9 (GDF9) also known as *FecG*, and bone morphogenetic protein 15 (BMP15 or GDF9b) also known as FecX (Galloway et al., 2000; Wilson et al., 2001; Hanrahan et al., 2004; Kumar et al., 2008). The mutations in two oocyte-derived growth factor genes including the X-linked *BMP15* (*FecX<sup>G</sup>* and *FecX<sup>B</sup>* mutations), *GDF9* (*FecG<sup>H</sup>* mutation) have and been associated with the exceptional prolificacy in Romney, Belclare, Cambridge, and Lacaune breeds (Mullen et al., 2013). BMP15 maps to the X chromosome and is specifically expressed in the oocytes. Mutations in this gene [Inverdale (*FecX*<sup>+</sup>), Hanna (*FecX*<sup>+</sup>), Belclare (*FecX*<sup>®</sup>), Galway (*FecX<sup>G</sup>*), and Lacaune (*FecX<sup>L</sup>*)] have been influentially associated with ovulation rate in various sheep breeds (Galloway et al., 2000; Hanrahan et al., 2004; Kumar et al., 2008). Among these mutations, the Galway mutation is a nucleotide alteration (C to T) at position 718 bp of the BMP15 gene, which introduces a premature stop codon in place of glutamic acid at amino acid residue 239 of the unprocessed protein (Kumar et al., 2008). All heterozygous ewes exhibit higher prolificacy than wild-type genotypes. The mutant type had a non-additive effect on ovulation rate and accordingly, the homozygotes are sterile (Galloway et al., 2000; Hanrahan et al., 2004). Based on the X-linked inheritance, rams carry only one copy and pass it to all daughters.

Kivircik sheep breed is one of the most important native livestock genetic resources of Turkey. It is a thin-tailed breed and its meat is preferred widely by the consumer because of superior meat quality characteristics. There is limited information about this fecundity genes in the Kivircik sheep breed. Taken together, this research was designed to evaluate the presence of the Galway (*FecX*<sup>6</sup>) mutation which is suggested to be associated with a high ovulation rate in Kivircik sheep.

#### MATERIALS AND METHODS

The study was conducted on a total of 91 Kivircik ewes. The animals were recorded for the Pedigree Project of the Turkish Ministry of Food, Agriculture and Livestock, and Cattle Breeders Association. All animals were housed and managed according to the standard farm procedures. Blood samples (~4mL) were collected in Vacutest tubes with a K<sub>3</sub>EDTA (0.2 mg/mL) anticoagulant (Vacutest Kima SRL, Arzergrande, PD, Italy). Genomic DNA was isolated using the standard phenol-chloroform method as described by Green and Sambrook (2012). The concentration of total DNA samples obtained and their quality was estimated using a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA).

The Galway (FecX<sup>G</sup>) mutation was screened in Kivircik sheep by the PCR-RFLP. Briefly, PCR amplified and digested DNA fragments were separated on 2% and 3% agarose gels, respectively, and stained with ethidium bromide. The gels were scored for the presence or absence of the mutations by a gel imaging system (DNR-Minilumi, DNR Bio-Imaging Systems, Israel). The primers, PCR conditions, and PCR mixture components are presented in Table 1. Hinfl was used as the restriction enzyme. In this respect, the PCR product from noncarriers (wild type genotype) has a Hinfl site, while carrier individuals (mutant genotype) lack this restriction site. After digestion, wild type individuals ( $FecX^{++}$ ) should have 111 bp and 30 bp fragments, heterozygotes should have (*FecX*<sup>G+</sup>) 141 bp, 111 bp, and 30 bp fragments, and homozygous individuals (*FecX*<sup>GG</sup>) are recognizable with an uncut 141 bp fragment (Kumar et al., 2008).

**Table 1.** Primers sequences (from 5' to 3'), PCR conditions, and PCR mixture components for the Galway ( $FecX^{G}$ ) genotyping

#### Primer sequences

#### F: CACTGTCTTCTTGTTACTGTATTTCAATGAGAC

#### R: GATGCAATACTGCCTGCTTG

#### PCR conditions

94°C 5' (94°C 30s, 62.3°C 30s, 72°C 45s) 35 cycles, 72 °C 5'

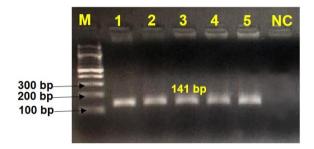
#### PCR mixture components

1 μL (0.025 μM) of each primer, 12.50 μL PCR master mix (OneTaq Quick-Load 2x MM with Standard Buffer, New England BioLabs Inc., Ipswich), 3 μL of the purified DNA sample, and 8 μL of nuclease-free water (Thermo Scientific)

F = forward, R = reverse.

#### **RESULTS AND DISCUSSION**

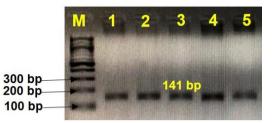
In small ruminant breeding, there has been increasing interest in the evaluation of prolificacy. The trends in selection procedures gradually changed from have traditional phenotype-based applications to genotypic considerations the by identification and utilization of major genes for prolificacy (Davis, 2004). The Booroola Merino was the first breed in which ovulation rate and litter size were shown to be affected by a segregating major gene in sheep (Piper et al., 1985). It was demonstrated that FecB, which is a dominant autosomal gene, has an additive effect on ovulation rate. On the other hand, an X-linked gene was associated with an increase in the ovulation rate and it was first described in Romney sheep and named the Inverdale gene (*FecX*). It is important to note that homozygous carrier females exhibit sterility. Therefore this gene has been suggested to be a pivotal genetic marker for prolificacy in sheep (Davis, 2004). This study focuses on the analysis of the Galway mutation (FecX<sup>G</sup>) in the Kivircik breed which is one of the most important sources of Turkey's national livestock. In this sense, the 141 bp fragment in the BMP15 gene [genomic location: X: 56594565-56601245 (-)] was amplified (Figure 1). In the present analysis, all animals remained undigested with the Hinfl restriction enzyme (Figure 2). This suggests that Kivircik sheep were found to be carriers for the mutation (*FecX*<sup>GG</sup>). Galway Hence, the corresponding genotype was fixed in studied ewes, and accordingly, population genetics parameters and Hardy-Weinberg equilibrium were not estimated. This result demonstrated that admissible prolific characteristics of the Kivircik breed can be partially explained by the presence of the FecX<sup>G</sup> mutation. However, it is worth noting that, undigested fragments in the PCR-RFLP analyses may cause unreliable or controversial results. To overcome this limitation, RFLP analysis was performed in duplicate in this study. Sanger sequencing confirmation was not possible because of the budget limitations of the study.



**Figure 1.** The electrophoresis pattern of PCR amplification for the Galway mutation (*FecX*<sup>6</sup>). M = marker (100 bp).

High litter size or twinning is a crucial economically important indicator that determines the productivity of the herd, concerning the number of lambs, meat, and wool. The presence of  $FecX^G$  mutation has been shown to be an important genetic factor to achieve high prolificacy in sheep (Davis, 2004; Kumar et al., 2008). Concerning different breeds of sheep, the results of *FecX<sup>G</sup>* mutation analyses revealed mostly controversial suggestions. But this situation is a common circumstance in genotypic evaluation. Different breeds or different individuals of the same breed may distinctive genotypic distributions. exhibit analyses Hereupon, previous revealed remarkable differences in genotypic frequencies in various breeds of sheep. Gürsel et al. (2011) found that all of the investigated Kivircik sheep were heterozygous for the  $FecX^{G}$  locus. These researchers suggested that Kivircik, Imrose, Awassi, and Chios breeds had an advantage for

fertility due to heterozygosity for  $FecX^G$ mutation. Moreover, the  $FecX^G$  mutation was identified in Belclare and Cambridge sheep (Davis, 2004). On the other hand, Dincel et al. (2015) suggested that the high prolificacy of the Sakiz breed does not result from  $Fec^B$ ,  $FecX^G$ , and  $FecX^I$  mutations.



**Figure 1.** The electrophoresis pattern of restriction enzyme digestion of PCR product with *Hin*fl for the Galway mutation (*FecX*<sup>6</sup>). M = marker (100 bp). The PCR product remained undigested, and hence, all ewes were genotyped as (*FecX*<sup>66</sup>).

There are certain limitations to the genetic studies conducted on native sheep breeds in Turkey. On one hand, unconscious crossbreeding and importation have resulted in a decrease or loss of diversity in Turkish native sheep breeds without genetic characterization. This situation has also resulted in difficulties in finding purebred individuals. On the other hand, the population sizes or the number of the genotyped individuals are rather low in these studies (also in the present study). These limitations prevent providing reliable suggestions or achieving the concrete data to use in gene-assisted selection. Moreover, pedigree data is mostly far from trustworthiness to be applicable in sheep breeding management. Kivircik breed is one of the most important native livestock genetic resources of Turkey and its meat is preferred widely by the consumer because of superior meat quality characteristics. Hence, further molecular genetic analyses should be performed in larger populations.

## CONCLUSIONS

The present analysis showed that all ewes were the Galway mutation ( $FecX^{GG}$ ) carriers. This suggests that selected Kivircik individuals have an advantage for fecundity due to the desired genotype. Consequently, ovine  $FecX^{G}$  may be considered to achieve high litter size in the Kivircik breed. Nevertheless, the other fecundity genes should be analyzed.

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## RARE HORSE POPULATIONS FROM GREEK ISLANDS

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#### Abstract

The study and preservation of indigenous horse populations, as part of animal genetic resources and as well as cultural world heritage is of national and global importance. Rare Greek island horse populations are of particular interest due to their unique characteristics and adaptations. Most are evolved isolated on island environments, surviving in harsh conditions, on rocky ground, with warm and dry climate, owning unique traits and extreme hardiness. Genetic characterization of these populations is lacking as well as systematic reservation efforts. Due to small population size, further difficulties arose on their recognition and proper management. Modern genetic tools such as microsatellites can play an important role on identification and reservation of populations or breeds. This preliminary report forms part of a PhD study regarding local Greek horse populations, aiming to support their reservation. For this report, we used 46 hair samples from local horse populations originating from Skyros, Rhodes, Lesvos (where two horse types are found, the traditional miniature "Midili" horse and the larger local Lesvos gaiter) and Creta island (Messara region). The samples were analyzed using 15 microsatellite markers (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, ASB23 and LEX33). From these populations, only Skyros and Messara horses are officially recognized as breeds. The studied horses form two phenotypes, the miniature horses with withers height (WH) ranging from 105-115 cm (Skyros, Rhodes and Midili) and the larger gaiters with a WH of 130 to 150 cm (Lesvos and Messara). A total of 120 alleles was found. Except from Midili, which due to insufficient sample number could not be reliably tested, the remaining populations show no significant deviation from Hardy-Weinberg equilibrium.

Key words: Horse breeds, Indigenous breeds, Microsatellites, Genetic equilibrium

#### COMPARE OF LACTATION MILK YIELD CHARACTERISTICS AND SOMATIC CELL COUNTS IN MORKARAMAN AND TUJ SHEEP

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#### Abstract

In this study was investigated of lactation milk yield, lactation length, average daily milk yield and somatic cell counts (SCC) in Morkaraman and Tuj sheep. It focuses on the effects of some environmental factors like breed and age that are affecting these characters. It was determined that breed was not significant on lactation milk yield statistically but significant differences were observed between age groups on lactation milk, lactation length and daily milk yield (p<0.05), and had influence on lactation length very significantly (p<0.01). According to the SCC, breed and age had no effect on SCC.

Key words: Morkaraman, Tuj, Milk yield, Somatic cell count

#### INTRODUCTION

In terms of human nutrition, milk has the high nutritional value among animal products. (Teker 2016). Consumption of milk and the products obtained from the milk is very significant for healthy life. Milk consumption preferences depend on socioeconomic and sociocultural conditions (Besler and Ünal 2006; Yerlikaya ve Karagözlü 2008).

Hygiene conditions must be performed in order to keep the quality of milk. Depending on the developing technology and the increasing level of prosperity, the quality of milk and dairy products has a significant effect on consumption preferences. One of the evaluation criteria used to determine whether milk is healthy and has high quality is the SCC that milk contains (Manlongat et al. 1998; Yöney 1998). The objective of this study was focused on milk yield characteristics and SCC of Morkaraman and Tuj ewes.

#### MATERIALS AND METHODS

This study was carried out on 31 Morkaraman and 36 Tuj ewes at Ataturk University Faculty of Agriculture Research and Application Farm. The birth season, which started in April, continued until May. About 2 months old lambs were weaned and all of flock started to pasture by June. In order to determine lactation milk yield characteristics, control milking was started after weaning lambs and continued until the end of pasture in 15-day intervals. The lactation length was determined by drying off the sheep which were below 50 g milk yield per day. Lactation milk yield was calculated using Trapeze II (Dutch Method) used by the International Registry Commission (ICAR) (Yakan 2012). Milk was analyzed by DeLaval DCC for the measurement of SCC. The GLM procedure of the SPSS (2011) 20.0.0 package program was used for the analysis of variance of the milk yield characteristics and SCC. Differences between groups were determined by the Duncan Multiple Comparison test.

#### **RESULTS AND DISCUSSION**

Lactation milk yield, lactation length, average daily milk yield and SCC of Morkaraman and Tuj breed are given in Table 1. It has been determined that breed is not statistically significant for lactation milk yield. The age of dam is significantly affected on lactation milk yield, average daily milk yield and lactation length. The similar results were reported by Küçük et al. (2000) and Abd Allah et al. (2011). It was observed that the values of lactation milk yield characteristics were below the average values reported in the literature. It was determined that lactation milk yield and average daily milk yield were the highest in Morkaraman; the length of lactation was highest in Tuj. The values for the lactation characteristics in the Morkaraman were found lower than the results Küçük et al. (2000), Özbey and Akcan (2000) and Kırmızıbayrak et al (2005). In this study, lactation milk yield found for Tuj breed is lower than that of Kırmızıbayrak et al. (2005); is higher than Karaoglu (1997); the length of lactation is similar to Kırmızıbayrak et al. (2005) and Karaoglu (1997). SCC is an important marker for udder health. Tuj sheep had higher SCC than Morkaraman sheep, but the influence of breed on SCC was not significant. SCC increased with age. However, SCC was not significantly affected by age. The mean SCC obtained in the study was determined lower than the SCC values in milk reported by Konar et al. (1994) and Huntley et al. (2012), less than by Yağcı (2005), Othmane et al. (2002) and Baro et al. (1994).

Sources	Lactation milk yield (kg)	Lactation length (day)	Average daily milk yield (kg)	SCC (cell*1000/ml)
Breed	ns	ns	ns	ns
Morkaraman	41.4 ± 5.0	128.2 ± 4.9	0.32 ± 0.2	285.1 ± 74.9
Tuj	40.2 ± 3.6	$131.6 \pm 4.6$	0.31 ± 0.2	327.8 ± 89.2
Age	**	**	*	ns
2	30.9 ± 3.9 <sup>c</sup>	119.8 ± 5.9 <sup>b</sup>	$0.26 \pm 0.2^{b}$	385.7 ± 158.5
3	$42.3 \pm 5.8^{abc}$	137.1 ± 7.2 <sup>a</sup>	$0.31 \pm 0.3^{ab}$	209.5 ± 100.5
4	$38.5 \pm 5.2^{bc}$	$126.9 \pm 7.4^{b}$	$0.30 \pm 0.3^{ab}$	899.8 ± 309.5
5	$53.0 \pm 5.2^{a}$	$146.6 \pm 6.9^{a}$	$0.36 \pm 0.3^{a}$	440.1 ± 239.4
≥6	$50.1 \pm 5.4^{ab}$	149.7 ± 7.2 <sup>a</sup>	$0.34 \pm 0.3^{ab}$	411.8 ± 196.6

Table 1. The least so	quares means and standard	errors of lactation v	vield characteristics and SCC
	quales means and standard	cirors or tactattori	

\*: Significant (p < 0.05); \*\*: Very significant (p < 0.01); ns: Insignificant

## CONCLUSIONS

There are limited researches of SCC in sheep and the studies concentrate on cow milk, so the quality standards have defined by limiting the SCC in the sheep milk. This case creates a gap in terms of quality standards for milk and dairy products obtained from sheep. This study has taken a step to fill this gap and it is suggested that new studies must be done to resolve this shortcoming.

#### ACKNOWLEDGEMENTS

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#### THE EFFECT OF BODY WEIGHT ON LAYING PERFORMANCE OF LAYING HENS

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#### Abstract

This study was conducted to investigate the effects of body weight on performance parameters of Lohmann white laying hens. At the beginning of the experiment, hens were weighed up individually and groups were classified as light (<1500 gr), medium (1500-1750 gr) and heavy (>1750gr) as to standard deviation of mean. The study was conducted over a period of 36 wks. Feed and water were offered ad libitum in the experiment. Considering the findings of performance traits differences among the groups were found significant. This study emphasized that body weight affected the laying performance except for FCR and cracked egg rate. Egg weight was determined to be lower in the group with light body weight than those of medium and heavy groups. However, egg production in the light group was higher (P<0.01) than that of heavy group. Feed intake was found to be higher in medium and heavy groups than that of light group.

Key words: Laying hen, Body weight, Performance parameters

#### INTRODUCTION

Human nutrition is a key factor in maintaining a healthy life. Egg is one of the most important food with its high protein value, rich in vitamins and minerals and low in calorie. In poultry farming, egg production, feed consumption and feed conversion ratio are the most important criteria for economic breeding (Akbay, 1980; Nazlıgül et al., 2001). In the flock, the heavier weight hens produce larger eggs than the other hens. However, the body weight of hens is not desirable to rise above a certain limit. Selection studies for increasing body weight decreased production (Özen 1986). eaa For the development of egg production, it is necessary to determine the nature of the relationship between body weight and egg production (Oke et al, 2004; Lacin et al., 2008). This study was conducted to investigate the effects of body weight on performance parameters of Lohmann white laying hens.

#### MATERIALS AND METHODS

Total of 288 Lohmann white layers, 44 wks of age, were allocated randomly three groups, each formed 24 replicate cages as subgroups, comprising of four hens. At the beginning of experiment, hens were weighed up individually and groups were classified to be light (<1500 gr), medium (1500-1750 gr) and heavy (>1750gr) as to standard deviation of mean. The study was conducted over a period of 36 wks. Feed and water were offered ad libitum in the experiment. Feed intake and egg production were recorded daily; egg weight was measured at 2-weeks intervals and body weight was measured monthly. Before determination of egg weight, a sample of 12 eggs from each experimental group was stored for 24 hrs in room temperature. Feed conversion ratio (FCR) was expressed as kilogram of feed consumed per kilogram of egg produced. The data were analyzed using a General Linear Model procedure (SPSS, 1996) for a completely randomized experimental design. Differences between means were determined by Duncan's multiple range test.

## **RESULTS AND DISCUSSION**

Tablo 1 shows laying performance parameters of hens in the various body weight groups (light, medium and heavy). During the experimental period, feed consumption was significantly different among the three groups. Feed consumption of hens also increased linearly as the body weight increased. Similarly to present study, some authors reported that there was a significant relationship between body weight and feed consumption (Lacin et al., 2008; Balcıoğlu et al., 2005).

	BW	FC	EP	FCR	EW	CE
	(kg)	(g)	(%)		(g)	(%)
Light	1572.5°	115.2 <sup>b</sup>	82.8ª	1.73	66.9 <sup>c</sup>	0.95
Medium	1710.5 <sup>b</sup>	116.6 <sup>ab</sup>	79.8 <sup>b</sup>	1.73	67.5 <sup>b</sup>	0.96
Heavy	1846.5ª	118.1ª	76.7 <sup>c</sup>	1.72	68.8ª	1.15
SEM	6.72	0.67	0.54	0.01	0.19	0.10
Anova						
Group(G)	**	**	**	ns	**	ns
Time (T)	**	**	**	**	**	**
G x T	ns	ns	ns	ns	ns	ns

Table 1. The Effects of Different Bod	w Weight on Laving	Performance Parameters of	Laving Hens
	y weight on Laying	j renomiance raiameters of	Laying Hens

BW= body weight ; FC= feed consumption (g/d); EP= egg production FCR= feed conversion ratio (kg feed consumed per kg egg produced) ; EW=egg weight ; CE=cracked egg (%)

Egg production was affected by body weight (P<0.01). The highest egg production was obtained from the light group. Egg production decreases as the body weight increases in the groups. Although heavy body weight group had higher egg weight than the other groups, egg production decreased (P<0.01) in this group. The effect of body weight on egg weight was found to be significant in light, medium and heavy body weight groups (P<0,01). It was found that the heavy group had a higher egg weight than the other groups (P<0.01). Lacin et al., (2008) and Summers and Leason (1983) reported similar results. The FCR and cracked egg rate were not affected by body weight. These results were similar to Harms et al., 1982; Leeson ve Summers, 1987).

## CONCLUSIONS

As a result, it was found that there was a positive relationship between the egg weight and the body weight of the hens and the heavier eggs were obtained from heavier chickens. The relationship between egg production and egg weight was negative and egg weight decreased as the egg production increased.

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## WHOLE-GENOME SEQUENCING AND GENOMIC ANALYSIS OF NORDUZ GOAT

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#### Abstract

Norduz goat is an important goat breed of Turkey and it is mainly reared in the Norduz area, located between 38.2 °N latitude and 43.25°E longitude, 1745 m above sea level, in Gürpınar district of Van province. Norduz goats are favorable for preweaning viability, high twin rate, and milk production level compared to the other breed in the region. In the study, it was aimed to obtain the wholegenome sequence of Norduz goat by using the next-generation sequencing technology. For this purpose, blood sample was taken from a Norduz goat and DNA extractions were carried out from the blood sample. Next-generation sequencing was carried out using the Illumina HiSeg X platform with 150 bp pair-end reads. After trimming and quality filtering, obtained read data was aligned on the reference goat genome and the Norduz goat genome was constructed with 33.16X coverage. Using a stringent filtering condition, 9.757.980 SNPs, 1.536.715 InDels, and 290 CNVs were detected. Variants causing radical changes in protein structure were selected and gene ontological analyses were carried out in these variants. In the Norduz goat genome, a high proportion of detected radical SNP variations were found in the classical complement activation biologic process which is an immunological response mechanism associated with native immunity. A high proportion of SNP and InDel variants detected in Norduz goat were found in the genes related the smell perception, sensory perception, and nervous system processes. CNVs detected in Norduz goat were found in genes related to defense to viruses, cell membrane transporters, immune response. According to gene ontology analysis results, it can be stated that Norduz goat has a unique genetic structure and it is an improved goat breed primarily based on natural selections.

Key words: Norduz goat, Genome, Next-generation sequencing, SNP, InDel, CNV

## EFFECT OF CENTRIFUGATION AT DIFFERENT LEVELS OF FREEZE-THAWED BLOOD ON DNA ISOLATION

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#### Abstract

DNA isolation from blood is a commonly used method to obtain nuclear DNA (nDNA) and/or mitochondrial DNA (mtDNA). For this purpose, both fresh blood and frozen blood can be used. After sampling, direct storing of blood in deep-freezes is a widely used application. However, this application leads to lysis of blood cells and prevent leukocyte preparation techniques. It was previously shown that DNA isolation could be performed from the pellet obtained by centrifugation of freeze-thawed blood, and this application had constructive results on DNA isolation. However, which centrifugation levels can be used for this application is unknown. Thus, in this study, DNA isolations were carried out from the pellet and supernatant obtained by centrifugation at different levels from freeze-thawed blood. Then, spectrophotometric, gel electrophoresis, and real-time PCR analyses were performed in the isolated DNA samples. Owing to these investigations, it was observed that the centrifugation process at 1.000 xg for 2 min was enough to harvest pellet from freeze-thawed blood. Furthermore, DNA isolations carried out from the pellet obtained by centrifugation process at levels equal to or greater than 1.000 xg for 2 min were resulted in high DNA yield. Centrifugation levels equal to or greater than 1.000 xg for 2 min had no distinguished effect on A260/A230 values. Agarose gel electrophoresis analysis showed no significant difference in DNA integrity in the isolated DNA samples. Real-time PCR analysis results showed that mtDNA ratio decreased in the isolated DNA samples from the pellet obtained by centrifugation of freeze-thawed blood at 100 xg and 500 xg for 2 min, but mtDNA/nDNA ratio did not change at centrifugation levels equal to or greater than 1.000 xg for 2 min. In conclusion, centrifugation at levels equal to or greater than 1.000 xg for 2 min is convenient for DNA isolations from the pellet obtained from freeze-thawed blood samples.

Key words: Blood, Freeze-thaw, DNA isolation, Centrifuge, DNA quality, mtDNA, nDNA

# EFFECTS OF ENVIRONMENT IN PRODUCTIVE PERFORMANCE OF HOLSTEIN DAIRY COWS IN ZIMBABWE

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#### Abstract

The external environmental stimuli (physical, chemical, climatic and biological) to which animals respond interact with their genotypes to determine level of performance. A research study was carried out to evaluate effect of environment for milk yield, milk components and somatic cell counts (SCC) on Holstein dairy cows in contrasting agro-ecological zones of Zimbabwe. Farm level data for the period 2003-2011 and subjected to Generalized Linear Model (GLM) analyses in Minitab 18.1(2017). Test day milk yield (TDMY), butterfat, protein, lactose, total solids, SCC and log-SCC were evaluated. There was a significant difference across agro-ecological zones ( $p \le 0.05$ ) in butterfat, protein, lactose, total solids, log-SCC and TDMY. Region II had higher least square mean values for TDMY, lactose, protein, total solids (TS) of 26.878kilogramms, 4.941 %, 3.756% and 13.175% respectively. Region IV had the highest least square mean values for butterfat of 3.964. TDMY was negatively correlated to butterfat, protein, TS, SCC with the correlation coefficient being (R=-0.210), (R=-0.176), (R=-0.125) and (R=-0.15) and positively correlated to lactose (R=0.420). Correlation between days in lactation and butterfat, protein, total solids and SCC was positive except for TDMY and lactose which was negatively correlated to days in lactation. Results from the research indicated that Region II gave high Holstein milk component traits performance.

Key words: Genotype by environment interaction, Dairy cattle, Agro-ecological zone, Correlation

## SAMPLING METHODS USED IN SCIENTIFIC RESEARCH AND IMPORTANCE OF SAMPLE SELECTION

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#### Abstract

While researching in any branch of science, it may not always be possible to deal with a situation or event in all its lines. For this reason, a narrower working environment is created within the framework of a certain direction. The work of identifying problems in a subject, finding solutions, evaluating and interpreting the results is called "scientific research". In scientific research, research using sampling methods is called "sampling research". Within the scope of this study, it is aimed to introduce sampling methods, to explain the importance and value of sample selection in scientific research, and to determine the advantages and disadvantages of sampling.

Key words: Sampling, Sampling methods, Sample selection

## EFFECTS OF DIFFERENT PROTEIN SOURCES ON GROWTH AND CARCASS PERFORMANCES OF DOMESTIC TURKEYS RAISED IN PELAGONIA REGION, R. N. MACEDONIA

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#### Abstract

The poultry production is a very delicate concept, they are very tender and sensitive to the lack of vitamins, minerals and proteins, and especially the amino acid composition. The appropriate amount of protein and balanced composition of amino acids are one of the prerequisites for intensive growth of young turkeys. A total of 30 heads of Domestic Turkey breed (white and black) was grown under the so-called indoor farming system in Pelagonia region, North Macedonia in the period of 2017-2018 year. The aim of this research was to determine the differences in the body weight, in the period of 10-90 days, and the carcass performances of turkeys fed with two different concepts in relation to the origin of feed proteins (group R - fed with fish flour and group S - fed with whey powder). In our study, regarding the live body weight of turkeys from both groups in the period of 90 days, a significant statistical difference between the two groups marked with R and S show that there is no significant difference between the two groups of turkeys (p > 0.05). The results obtained from our study would be an initiative for a further research of this type and will be of great benefit to farmers in our country.

Key words: Domestic Turkey, Growth, Fish flour, Whey powder, Pelagonia region

## INTRODUCTION

Turkey meat contains all the essential amino acids necessary for the human body such as: lysine, leucine, methionine, tryptophan and others. Turkey meat contains less fat, but high quality essential fatty acids such as linolenic, arachidonic and linoleic. It also contains a small percentage of cholesterol, but also a large percentage of B vitamins, which makes it very suitable for modern and dietary nutrition (Petrovic. V., 1988; Caballero B, 2005). The amount of nutrients and the quality of poultry fat largely depend on its genetic potential as well as the diet of the poultry, especially specific breeding technologies (organic production, extensive breeding system) have been shown to affect

the composition of poultry meat ( Caballero B. et al., 2005).

The production of domestic poultry is a very delicate matter; they are very tender and sensitive to the lack of vitamins, minerals and proteins, and especially the amino acid composition. They are also sensitive to changes in weather conditions, humidity and gases, to some stresses such as: noise, vaccination, capture and regrouping, etc. (Peric 2004). An adequate amount of protein and a balanced composition of amino acids are one of the prerequisites for intensive growth of young turkeys. In the first month of growing the required content of total protein in the diet is 28%, and in the following month they are only lower by 2% (Baker, D.H., 2000). Fish meal contains higher levels of the amino acids lysine, methionine and cysteine (Swick, 1999). With a favorable amino acid composition, fish flour contains both Ca and P (Hendriks et al., 2002) in a favorable ratio, micronutrients - Se and I, contributes to which easily available minerals that are used efficiently, reducing environmental pollution and reducing costs for additional minerals in the diet (FIN., 2001). Due to the fat content of 2% - 12% it is also used to meet the energy needs of animals (Windstor, 2001). It contains a very long chain of polyunsaturated fatty acids (omega-3) that help the body prevent disease and maintain the immune system and is a natural source of vitamins (including choline, biotin and vitamins B12, A and E). Whey is produced in the process of separating lactic acid. Lactic acid bacteria break down lactose (milk sugar) into glucose galactose, and then glucose and is converted to L (+) lactic acid during lactic fermentation (Pijanovski, acid 1971). According to Scingoethr, (1976) and Morista et al. (1982) whey contains unidentified growth factors, while the protein content of and whev includes alpha beta-lacto globulins and can be used as a valuable source of protein in animals (Brunner, 1981). From milk to whey production pass watersoluble vitamins, while fat-soluble vitamins only partially.

Growth as a basic function and production in fat animals involves continuous increase in body weight (Hurwitz & Talpaz, 1997). Different tissue and hormone responses, on the one hand, and growth factor activity, on the other, result in different growth patterns in different organs. Gray et al. (1983) therefore determined different growth rates for different organs of birds. Sanduky & Heath, (1988) observed differences in the growth of different muscles, and Iwamoto et.al (1993) even found different growth rates in different muscle fibers. The aim of the research in this paper is to determine the live body weight and slaughter characteristics of turkeys fed with two different mixtures in terms of protein origin (fishmeal and whey powder).

# MATERIALS AND METHODS

A total of 30 heads of Domestic Turkey breed (white and black) was grown under the so-called indoor farming system (controlled room temperature, ventilation and light) in Pelagonia region, North Macedonia in the period of 2017-2018 year. Turkey's feed ration consisted of the following components: corn, soybean meal, wheat, sunflower husk, fishmeal / whey powder, fodder yeast, lard, chalk, salt as well as dicalcium phosphate and mineral order supplement in to meet the requirements of poultry nutrition (according to NRC, 1994). The composition of fish flour added to the feed mixture of turkeys from group "R" was as follows: lysine 28,000 mg, methionine 12,000 mg, Ca CO3 = 2,000 mg, NaCl = 4,000 mg, acidifier 2000 mg, aroma 2000 mg, carrier 2000 mg, soy protein isolate, peeled soybean flour, amino acids, antitoxin. Whey powder that was added to the feed mixture of turkeys from group "S" was composed of 80% whey protein (cow, sheep and goat), 11.9 g. carbohydrates and low content of saturated fat (11.9 g out of a total of 3.4 g / 100 g whey powder). Food intake was followed by daily measurement of food content and subtraction of the remaining amount of food from the previous day. The live weight of the turkeys was measured every ten days and the increase in live weight was calculated as the difference between two consecutive measurements. Turkeys were slaughtered at the age of 100 days of rearing. After a previous twelve (12) hours of starvation and physical stunning, slaughtered the turkeys were and the process of skinning subsequently (dressing), exenteration, evisceration and dissection was performed manually in a specialized workshop for domestic animals slaughtering. The turkeys were also given the preparation ŽIVIMICIN® 20 g (antibiotic with vitamins). Juvenile turkeys were constantly monitored by a veterinarian at the time of fattening and were actively immunized against Newcastle disease (strain La Sota ATCV et: QI01AA02). The live weight of the turkeys was measured every ten days and the increase in live weight was calculated as the difference between two consecutive measurements. In order to obtain a relevant data on live weight and slaughter parameters at turkevs, the following measurements were performed, which include:

1) Measurement of live weight every ten days from the first measurement which will be at 10 days of age;

2) Measurement of carcass parameters on the day of slaughtering: mass measurement of slaughtered carcasses without skin and feathers, measurement of edible parts, measurement of inedible parts and calculation of the yield of a classically processed carcass.

## **RESULTS AND DISCUSSION**

The results of live mass measurements in juvenile turkeys in the period from the  $10^{th}$  to the  $90^{th}$  day.

**Table 1.** Body weight (g) variation of turkey with respect to feeding intervals (days) over a period of 90 days (n = 8; 95% Confidence Interval for Mean

Treatment	Meal type	Mean ± Std. Deviation	Minimum	Maximum
T10	R	136.2500b±2.91548	132.00	140.00
	S	130.1250a±1.72689	127.00	132.00
T20	R	296.3750b±4.50198	290.00	302.00
	S	277.8750a±3.44083	272.00	283.00
Т30	R	477.7500b±4.65219	471.00	484.00
	S	445.5000a±5.37188	439.00	454.00
T40	R	703.0000b±4.24264	698.00	709.00
	S	650.5000a±4.14039	644.00	657.00
T50	R	974.8750b±4.73400	969.00	982.00
	S	905.7500a±3.32738	901.00	910.00
T60	R	1397.5000b±8.41767	1380.00	1405.00
	S	1107.7500a±3.91882	1101.00	1113.00
T70	R	1726.5000b±8.73417	1711.00	1738.00
	S	1488.7500a±5.84930	1481.00	1496.00
T80	R	2198.5000b±4.30946	2191.00	2206.00
	S	1824.7500a±8.51469	1813.00	1836.00
Т90	F	2809.8750b±7.03943	2801.00	2819.00
	S	2475.2500a±9.75046	2465.00	2492.00

T10 = 0 to  $10^{th}$  day of feeding; T20 =  $10^{th}$  to  $20^{th}$  day; T90 =  $80^{st}$  to  $90^{th}$  day of feeding. For each treatment, means in the same column with different letters are significantly different from each other. Table 1 indicates that there are significant differences of body weights between the two groups for different feeding intervals (p<0.05).

In Table 2 are shown the obtained results about carcass traits at both groups (R-group and S-group). Therefore, before slaughtering, a live body weight at the R and S groups is 2.78 kg and 2.43 kg, while the average body weight of the classically

processed carcasses is 1.77 kg and 1.52kg respectively. The mean value of meat yield (rendement) of the carcasses at both groups slightly differs 63.50% and 62.72%. There is a slight difference in the mean value of the non-edible internal organs (intestines, etc.)

and it is 0.15 kg in group R turkeys and 0.14 kg in group S turkeys.

Table 3 shows that there is no significant difference between the two groups of Turkeys (Fish flour fed and Whey powder fed) for all the parameters since p value is greater than 0.05 (p>0.05). The growth and carcass quality of slaughtered turkeys can be influenced by many factors, including: the way of breeding, the composition of the

food, the final body weight, age, sex, as well as the genotype of the individuals. Our results are consistent with the results of many authors from the world literature (Halvorson et al., 1991; Brake et al., 1994; Waldroup et al., 1997; Roberson et al., 2003; Bogosavljević-Bošković et al., 2005; Nestor et al., 2005; Hulet, 2006; Laudadio et al., 2009; Medić et al., 2009; Sogut et al., 2010).

(kg) skin (kg) $^{\text{carcass (kg)}}$ (%) (%) (%) R 2,78±0.70 2,18±0.52 1,77±0.46 0.22±0.05 0.15±0.03 63.50					-			
$\mathbf{v} + \mathbf{v}$	(N=8)	Group	before slaughter	without feathers and	processed	portions	portions	Meat yield (%)
	x ±SD	R S	2,78±0.70 2,43±0.28	2,18±0.52 1,87±0.22	1,77±0.46 1,52±0.18	0.22±0.05 0.19±0.05	0.15±0.03 0.14±0.04	63.50 62.72

Table 2. Carcass	traits of Turkey	obtained from	arouns R and S
Table 2. Carcass	s traits of Turkeys	Sobramed nom	groups r and s

**Table 3.** ANOVA for weight of body parts of Turkeys fed with two kinds of meals (fish flour & whey powder) for 90 days

Parameters		Sum of Squares	Df	Mean Square	F	Sig.
Body W	Between Groups	507733.516	1	507733.516	1.673	.222
	Within Groups	3338035.714	11	303457.792		
	Total	3845769.231	12			
Carcass W	Between Groups	250714.286	1	250714.286	1.909	.194
	Within Groups	1444285.714	11	131298.701		
	Total	1695000.000	12			
Caracass WFS	Between Groups	385073.260	1	385073.260	2.305	.157
	Within Groups	1837619.048	11	167056.277		
	Total	2222692.308	12			
LHG	Between Groups	3191.209	1	3191.209	1.133	.310
	Within Groups	30985.714	11	2816.883		
	Total	34176.923	12			
guts	Between Groups	595.055	1	595.055	.477	.504
	Within Groups	13735.714	11	1248.701		
	Total	14330.769	12			

Df: degree of freedom

# CONCLUSIONS

In our research, regarding the live weight of turkeys in both groups it was concluded that the obtained statistics (ANOVA) for live weight of turkeys up to 90<sup>th</sup> day of age, show significant differences between the two groups labeled S (whose mixture contained whey protein) and R (whose

mixture contained fishmeal protein) for different feeding intervals (p < 0.05). According to the obtained results we can conclude that the group of turkeys that were fed with whey powder (group S), has a lower live body weight compared to the group of turkeys fed with fish meal and the average live weight on the 90<sup>th</sup> day of fattening is 2.475 kg, while the average live weight of turkeys in group R is 2.807 kg. There is a slight difference in the mean value of the non-edible internal organs (intestines, etc.) and it is 0.15 kg in group R and 0.14 kg in group S. Slaughtering turkeys values of the two groups R and S show that there is no significant difference between the two groups of turkeys because p value (Sig.) Is greater than 0.05. The results obtained from our study would be an initiative for a further research of this type and will be of great benefit to farmers in our country.

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## PERFORMANCE TRAITS OF BALOCHISTAN CAMEL BREEDS OF PAKISTAN

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#### Abstract

This study is amid to evaluate the qualitative and quantitative performance of camels of Balochistan Pakistan. Camel is one of the major and abundance species of province with 41% of total camel population of Pakistan. The recognized camel breeds of Balochistan are eight (Brahvi, Kachhi, Kharani, Lassi, Makrani, Rodbari, Pishin and Kohi breed) out of twenty (n=20) breeds of Pakistan. This study discovered that Kachhi breed is a riverine camel while other is mountainous camels. Rodbari breed had highest birth weight (48.58 kg) among other breeds while the weaning weight (118.83 kg) is high in Kharani breed and Adult weight (712 kg) is high in Pishin breed. The milk yield per lactation (2049 liter) is high in Kachhi breed and lactation length (579.18 days) is high in Brahvi breed. Age of puberty female camel (1282.6 days) and age of first breeding (1575.7 days) are delay in Brahvi breed. Gestation period (405.53 days) is high in Makrani breed and dry period (369.12 days) is long in Brahvi breed. Calving interval (787.65 days) is long in Rodbari breed. Age at puberty for male camel is long in Pishin breed and peak rutting vigor (128.54 days) is more in Kohi breed. Duration for copulation (25.38 mints) is high in Brahvi breed. The camel of this province can be improved with application of specific latest molecular design regarding selection breeding programs which can cover the delay maturity, low conception rate and long calving interval period.

Key words: Camel, Management, Performance, Pastoral, Breeds, Balochistan

#### INTRODUCTION

The conservation, protection, reproduction and nurturing of indigenous livestock breeds are necessary, as they are assumed to be the most important national assets of each country (FAO, 2007). In survival strategies keeping of multipurpose livestock species are essential component which communities have developed to deal with extreme climatic and environmental condition. Keeping a verity of local livestock breeds is decisive to food security, reducing poverty and sustainable development. The conservation of these locally adopted livestock a breed is very much in the interest of developing countries both for present and future food Growing incomes security. is directly proportional to demand for specialized foods produced by diversification of animal's production systems (Oldenbroek, 2007). Arabian or one humped (Camelus dromedarius) is a precious livestock species particularly adapted to hot and arid environment. It is a multipurpose animal provides milk, meat, wool, hair and hides. Camels are also used for ridding, as a beast of Burdon and a draught animal for agriculture operations and short distance transports. They are suitable domestic animals in the desert with long, dry hot periods of eight months and limited erratic rainfalls between 50 to 55 mm (Ramet, 2001). Conversion of scanty plants in to milk, meat and fiber represent it versatility. In comparison to other animals, camel eats less, sleeps in short intervals and has a long-lasting memory (Faraz et al., 2019 a, b, c). Camels are ideal for pastoralist as they yield milk in all seasons when other animals stop milking during dry periods. For per kg milk production the intake of camel is very low, requires only 1.9kg of dry matter as compared with cows requires 9.1kg of dry matter for production of per liter milk (stiles, 1983).

Camels concerned with family "Camelidae" which possess three Geniuses, 1-Camelus, 2-Lama, 3-Vicugna and seven species. Genus *Camelus* 

contributes three species; the 1<sup>st</sup> one is the one humped or Arabian or *Dromedary* camel while the 2<sup>nd</sup> is called two humped or *Bactrianus* camel, 3<sup>rd</sup> one is *Bactrian ferus*. Genus Lama comprises two species, a-*Lama glama*, b-*Lama guanaco*. The Genus *Vicugna* has also two specie called *Vicugna vicugna* and *Vicugna Pacose* (Amanat *et al.*, 2019).

Worldwide camel population is about 35 million (FAOSTAT, 2019). Out of these Africa accounts 85% camels while Asia shares 15% camels. From African camels, 70% found in Somalia, Sudan, Ethiopia and Kenya, 15% of African camels roamed in Chad. Some camels also found in other regions of the world which may be few thousand. Dromedary or one humped camel contributes 94% of total world camel population whereas Bactrian or two humped camels participate only 6% which are roamed specially in Asia (FAO, 2013). Asia is the 2<sup>nd</sup> largest host of camel population in the world. Both Dromedary and Bactrian camels found in Asia. Pakistan and India comprise 70% of Asian camels. Pakistan is the house of about 1.1 million camels and Balochistan is the richest province with 41% of camel population while in 2<sup>nd</sup> number Sindh stands with 30% of camel heads followed by Punjab and KPK with 22% and 7% respectively (GOP, 2019-20; Faraz, 2020).

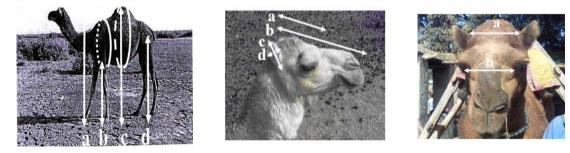
## MATERIALS AND METHODS

Present study was carried out on Balochistan camel breeds to observed phenotypic characteristics and to assess their performance traits in Balochistan province of Pakistan.

## Selection of Animals

Total (n=240) camels were selected from all 8 breeds (Brahvi, Kachhi, Kharani, Lassi, Makrani, Rodbari, Pishin and Kohi breed) of Balochistan. About 30 camels of each breed from their home tracts include 5 camels from each herd of different villages of same Districts. Camels were selected randomly from 8 home tract districts (Chaghi, Kharan, Lassbela, Punigoor, Gawdar, Pishine). Kohlue, Dader and А verv comprehensive information regarding their habitats, feeding, breeding, housing, management, production system, health/vaccination, utility patterns and constraints like poor selection, feeding and management system was collected. Qualitative (morphological) characteristics included sex, coat colour, body shape, head, ears, eyes, muzzles, mouth, hind quarter, shoulder, chest, legs and foots. Quantitative (physiological) characteristics comprises growth traits of female camels (n=136) and males (n=104), somatometric measurements include girth, height, length, fore leg (length) and hind leg (length). Production traits, e.g. milk yield per lactation (lit), lactation length (days), quantity of hair production (kg) and meat production (dressing %). Female reproductive traits consist of age at puberty (days), age at first breeding (days), gestation period (days), dry period (days), number of calves during life, calving intervals (days) and reproduction span (yrs.).

The biometry (body parts measurements) was conducted with a tape meter of the mature animals of each breed in the morning time when their bellies were empty. No pregnant animal was included in this study. Each animal was measured with in a limit of 10 minutes and the other person was writing the measurements. The animals were measured from the left side to keep the data homogenized. The animals were placed on a smooth place and were measured with a tape measure.



**Figure 1.** Plate 1; a=Shoulder height, b=Fore leg length, c=Height, d-Hind leg length, Girth=Square dots and hump girth = desh, Plate 2; Morphological characteristics (a - Head length, b - Face length, c - ear length and d - ear width), Plate 3; Morphological characteristics (a, b - Head and Face width, respectively).

#### **RESULTS AND DISCUSSION**

The results of qualitative traits of Balochistan camel breeds are mentioned in table 1. This study revealed that 'Brahvi' camel breed has a compacts and smaller body size with short and fine legs, but chest is wider. The whole body (neck, belly and legs) is covered with dense wooly hair in cold season. 'Kharani" camel exists in three verities of colours locally called Bor (brown), sore (red) and zard (yellow). Body is compact and medium size, neck is short. This breed is resembled to Brahvi camel breed. 'Kachhi' camel breed has a compact body, short neck, Small legs and small rounded, hard feet pads. "Lassi" camels have a medium sized body, muzzle is pointed and face is longer. "Rodbari" camel breed has slim body, long chin, short neck and body is covered with dense growth of hair. "Kohi" camel "Pishin" camel breed has a large body, long eye lashes, black nail and medium legs.

Camel breed	Habitat	Characteristics	Ecological zone
Brahvi	Chaghi District central home but extend to brahvi mountains and Bolan to Jacubabad, Further found in Shadad kot , Garhi kharo and Larkan Districts of Sindh Province.	Coat colour is light to dark brown but in dim colour, Small compact body, short but strong legs, hind quarters are massive and well developed, dense long wooly hair over neck, shoulder and belly. Protrusion of tongue from mouth is command. This is a hilly and baggage camel breed.	Central Brahvi Highland (CBH). This region comprises high and arid mountains with very hot (49°C) summers and very cold (below zero) winters. The rain fall of the regions is low and erratic (100-200mm per year).
Kharani	Khran, washuk, Nushki, Khuzdar, Kalat districs	Exist in three coat colour (red, yellow and brown) compare to Brahvi camel colour Kharani camel colour is shiny. Large round head, ears are small and flat. Big hump, hair is straight; protrusion of tongue from mouth is less. This is a pour ridding and desert camel breed also found in mountainous areas.	Chaghi Kharan Deserts (CKD).This region includes deserted plains, steppe and mountainous deserts. This region is very dry and hotter (40°C) in summers with rare rain fall. Gets less precipitation in winters and springs from Mediterranean winds.
Lassi	Lessbela, Awaran, Karachi and Khuzdar districts	Generally coat colour is brown but also found in red brown. Body is comparatively large than makrani, large legs, neck, ears, eyes, foot, hump and mouth. Pointed muzzle, long head, straight forwarded neck and straight hair. Hump, udder and hind quarters are well developed. This is a dual purpose, used for ridding and warking.	Balochistan Coastal region (BCR). The climate of this region is hot (40°C) in summer and in winter temp fall down to 6°C in winters. Annual rain fall is very low and precipitate about 125mm per year.
Makrani	Makran, Lessbela, Jalwan, sarwan areas.	Coat colouer is light brown to dark brown. They have slim and small body, small neck, head, legs, foots compare to Laasi camels. Neck is bending like, hump is medium, and Hair is curling style. Makrani camels found in coastal region are thinner and larger in size. Hilly and bagged type camel breeds.	Balochistan Coastal Region (B C R)
Rodbari	Makran, Turbat, Punjgoor, Passni, Gowder areas.	Coat colour is dirty gray to light red. Body is slim, long chin, short neck; body is covered with dens growth of hair. Hump is well developed. Desert and bagged type camel.	Balochistan Coastal Region (B C R)
Kachhi	Originally exist between Sibi to Jacobabad, but also found in Shikarpur and Sukkar.	Coat colour is slightly darker brown. Body is compact with short neck and small legs. Medium head, lower lips droops, hairless ears and eyelids, Hind quarters are well developed. Feet pads are smaller, hard and rounded. Baggage type camel but also used for ridding. It is a riverine type camel	Kachhi Basin Region (KBR). The climate of this region is very hot and humid in summer, harshness of summer prolongs over the months of April, may, June, July, August, September and October. In winters the weather becomes pleasant. The humidity is highest in summers.
Kohi	Dera Bugti, Kohlu, Barkhan, part of Loralai and Zhob, Musakhai districts.	Coat colour is white but also found in white brown colour. Compact body, big head, short and thin neck, white nail, long legs, medium face and small hump is placed in centers. Foot are large, tail is medium not fatty, udder is large; eyes are white and red in colour. It is a mountainous and bagged type camel.	Suleiman Mountainous Region (SMR). The climate of the region is mild in summer because of the high altitude and rains in monsoon time. Temperature reaches to 32 C <sup>e</sup> in summer and drops below zero in winter. The annual precipitation ranges from 300-600 mm per year and the main source of rain is monsoon
Pishin	Pishin, Qillaabdullah and Quetta districts	Coat colour is between light brown to dark brown. Resemble to Brahvi camel. Compact body, short stature, long eye lashes; they have thick set of bones and carry heavy weights. Hilly and bagged type animal.	Northern highlands (NHL). The climate of the region is mild in summer because of the high altitude and some eastern part of the region receives rains in monsoon time. The temperature reaches to 30 C° in summer and drops below zero in winter. The region is the coldest region of the province. The annual precipitation ranges from 250-600m mm per anum

Comparison of Kharani and Brahvi camel breeds in Kharan District where they overlaps is observed that the coat colour of Kharani camels exist in three pattern locally in Balochi language called (brown), zard (yellow) and sor (red) while the coat colour of Brahvi camel breed is light to dark fawn but dim in colour compare to Kharani breed. Head of this breed is large. Hump is prominent and big; neck is large while Brahvi breed has round head, small hump and small neck. Kharani camels are less dangerous and mostly docile while Brahvi camels are more dangerous and harmful, protrusion of tongue is command. They have strong, massive hind quarters. Compare to Kharani camels Brahvi camels have wider chest.

Comparison of Lassi and Makrani camel breeds in Lassbela District, Lassi is found comparatively large body size with large legs, foots, neck, ears, eyes, lips and mouth, while hump, udder and nostril are well developed. They have straight and forwarded neck, straight hair. Makrani breed has small legs, foot, hump, ears, eyes, lips, mouth, and nostril and shoulders while neck is bend like and hair is curling style. Makrani camel colour is brown to dark brown and colour of Lassi camel is brown with reddish tinge.

Regarding to draught camels are divided two categories, "Baggage" and "Riding" types. Baggage type further divided into "Hilly" and "Plains", hilly type camels in context to habitats divide into "Lowland" and "Mountain". Lowland has two components "Riverine" and "Desert", Mountains type camels are divided into "Ridding" and "Pak" type camels (Wilson, 2000). The native camel breeds of Balochistan is generally fall in three components They are hilly and baggage type (Brahvi, Makrani, Pishin, Rodbari). Kharani camel breed is purely ridding breed. Kachhi and Lassi breeds are dual purpose breeds camel, so they can be used for weight carrying and as well as for ridding. Baggage type camels in Balochistan are used for ridding but ridding of these camels is not comfortable. Hilly are baggage type camel breeds have comparatively small, compact, massive body, short neck and legs, wide chest, well developed hindquarters.

The overall means and std. error for growth traits of Balochistan camel breeds are mentioned in table-2.

Table 2 Least means square of growth	characteristics of Balochistan camel breeds.
Table -2. Least means square of growth	

Camel	No of	Birth weight	Weaning weight	Adult	Age of ridding	No of	Age of loading
breeds	camels	(kg)	(kg)	weight(kg)	(days)	camels	(days)
Brahvi	30	47.33±0.50	118.57±0.91	648.83±3.33	1296.4±27.74	13	1664.5±4.34
Kharani	29	46.96±0.39	118.83±1.06	698.72±4.69	1192.9±6.78	13	2487.6±26.07
Lassi	30	41.36±0.64	111.43±0.92	598.07±5.71	1118.6±4.90	13	1669.6±5.86
Makrani	30	46.96±0.75	115.87±0.88	684.37±4.44	1280.9±4.32	13	1664.5±4.34
Roadbary	30	48.58±0.43	118.37±0.83	696.37±3.67	1429.8±1.06	13	1672.0±2.71
Kachhi	30	45.63±0.39	115.47±0.81	656.30±4.08	1195.6±2.04	13	1601.5±17.66
Kohi	30	38.46±0.52	110.50±1.42	657.00±3.77	1389.3±7.25	13	5675.8±2.93
Pishin	Зо	48.40±0.41	117.97±0.41	712.00±3.46	1273.9±2.04	13	1719.9±43.82
Total	239	45.44±0.28	115.86±0.39	668.83±2.66	1285.1±7.10	104	1630.9±48.2

In both production (sedentary and transhumant) systems, sex had a significant effect on Growth characteristics. The findings of present study are in horizontal line with the findings of Waheed and Tariq (2014). Different reports are present in camel world. In generally the average birth weight of camel is mentioned about 37kg, but it may differ extensively among regions, breeds and within the same breed and it may range between 27kg to 39kg (Bakheit et al., 2012). In India the birth weights of camels range from 26.3 to 51.2 kg, with mean of 37.3 kg (Bhargava et al., 1965). Sabahat (2014) reported that the average birth weight, weaning weight and adult weight of male and female lassi breed camels was 41kg and 39kg, 68kg and 65kg, 570kg and 550kg respectively. The overall means and std. error for sometometric measurements of Balochistan camel breeds present in table-3. The finding of this study for sometometric measurements are in agree with the findings of G.B.isani and M.N.Baloch (2001), for same breeds of camels . In sometometric measurement's males had a significant difference for girth, height and length than female's camels. Biometry is most important section of physical traits (Sarkar et al., 2007). In Pakistani camels, Marecha, Dhatti, Larri, Kohi, Sakrai and Campbelpuri breeds sometometric measurements calculated for Heart girth 211.9±15.8, 211.1±12.9, 210.3±16.6, 200.4±8.3, 199.6±12.5, Height, 216.8±15.9, 228.4±10.5, 216.6±12.7, 189.1±7.1, 216.4±22.9, 201.3±22.6 Length 165.5±9.9, 176.8±9.8, 174.5±13.7, 147.2±8.5, 194.9±13.9, 163.4±24.6, Fore leg 120.3±6.9, 134.2±3.2, 133.8±11.6, 123.8±3.1, 124.1±2.8, 117.1±4.9 Hind leg 150.9±8.7,  $159.9\pm6.1$ ,  $158.9\pm5.6$ ,  $141.2\pm5.0$ ,  $158.8\pm6.0$ , and 152.9±7.2 respectively (Shah, 2014).

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Camel	No of	Heart girth	Height	Length	Fore leg	Hind leg
breeds	camels	(cm)	(cm)	(cm)	length (cm)	length (cm)
Brahvi	30	210.80±1.93	203.90±2.23	204.87±2.23	148.77±2.41	158.07±2.43
Kharani	29	224.79±1.00	218.86±1.24	184.66±5.41	161.62±2.20	169.59±2.46
Lassi	30	224.50±2.01	215.30±1.65	173.57±0.97	153.03±0.90	162.27±0.93
Makrani	30	212.90±0.93	206.10±0.96	205.40±0.91	149.87±0.57	157.13±0.73
Rodbari	30	218.18±6.77	212.83±0.76	178.87±0.75	162.62±0.60	162.62±0.60
Kachhi	30	228.43±1.63	237.00±1.38	190.70±2.06	160.83±1.65	171.23±1.52
Kohi	30	204.13±2.85	200.97±2.94	144.93±3.64	98.43±1.54	108.67±1.50
Pishin	30	232.38±0.86	223.82±1.19	158.28±1.29	155.90±1.11	165.67±105
Total	239	219.49±1.17	214.83±0.92	180.34±1.57	147.60±1.34	156.85±1.33

Overall means and std. error for productive characteristics (Milk yield, Lactation length, Hair production and meat production) are displayed in table-4. The results of this study for productive traits are closely confirmed the finding of G.B. Isani and Baloch (2001). Connection to milk yield per lactation Pakistan stands first among camel rearing countries (India 2482; Somalia 1825, Tunisia and Algeria 1460; Ethopia 1825) with an average milk yield 2920 liters. (Faraz *et al.*, 2013). In a study regarding to hair production of Kharai, Dhatti, Larri and Sukari camel breeds of Sindh province of Pakistan are 1.63, 1.62, 1.47and 1.36 respectively (Kaurajo *et al.*, 2020). According to (Khan *et al.*, 2003) average slaughter weight of Pakistani camels ranged from 395/660 kg and dressing percentage (%) ranged from 45 to 55% (exceptionally 60%), while hair production ranged from 1-3kg. The result of present study regarding to meat and hair production are in line with these findings.

Camel breeds	No of camels	Milk yield per lactation (lit)	Lactation length (days)	No of camels	Hair production	Meat production dressing % (kg)
			- 9- ()-)		(kg)	
Brahvi	17	1658.4±7.86	579.18±4.06	30	2.38±0.02	49.90±0.79
Kharani	16	1680.6±5.93	549.8±5.02	29	2.32±0.03	54.58±0.07
Lassi	17	1335.2±4.13	318.00±4.65	30	2.26±0.05	51.56±0.93
Makrani	17	1929.0±9.20	526.76±2.79	30	2.62±0.42	50.96±0.82
Rodbari	17	1703.0±2.65	461.94±1.11	30	2.47±0.08	5290±0.90
Kachhi	17	2049.3±4.21	542.06±4.05	30	2.12±0.03	51.50±0.83
Kohi	17	1837.8±3.86	377.06±7.71	30	2.83±0.67	48.96±0.74
Pishin	17	1714.9±8.20	368.00±4.28	30	$1.58 \pm 0.04$	52.33±0.88
Total	135	1739.0±17.38	464.73±8.16	135	2.32±0.10	51.70±0.31

The overall reproductive traits in female camel breeds of Balochistan are present in table-5. The results of present study are nearly parallel with the findings of Isani and Baloch (2001) and Shah (2014). The gestation period ranged between 377 to 390 days. The result of present study regarding to average gestation period (389 days) is little bit higher than the average gestation period of 380 days calculated by the results of many other researchers (Wilson, 1986; Richard, 1989; Khanna, 2004 and Musa *et al.*, 2006; Mehta and Sahani, 2009).

#### CONCLUSION

Camel is most common and abundant specie of Balochistan province of Pakistan. The popularity of camel in Balochistan can be image by the fact that camels represent the provincial monogram of Balochistan. The study concluded that camels proved their sustainability in arid, semiarid, deserts and periphery arias of Balochistan. Generally, livestock, especially Camel is playing as back bone role regarding to food security and live hood of nomadic and pastoral people. Beside these huge advantages the provincial department government and livestock completely neglected this species which is bestowed with unearth potential. In this study camels of Balochistan were found badly suffered with poor health and nutrition conditions. Camels were found with high ratio of ecto and endoparasites and there is no concept for schedule vaccination programs due to completely UN educated camel owners. It is greet need of time that provincial government and especially livestock department arrange mobile teams for health care programs of camels. Present study disclosed that the birth weight, adult weight and milk yield of Balochistan camels are higher than the many countries (Somalia, Sudan, Ethiopia, Tunisia, Iran and Suede Arabia). However reproductive performance is slightly durable which can be improved with the implementation of latest breeding methods. Intensive commercials farming, developing of marketing system and installing of milk processing units are the ways to attained the unearth potentials of camels.

Camel	No of	Age of Puberty	Age At 1 <sup>st</sup>	Gestation	Dry Periods	No of	Calving	Reproductio
Breeds	Camels	(Days)	Breeding	Period	(Days)	Calves	Intervals	n Span (Yrs)
			(Days)	(Days)		During Life	(Days)	
Brahvi	17	1282.6±6.99	1575.7±12.79	379.88±0.94	369.12±3.11	17.35±0.28	719.82±4.72	19.64±0.67
Kharani	16	1115.9±6.86	1509.1±14.72	382.81±2.83	351.31±3.22	9.37±0.61	768.75±6.86	20.49±0.49
Lassi	17	1232.4±13.12	1513.8±7.16	399.35±5.59	338.18±3.96	18.41±0.34	778.24±4.18	21.52±0.83
Makrani	17	1203.6±15.18	1453.9±7.23	405.53±3.84	354.59±4.29	17.64±0.27	776.53±3.85	19.58±0.57
Rodbari	17	1024.6±13.51	1206.9±13.46	377.00±2.23	282.77±2.28	17.94±0.31	787.65±1.65	20.05±0.60
Kachhi	17	1278.4±20.31	1529.6±20.33	394.24±6.34	313.65±4.98	15.52±0.35	727.Oo±4.98	17.17±0.43
Kohi	17	1297.5±22.50	1522.8±18.51	389.41±3.65	384.88±5.73	14.11±0.54	726.82±11.69	16.23±0.65
Pishin	17	1231.0±16.36	1325.8±16.81	388.12±3.26	326.76±4.74	18.35±0.36	795.18±3.33	20.64±0.57
Total	135	1208.9±9.25	1452.0±11.13	389.59±1.57	340.07±3.02	16.14±0.28	759.93±3.19	19.68±0.27

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## **AUTHORS CONTRIBUTION**

All authors contributed to make this manuscript possible. Abdul Fatih conducted research. Muhammad Masood Tariq and Masroor Ahmad Bajwa supervised the research. Abdul Waheed and Ecevit Eyduran helped in analysis. Asim Faraz wrote the paper, Majed Rafeeq, Illahi Bakhsh Marghazani and Jameel Ahmed helped in writeup, Mudassar Jehan reviewed the article.

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## MAY THE FETAL GENDER EFFECTS THE MATERNAL BODY CONDITION?

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#### Abstract

Body fat reserve is important in milk production, fertility, feed consumption and general health status in dairy goats. Body Condition Score (BCS) is an indicator of the degree of total muscle and fat depth of a live animal. It also reflects, to some extent, internal body fat reserves. It is known that there is a positive correlation between reproductive efficiency and BCS. An optimum BCS is defined for each stage of the reproduction cycle. The elevated BCS in pregnancy leads to pregnancy toxaemia, fatty liver, placental retention, and dystocia while the poor BCS leads to poor kid survivability and milk production. The aim of the study was to determine the effect of foetus number and foetal gender on the change of body condition scores from the measurements at the end of the second trimester of pregnancy to the measurements at the time of birth in dairy goats. The animal material of the study was 70 Damascus goats whose pregnancies were determined by real time ultrasound examination on the day 50 after mating. Goats were breed intensively in a commercial dairy farm, aged 2-5 years, had given at least one birth and became pregnant during the breeding season. Goats were fed with concentrate having 16% CP and 2800 Kcal / kg metabolizable energy (ME) and good quality roughage during study. Water and mineral blocks were supplied ad libitum. Body condition was scored on the 100th day of pregnancy and at the time of parturition with a 4-point scoring system (according to Mitchell, 1986) in all pregnant goats. Six goats aborted due to different reasons during the study were excluded and 5 study groups were designed from 64 goats. BCS changes in goats who conceived single female foetuses (Group 1, n=9), single male foetuses (Group 2, n=11), twin female foetuses (Group 3, n=11), twin male foetuses (Group 4, n=15), multiple cross gender foetuses (Group 5, n=18) were examined. The relationship between maternal BCS and foetal gender was measured with SPSS 23.0 with repeated measures ANOVA. Mean BCS in groups (group1, group2, group3, group4 and group 5) at day 100 was 2.78±0.83, 2.91±0.54, 2.64±0.67, 2.73±0.70 respectively, while the mean BCS at parturition was 2.83±0.79, 2.61±0.89, 2.68±0.87, 2.64±0.71, 1.97±0.81, 2.39± 0.78 respectively. When the effect of foetal gender on maternal BCS was examined, the difference between day 100 BCS and on parturition BCS was statistically significant only in Group 4 and Group 5 (p<0.05). While there is no significant effect of gender on maternal BCS in single pregnancies, carrying at least one male foetus in multiple pregnancies seems to significantly reduce the maternal BCS. But It is recommended supporting the obtained results with blood parameters which reflecting the loss of BCS in circulation in larger study groups. If it is confirmed by future other studies that the male gender has an obvious effect on maternal BCS loss, those dams should be subject to a special feeding program in the early period of pregnancies, before BCS loss has yet occurred. This situation may make it necessary to determine fetal gender during pregnancy in whole flock.

Key words: Goat, Body condition score, Pregnancy, Foetal gender, Male foetus

## IN-DEPTH BRAIN PHOSPHOPROTEOME STUDY REVEALS NEUROBIOLOGICAL UNDERPINNINGS FOR NURSE HONEYBEE WORKERS (APIS MELLIFERA LIGUSTICA)

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## Abstract

The nurse bees perform many tasks inside the hive in the order of their roles in honeybee coloney servival. There is a lack of knowledge about how the neurobiological activities during protein phosphorylation in the brains of nurse honeybee workers align with their tasks performances. The phosphoproteome in the brains of nurse honeybee workers was characterized using Ti4+-IMAC phosphopeptide enrichment, shotgun proteome, label-free quantitation, and bioinformatics. The identified 1,058 phosphoproteins in the nurse bee brain were involved in a wide spectrum of biological functions, metabolic pathways and kinase activities, indicating their pivotal roles to drive the brain development and maturation, the neurobiological activities, learning, and the cognition of the nurse bee brain during nursing tasks performance. The more strongly represented phosphoproteins in the nurse bee brains were intensively involved in the biological pathways of phosphatidylinositol signaling system, inositol phosphate metabolism, phototransduction, and wnt signaling. While the most enriched kinases in the nurse bee brain were CDK2\_CDK3, p38, СК2, JNK. ACTR2\_ACTR2B\_TGFbR2, CLK, PKC, and PKA, suggesting their vital roles in the regulation of biological processes, signal transduction, and olfactory learning processes to make possible the mission of nurse bees through the brain maturation and the development of cerebral structures. Our work is the first indepth and comprehensive phosphoproteome report on nurse honeybee worker brains and provides novel insights into the molecular details of phosphoproteins that tune protein function to the needs of nurse honeybee workers. These data provide a firm basis for future mechanistic research to better understand the neurobiological roles of targeted proteins in the nurse bee brain.

Key words: Brain, Nurse bees, Honeybee, Phosphoproteome

## FORAGE WATERMELON AND USAGE IN ANIMAL NUTRITION

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## Abstract

There are some morphological differences between forage watermelon and edible watermelon. On first sight, the fruit structures are different. The fruit skin of the forage watermelon is thick, hard and durable; fruit flesh is yellow-white or greenish in color. Fruit structure is elastic and hard. The flesh is firm and the fruits preserve their juice for a long time after riperning. Forage watermelon has a structure that changes from oval to cylindrical in shape. Forage watermelon has a yield of 8-9 tons per decare. Especially the fruit part of the fodder watermelon is widely used in animal feeding in countries such as Turkmenistan, Russia, Africa and Brazil. The fruit part can be given alone or mixed with various dry forages, corn stalk and straw. In addition to this, silage can be made with green plants, except for the fruits, the green parts that remain above the ground. Forage watermelon can be added to cattle and sheep rations between 20-80%. In this review, the feed value and usage of forage watermelon (Citrullus lanatus var. citroides) in animal nutrition are focused on.

Key words: Forages, forage watermelon, ruminant nutrition

## INTRODUCTION

Forage watermelon is known by different names (citron watermelon, protective watermelon, tsamma, warehouse watermelon etc.) in different parts of the world (Laghetti and Hammer 2007; Mujaju et al., 2010). Forage watermelon (Citrillus lanatus (Thunb.) Matsum and Nakai var. citroides (Balley) Mansf.) is a new species for agricultural potential in Turkey or some European countries like Serbia, Bosnia and Herzegovina and resembling ecologies (Acar, 2009). The fodder watermelon is a cucurbitaceous originating from Africa, introduced in the Northeast by the slaves, that through hybridization with other species of the genus is being diffused until today (Silva 2003).

Edible watermelon and forage watermelon are the same in genus and species but different varieties and their chromosome numbers are the same. Hybridization can make between them (Kobitev 1956, Övezmuradov 1972, Stephens 2009, Bullitta et al. 2008, Nesom 2011). Forage watermelon has some morphological differences from edible watermelon. For example, fruit structures are different. In forage watermelons, the fruit skin is hard, thick and durable, the fruit flesh is yellow-white or greenish, and the pulp is firm and preserves its juice for a long time after ripening (Acar 2009).

It is used for a long period in Turkmenistan with some advantages such as easy transportation and long time storage capacity without deterioration in some cases up to next year even the year after next year (Övezmuradov 1972).

# GENERAL FEATURES OF FORAGE WATERMELON

Forage watermelon has a lower sugar content than edible watermelon and has higher dry matter. With its elastic structure and hard shell, it does not break easily and can be stored for more than one year at room temperature. It has a structure that changes from oval to cylindrical in shape. At the same time, the amount of pectin is high (Kobitev 1956, Popov et al. 1957, Laghetti and Hammer 2007, Acar 2009, Stephens 2009, Mujaju et al. 2010, Bullita et al. 2008, Wehner 2011). It is stated that it is added to fruit juices to gel it faster due to its high pectin content (Acar 2009).

Fruit shape of forage water melon is circular and has 15 cm in length. Forage watermelon can be

grow at similar conditions with edible water melon (Stephens 2010). It is also highly resistant to root nematodes and some disease as well as root stock so it is a good material for obtaining high amount of fruit yield (Laghetti and Hammer 2007, Silva et al. 2009, Acar 2009).

Forage watermelon is possibly a promising crop for growing in Turkey and other countries because of the following:

1. A good stogare quality of the fruit: Due to a high content of pectins, its fruit can be stored for one year.Large fruit size and high yields: The fruit can reach a weight of 15-20 kg;

2. Resistant to a number of diseases and lesser demands on cultivation conditions (Simić et al., 2013). However, together with these mentioned qualities, there are also certain shortcomings: fairly late ripening and a fruit pulp which is not sweet and is too compact (Simić et al., 2011).

3. The fruits of forage watermelon used as a flavourant especially for straw in winter-feeding of animals and their hulled kernels are also a high protein (18.1%) and oil (23.3%) source (Acar et al., 2012).

#### AGRICULTURE OF FORAGE WATERMELON

Sowing of seeds are done when the soil temperature is 10-14 °C. The suitable season for sowing is the beginning of spring. Sowing is done in two ways: Quarry method and row sowing. The row planting method is more commonly used. It is adjusted to be 1.5-2 meters between rows and 0.5-0.8 meters above the row. Seeds are buried 2-4 cm deep. It is possible to sow directly with a seeder or by using single or double seeders in the furrows opened with furrow openers. The quarry planting is done by burying 2-3 seeds in each guarry in small lands and filling the straits (Acar 2009). Post-planting care procedures are the same as for edible watermelon. Forage watermelon is more resistant to diseases and pests when compared to edible watermelon. Chemical control should be done taking into account the harvest date. Harvest time varies according to the type of forage watermelon. (Acar 2009).

# USAGE OF FORAGE WATERMELON IN ANIMAL NUTRITION

The storage time and ease of storage of ripe fruits provide advantages in terms of use. It was

also stated that there was no loss of nutritional value of forage watermelon during storage (Kobitev 1956). Especially the fruit of Citrullus lanatus var. citroides is used in animal nutrition in former Soviet Republics, eastern bloc countries and some countries of the world such as Africa, Brazil, Turkmenistan (Kobitev 1956, Popov et al. 1957, Övezmuradov 1972, Silva et al. 2009).

Forage watermelon fruits can be given solely or mixed with straw, cornstalk and dry forages (Kobitev 1956, Övezmuradov 1972, Acar 2009, In addition to the fruits (Kobitev 1956, Övezmuradov 1972) and the green aboveground parts other than the fruits, they are also in silage with silage plants. used (Övezmuradov 1972, Acar 2009). In addition their fruits; forage watermelons green above-ground parts can be silage with other silage plants.

Forage watermelon contains an average of 8% dry matter. It contains 12% crude protein, 8% crude oil, 7% crude ash and 30% crude cellulose in its dry matter (Acar et al. 2015). It is stated that forage watermelon is grown abundantly in farms in the south and southeast of Russia and 10 kg/day is given to cattle and 3-4 kg/day to sheep (Laghetti and Hammer 2007). In the north of Brazil, it was stated that the forage watermelon is one of the feed sources in the region's livestock and provides convenience in terms of easy storage and fruit yield of 6500-7000 kg/da is obtained (Silva et. al. 2009). In the research carried out in İzmir between 2009-2010, an average fruit yield of 8761 kg/da was obtained from forage watermelon grown as a second planting (Geren et al. 2011). The fruit characteristics of forage watermelon are given in Table 1 and the feed value of forage watermelon is given in Table 2.

Due to the high oil (28-30%) and protein content of the seeds, seeds of forage watermelon are using in animal nutrition together with fruits to increase the nutritional value of the fruits (Acar et. al. 2012) and the sizes of the seeds vary (Zorobi et al. 2006). The feed value of forage watermelon seeds is given in Table 3.

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Groups by Fruit Weight (gr)						
Parameters	Small (X<2kg)	Medium (2 <x<4 kg)<="" th=""><th>Large (4<x kg)<="" th=""><th>Avarage</th><th>SEM</th></x></th></x<4>	Large (4 <x kg)<="" th=""><th>Avarage</th><th>SEM</th></x>	Avarage	SEM	
Fruit Weight (gr)	1614.01	3466.68	6163.35	3748.01	918.3**	
Shell Weight (gr)	861.18	1784.00	2740.00	1795.06	534.6*	
Seed Weight (gr)	89.52	159.56	214.31	154.46	14.37**	
Pulp Weight (gr)	663.32	1523.12	3209.04	1798.49	812.0**	
Shell Ratio (%)	53.56	51.37	44.09	49.67	-	
Seed Ratio (%)	5.54	4.59	3.49	4.54	0.5460	
Pulp Ratio (%)	40.90	44.04	52.41	45.79	-	
Perimeter of Fruit (cm)	38.10	48.93	57.10	48.04	2.425**	
Fruit Height (cm)	29.23	36.43	49.07	38.24	3.740**	
Shell Thickness (cm)	1.18	1.50	2.20	1.62	0.2912*	

Table 1. Fruit	Characteristics o	of Forage	Watermelon	(Acar et al.,	2015)

SEM: Standart error mean, \*P<0,05, \*\*P<0,01

Table 2. Change in Feed Value of Different Sizes of Forage Watermelon (Acar et. al. 2015)

Fruit Size		DM (%)	CA (%)	CP (%)	CO (%)	NDF (%)	ADF (%)	NFC (%)
Small Fruit	Seedless	4.32	10.33	10.76	4.46	35.04	29.88	39.41
	Seedy	6.65	7.07	13.58	11.69	51.84	41.85	15.82
Middle	Seedless	5.38	10.89	9.85	3.6	33.07	27.19	42.59
Fruit	Seedy	8.25	6.35	11.33	8.29	46.46	37.25	27.57
Large Fruit	Seedless	5.66	8.45	7.47	1.77	33.6	27.53	48.71
	Seedy	8.27	7.9	11.24	4.14	35.19	28.75	41.53
Avarage	Seedless	5.12	9.89	9.36	3.28	33.9	28.2	43.57
	Seedy	7.72	7.11	12.05	8.04	44.50	35.95	28.30

DM: Dry matter, CA: Crude ash, CP: Crude protein, CO: Crude oil,

NDF: Neutral detergent fiber, ADF: Acid detergent fiber, NFC: Non fiber carbonhydrate.

Table 3. Feed Value of Forage Watermelons Seeds (Acar et. al. 2012)

Properties	Kernel	Hulled Kernel
Moisture (%)	3.61±0.18	4.55±0.27
Crude Protein (%)	32.71±2.32	18.13±0.87
Crude Oil (%)	43.32±2.17	23.31±1.08
Crude Ash (%)	3.76±0.89	2.59±0.13
Crude Fiber	3.04±0.32	44.70±3.71
Total Phenol (mgGAE/g)	0.24±0.03	0.30±0.008
Antioxidant Activity (%)	5.06±0.68	13.90±1.2

mgGAE/g: Milligrams of gallic acid equivalents/gram

#### **OTHER USAGES OF FORAGE WATERMELON**

In addition to animal nutrition, forage watermelon can be used as human food. Due to the low sugar content of forage watermelon, it is also known as diet watermelon by people. It is used in jam making due to its pectin content. Pectin has a wide range of uses. Fresh and small fruits are used to make jams and pickles. (Acar et al. 2019).Its seeds are also used in human consumption in the snacks, oil, medical and cosmetic industries. The seeds can also be used as human food and the oil obtained can be used as a skin care product in the cosmetic industry. At the same time, the seeds can be used in alternative medicine. (Kobitev 1956, Övezmuradov 1972, Laghetti and Hammer 2007, Lauoku et al. 2007, Minsart and Bertin 2008, Acar 2009, Wehner 2011). Since forage watermelon is resistant to root diseases and pests seen in table watermelon, it can also be used in breeding table watermelon and as rootstock grafting material (Acar et al. 2019).

## CONCLUSION

Forage watermelon, which is used as a forage plant in the world, can be considered as a new roughage source in Turkey. Forage watermelon can be used in other fields besides animal feeding. Groundwater has been used extensively in irrigation and has declined gradually every year due to the over water extraction. Less water consumption crops such as forages water melon can be a good alternative for sustainable irrigation and animal production. Forage watermelon is a good alternative roughage; considering its high productivity, high resistance to environmental conditions and low water requirement.

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## PRINCIPAL COMPONENT ANALYSIS OF MORPHOLOGICAL TRAITS IN ROSS 308 BROILER CHICKEN BREED

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## Abstract

Morphological characterisation of animal breeds is a crucial stage in animal development program as it allows livestock farmers to identify and select superior lines for advancement. Principal component analysis is a valuable instrument in multivariate methodology and it is very useful when characteristics are interrelated. The objective of this study was to explore the relationship amongst morphological traits and body weight (BW) of Ross 308 chicken breed. Morphological traits were recorded on one hundred Ross 308 chickens (male = 50, female = 50) at the Broiler Production division of Potchefstroom College of Agriculture, South Africa. The data was analysed using Principal Component Analysis (PCA) and stepwise regression. The PCA results extracted only three and two components in males and female chicken respectively, which contributed a remarkable 67.78% and 57.15% of variation. The specified principal components extracted contributed significantly to describe the whole Ross 308 chicken breed structuring. Regression results showed that the use of components was appropriate in predicting BW. Therefore, components found might be valuable in breeding programmes with essential reduction in morphological traits to be documented to explain the morphological structuring in Ross 308 chicken breed.

Key words: Body weight, Phenotypic characterization, Regression analysis

## IDENTIFYING SELECTION CRITERIA OF GOAT FARMERS IN MADIGA VILLAGE, LIMPOPO PROVINCE, SOUTH AFRICA: IMPLICATIONS TO DESIGN COMMUNITY-BASED BREEDING PROGRAMME

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## Abstract

Understanding the selection criteria of farmers would provide information to deciding how to approach farmers' herds in order to design community-based breeding programmes for genetic improvement. The study was conducted to investigate the selection criteria used by goat farmers in Madiga village. The study was conducted at Madiga village, Polokwane Local Municipality, in Limpopo Province, South Africa. The village is situated about 12.2 km west of the University of Limpopo. The village was purposively selected, out of 54 farmers, 26 (13 males and 13 females) goat farmers were randomly selected and given well-structured questionnaires to identify selection criteria of breeding stock. Chi-square test was used for data analysis, for comparison between males and females. The results indicated that there is no significant (P> 0.05) variation between selection criteria (growth rate, coat color, conformation and others) amongst the male and female farmers with P-value of 0.446, and the selection criteria values: 2 (15.4%), 5 (38.5%), 4 (30.8%), 2 (15.4%) and 3 (23.1%), 5 (38.5%), 1 (7.7%), 4 (30.8%), respectively. The present study suggests the importance of growth rate, skin color, conformation and others in the selection of goat breeding in Madiga village. However, the selection of traits for breeding were not different amongst the male and female farmers. Developing a communitybased breeding programme will benefit both male and female farmers since, there is no difference in selection criteria.

Key words: Growth rate, Coat color, Conformation, Chi-square test

## GENETIC DIVERSITY OF MTDNA IN GREEN JUNGLEFOWL (GALLUS VARIUS) OF INDONESIA: A META-ANALYSIS STUDY

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#### Abstract

Green junglefowl (Gallus varius) was originated from Southeast Asia, mainly Indonesia. A meta-analysis study was conducted in this research to observe the genetic diversity of whole genome mitochondrial DNA (mtDNA) of Green Junglefowl. Thirteen (13) mtDNA sequences of Green junglefowl from Indonesia were analyzed in this study and obtained from GenBank. Research revealed that total twenty-nine (29) polymorphic site and thirteen (13) haplotypes were detected among birds' study. Thus, the haplotype diversity (Hd), nucleotide diversity (pi) and Average number of nucleotide differences (k) were 1.00±0.03; 0.0003 and 4.821 respectively. The phylogenetic tree based on the whole genome mtDNA (16,841 bp) reveals that Green junglefowl of Indonesia were classified into five clusters. In addition, the Geographical populations were not contributed to mtDNA sequence variaton in birds' study. It can be concluded that the Green junglefowl from Wallacea region (Sulawesi and Molucca) was detected as the common ancestry of Green junglefowl of Indonesia based on the whole genome mtDNA information.

Key words: GJF, Indonesia, mtDNA, phylogenetic tree, polymorphic

#### INTRODUCTION

Green junglefowl (Gallus varius) is one of wild species that spread in Indonesia (Fig. 1). Therefore, the other junglefowl species of Red junglefowl (Gallus gallus), Grey junglefowl (Gallus sonneratii) and Ceylon junglefowl (Gallus lafayetii) were spread in Southeast Asia, Southern India and Sri Lanka respectively (Desta, 2019). Red junglefowl was known as the common ancestry of chickens in the world (Lawal et al., 2020). Recently, Red junglefowl was classified into five subspecies of G. g. gallus, G. g. jabouillei, G. g. murghi, G. g. spadiceus and G. g. bankiva (Fernandes et al., 2019). In Bengkulu Province (Indonesia), Red junglefowl was used for crossbreeding program with local hens (Gallus domesticus) to produce Burgo chicken. Hence, Burgo chicken is an exotic pet animal that kept for crowing contest purposes (Setianto et al., 2019).

In Java Island (Indonesia), Green junglefowl was kept for crossbreeding program with local hens to produce unfertile Bekisar chicken. Similar to the Burgo chicken, Bekisar chicken is an exotic pet animal that kept for crowing contest purposes (Hidayat and Asmarasari, 2015). As the endemic wild bird of Indonesia, study to observe the genetic diversity among Green junglefowl from several paleces of Indonesia is important for the conservation program. Therefore, the genetic diversity in the mitochondrial DNA (mtDNA) of Green junglfowl can be used to characterize Green junglefowl of Indonesia. Furthermore, characterization of Green junglefowl may be used for producing Bekisar chicken with desirable characteristics. The genetic relationship in the animal can be conducted with mtDNA information (Bekker et al., 2016). This study was aimed to observe the genetic diversity of mtDNA in Green junglefowl of Indonesia based on the reference sequence (GenBank). The results study can be used as the basic information to conserve Green junglefowl in the future.

#### MATERIALS AND METHODS

Amount thirteen (13) whole genome mtDNA sequences (16,841 bp) of Green junglefowl (Gallus varius) were used in this study and referring to the reference sequence (GenBank) as presented in Table 1. Thus a bioinformatic analysis were performed to evaluate the mtDNA of birds' study. A BioEdit software Hall (2001) was was for alignment in observed sequences. A

MEGA-X software Hall (2013) was used to construct a phylogenetic tree based on the observed sequences. A DNAsp software Rozas et al. (2016) was used to perform statistical analysis in mtDNA diversity. A NETWORK software was used to construct a median joining network. Thus, Arlequin software Excoffier and Lischer (2010) was used to obtain analysis of molecular variance (AMOVA).

<b>Table 1.</b> The accession number of mtDNA sequence in Green junglefowl (Gallus)	
varius) of Indonesia based on GenBank	

No	GenBank	Sample location		
1	KY039425	Molucca 1		
2	KY039395	Molucca 2		
3	KY039427	Manado 1, Sulawesi Island		
4	KY039428	Manado 2, Sulawesi Island		
5	KY039420	Kendu 1, Java Island		
6	KY039419	Kendu 2, Java Island		
7	KY039424	Manokwari 1, Papua Island		
8	KY039423	Manokwari 2, Papua Island		
9	KY039418	Garut, Java Island		
10	KY039429	Goma, Sulawesi Island		
11	KY039426	Lombok Island		
12	KY039422	Nunukan, Borneo Island		
13	KY039421	Tarakan, Borneo Island		

#### **RESULTS AND DISCUSSION**

According to the thirteen (13) whole genome mtDNA sequence, the Green junglefowl of Indonesia can be classified into 13 haploypes with 29 polymorphism sites (Table 2).

**Table 2.** The statistical data calculated based on whole genome of mtDNA (16,841 bp) in Green junglefowl (Gallus varius) of Indonesia

Parameter	Value
Total number of site	16,841
Number of polymorphic	29
site	
Number of haplotype (h)	13
Haplotype diversity (Hd)	$1.00 \pm 0.03$
Nucleotide diversity (pi)	0.0003
Average number of nucleot	4.821
differences (k)	

Therefore, the haplotype diversity in birds' study was high  $(1.00\pm0.03)$ . Meanwhile, the nucleotide diversity (pi) in birds study was low (0.0003).

Despite, the AMOVA with three geographical factors of Sundaland (Java, Lombok, Borneo), Wallacea (Sulawesi, Molucca) and Nahuland (Papua) reveals that the genetic diversity of mtDNA in study birds was not affected by geographical factor (-4.40%) as presented in Table 3. In the glacial period (pleistocene) of 110,000 to 20,000 years ago, Borneo and Java islands were part of a large subcontinental namely Sundaland (Hall, 2013). While, Sulawesi and Molucca islands have been existed in this period namely Wallacea area. While, Papua island was part of a large continental namely Sahuland (Australia).

Hence, the geographical area of Sundaland, Wallacea region and Nahuland was closed and may be given a low effect to the genetic diversity of mtDNA in birds' study. In addition, these geographical areas were under tropical climate with similar environment. Previous studies reported that the contribution of geographical area in the D-loop region (mtDNA) diversity of native chicken in Indonesia and Nigeria were 5.82% and 2.68% respectively (Sulandari et al., 2008; Adebambo et al., 2009). In addition, the pairwise distance among Green junglefowl of Indonesia was ranged from 0.0001 to 0.0007 (Table 4).

Hence, the Green junglefowl of Indonesia were showed under close relationship. According to the phylogenetic tree, the Green junglefowl of Indonesia was classified into five (5) clusters of cluster 1 (Lombok), cluster 2 (Goma), cluster 3 (Manado 1), cluster 4 (Garut and Kendu 1) and the others are in cluster 5 (Fig. 1). According to the median joining network, the Green junglefowl from Wallacea area (Sulawesi and Molucca) was showed as the common ancestry for Green junglefowl of Indonesia (Fig. 2).

**Table 3.** The analysis of molecular variance (AMOVA) of mtDNA in Gallus varius with three geographical areas of Sundaland (Java, Lombok, Borneo), Wallacea (Sulawesi, Molucca) and Nahuland (Papua)

Source of variation	Degree of Free	dom Sum of squares	Variance	Percentage of
			components	variation
Among populations	2	4.12	-0.10	-4.40
Within populations	10	24.80	2.48	104.40
Total	12	28.92	2.38	

Note: Fixation index (FST) = -0.04

**Table 4.** Pairwise distance among Gallus varius of Indonesia based on the whole genome of mtDNA (16,841 bp)

Location (GeneBank)	1	2	3	4	5	6	7	8	9	10	11	12
1. Molucca 1 (KY039425)	-											
2. Molucca 2 (KY039395)	0.00	-										
	02											
3. Manado 1 (KY039427)	0.00	0.00	-									
	04	04										
4. Manado 2 (KY039428)	0.00	0.00	0.00	-								
	02	02	04									
5. Kendu 1 (KY039420)	0.00	0.00	0.00	0.00	-							
	03	03	04	03								
6. Kendu 2 (KY039419)	0.00	0.00	0.00	0.00	0.00	-						
	03	01	04	03	04							
7. Manokwari 1	0.00	0.00	0.00	0.00	0.00	0.00	-					
(KY039424)	02	02	04	02	03	03						
8. Manokwari 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-				
(KY039423)	03	01	04	03	04	01	03					
9. Garut (KY039418)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-			
	04	04	05	04	03	04	04	04				
10. Goma (KY039429)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-		
	05	05	06	05	04	05	05	05	05			
11. Lombok (KY039426)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	
	04	04	07	05	06	05	05	05	07	08		
12. Nunukan (KY039422)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
	01	02	04	02	03	03	02	03	04	05	04	
13. Tarakan (KY039421)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	03	02	05	02	04	02	04	02	04	05	05	03

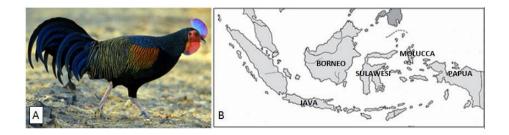
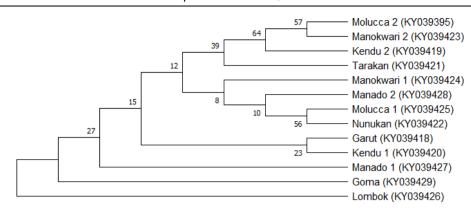


Figure 1. A Green Junglefowl (Gallus varius) (A) and its habitat in many places of Indonesia (B)



**Figure 2.** The circular phylogeny tree of the relationships among Green Junglefowl (Gallus varius) of Indonesia based on the whole genome of mtDNA (16,841 bp)

## CONCLUSION

The genetic diversity of whole genome mtDNA in Green junglefowl of Indonesia was high with 29 polymorphic sites. Thus, the mtDNA diversity in birds' study was not affected by geographical area. Thus, the gene flow in the Green junglefowl of Indonesia was originated from Wallacea area (Sulawesi and Molucca). However, the depth study with a large number of sample from live birds and fossils are important to evaluate the mtDNA diversity of Green junglefowl in Indonesia accurately.

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## THE RELATIONSHIPS BETWEEN LEPTIN GENE POLYMORPHISM AND SOME PERFORMANCE TRAITS IN SIMMENTAL AND BROWN SWISS CATTLE

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## Abstract

It was aimed to investigate the Leptin gene Sau3AI polymorphism in Simmental and Brown Swiss breed cattle raised in the province of Erzurum in a private enterprise, to determine the genotype and allele frequencies of the cattle and to associate these genotypes with some performance characteristics. In the study, leptin/Sau3AI gene polymorphisms in DNA isolated from blood samples obtained from Simmental and Brown Swiss cattle were determined using PCR-RFLP method. As genotype frequencies of leptin/Sau3AI gene in the population, in Brown Swiss cattle, 88,7% with genotype AA, 9,7% with AB and 1,6% with BB genotypes were determined, Simmental breed cattle, 78,3% with genotype AA, 16,7% with AB and with 5,0% BB genotypes were determined. AA genotype frequencies were the highest in the population, and those with BB genotypes had the lowest frequency in both breeds. When the population was examined in terms of allele frequencies, the A allele was 0,87 and B allele was 0,13 in the Simmental cattle, and the A allele was 0,94 and B allele was 0,06 in the Brown Swiss breed. According to Hardy-Weinberg genetic balance test, the distribution of genotype frequencies was balanced (P>0.05) in the Brown Swiss breed but not in the Simmental breed in the population studied. As a result of the analysis performed in the Simmental breed, the general averages were found to be 5422,4 ± 1901,74 kg for actual milk yield, 5626,6 ± 1475,85 kg for 305-day milk yield, 298,7 ± 84,80 days for lactation duration and 18,5 ± 4,84 kg for daily milk yield. According to the statistical analysis results, the effect of genotype on the actual milk yield during lactation, lactation duration and daily milk yield was significant. As a result of the analysis made in the Brown Swiss breed, the general averages were 3917,8 ± 1584,38 kg for actual milk yield, 4614,3 ± 982,62 kg for 305 days milk yield, 254.9 ± 99.88 days for lactation duration and 16,0 ± 3,82 kg for daily milk yield. According to the statistical analysis results, the effect of genotype on performance characteristics was insignificant in Simmental and Brown Swiss cattle.

Key words: Leptin, Polymorphism, Performance traits, Simmental, Brown Swiss

## INTRODUCTION

The livestock sector, which has an important place in meeting the animal-based nutrient needs of human beings, shows a rapid development with the effect of technology and other factors. In parallel with the technological developments, new breeding methods are applied to meet the demand for animal products due to population growth. Two factors are effective on the phenotypic value of animals in terms of any trait. One of them is genotype and the other is environment (Akman, 2016). While determining the quantitative characteristics of animals in the livestock sector, it is impossible to determine genotypes with ideal alleles by looking only at phenotypes, but phenotypic values do not always reflect genotypic values. Creating the superior phenotype in animals is only possible by identifying good and efficient genes that affect the character, bringing together the desired genes in the genotype and benefiting from the interaction between genes. One of the candidate genes for marker assisted selection is the leptin gene. The leptin gene is expressed in a variety of tissues, including adipose tissue, placenta, mammary glands, skeletal muscles, gastric mucosa, brain, and pituitary glands. Leptin appears to have a major effect in coordinating whole body energy metabolism and can be classified as a metabolism modifier (Houseknecht et al., 1998). In cattle, the leptin gene is located on the 4th chromosome and consists of three exons (Pomp et al. 1997). Leptin is a 16 kDa protein synthesized by adipose tissue and plays a role in the regulation of feed intake, energy balance, fertility and immune functions (Fruhbeck et al., 1998).

The leptin gene is associated with single nucleotide polymorphisms and also with many quantitative characters such as carcass fat, feed consumption, and milk yield (Kök et al., 2015). Polymorphisms in the leptin gene have been associated with milk performance (Liefers et al., 2002; Heravi et al., 2006), increased perinatal mortality in dairy products (Brickell et al., 2010), calf birth and weaning weights in beef and dairy products (Almeida et al., 2003; Nkrumah et al., 2005; DeVuyst et al., 2008) and reproductive characteristics in dairy cattle, includina gestational age (Komisarek and Antkowiak, 2007).

Leptin is a protein that has an important role in the realization of many functions in animals. In addition to taking part in the metabolism and growth of animals, leptin has important duties on features such as feed utilization, energy metabolism and reproductive efficiency. The leptin gene, which has an important role in all of these economically important mechanisms, is an excellent candidate gene for studies of polymorphism and association of these polymorphisms with economic characteristics (Ninov et al., 2008; Özdemir, 2008). Therefore, the objective of the present investigation was to investigate the genotypic structures of the Leptin gene Sau3AI (LEP/Sau3AI) polymorphism, to determine the genotype and allele frequencies of the cattle and to associate these genotypes with some performance characteristics in Simmental and Brown Swiss cows in Turkey.

# MATERIAL AND METHODS

The animal material of this research consisted of genomic DNA samples obtained from blood samples taken for previous studies (Ünal, 2020)

of 62 Brown Swiss and 60 Simmental breeds, which were raised in two different farms intensively produced in Erzurum.

The Leptin gene specific primers (5'-TCT TAA GCT AGT CAG GTT CCA CAA GGT-3' and 5'-TGC TCC ACG CAG GTG AGC AAG-3') designed by Ozdemir (2011), were used to amplify a 495 bp Fragment in cattle. Amplification reactions were performed in a final volume 25 µl containing approximately 3  $\mu$ l genomic DNA, 1  $\mu$ l dNTP (D7595: Sigma, St. Louis, MO, USA), 0.5 U of Tag DNA Polymerase (D1806: Sigma, St. Louis, MO, USA), 1 µM of each Primer, 3 µl of 10x PCR Buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2 and 0.01% gelatin), 1 µl of 25 mM MgCl2 and ddH2O. After 10  $\mu$ l mineral oil in tubes. PCR amplifications were performed in 2 min at 94°C, 30 cycles of 45 s at 94, 60 and 72°C, which w as followed by final extension at 72°C for 5 min. After the PCR process, 8 µl of each PCR product was run on a 1.2% agarose gel at 80 V for 25 minutes to determine whether amplification occurred. Products with completed amplifications were stored in an environment at -20°C until the next step. To genotype animals for the RFLP, 7-9 µl PCR reaction mix was used for Sau3A1 enzyme digestion which was performed in 20 µl volume in 0.2 ml sterilized eppendorf tubes and incubated at 37 °C, 12 h. Each 20 µl digestion mix was electrophoresed in 2.5% agarose gel at 30 V for 2.5 h and DNA was visualized by staining with ethidium bromide under UV light. A standard DNA marker (P1473: Sigma, St. Louis, MO, USA) was used. The digested AA PCR product exhibited two fragments of 299 and 196 bp. For the BB genotype exhibited 214, 196 and 85 bp (Table 1). For each cattle breed, Leptin allele frequencies were determined by gene counting. The Chisquare ( $X^2$ ) test was used to check whether the populations were in Hardy-Weinberg equilibrium or not.

Tablo 1. Restriction enzyme, the cutting region and fragment size of genotypes

RE	PCR product (bp)	Cutting region	Genotype and fragment size (bp)
			AA:299/196
Sau3AI	495	5'-/GATC-3'	AB: 299/214/196/85
			BB:214/196/85

In the statistical analysis; Yield records of 60 Simmental and 62 Brown Swiss animals raised in private enterprises in Erzurum were used and milk yield characteristics such as actual milk yield, 305-day milk yield, lactation period and daily milk yield were examined as performance characteristics. Intermittent environmental factors such as genotype, lactation order and calving season, which are effective on these yield traits, are emphasized. According to the yield characteristics in the study, the following statistical model was used.

 $Y_{ijkl}: \mu + a_i + b_j + c_k + e_{ijkl}$ 

Y<sub>ijkl</sub>: The value of any Simmental or Brown Swiss cow in terms of performance (actual milk yield, 305-day milk yield, lactation period and daily milk yield) characteristics considered,

μ: Population mean

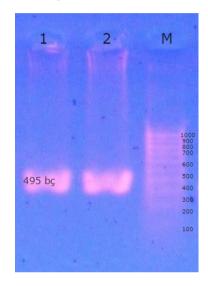
ai: i. genotype effect (i:3; 1:AA; 2:AB; 3:BB)

bj: j. effect of lactation order (2.-7),

 $c_k$ : k. effect of calving season (k:2; 1: Kış ve ilkbahar, 2: Yaz ve Sonbahar)

e<sub>ijkl:</sub> I. Marginal error.

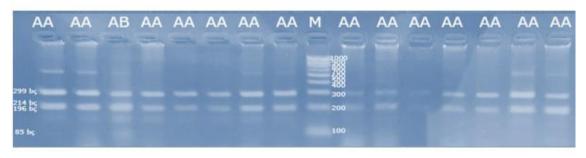
In the model used, all the factors except the error were considered constant, and the error was accepted as randomized. out on a 1.2% agarose gel by PCR and PCR products were obtained. Figure 1 shows the agarose gel image of the PCR products. DNA samples obtained from Simmental and Brown Swiss cattle were amplified in a PCR device and as a result of cutting the DNA fragments of 495 bp long PCR product with restriction enzyme, 299 and 196 bp for AA genotype, 214, 196 and 85 bp for BB genotype and 299 for AB genotype. It has been determined that they form 214, 196 and 85 bp long bands.



#### **RESULT AND DISCUSSION**

DNA samples obtained from the blood of Simmental and Brown Swiss cattle were carried

**Figure 1.** Agarose gel image of PCR products (M: marker,1000-100 bp; leptin PCR product: 495 bp)



**Figure 2.** PCR-RFLP gel image of LEP/Sau3AI polymorphism (M:DNA marker, AA: 299/196 bp, AB: 299/214/196/85 bp, BB: 214/196/85 bp)

Capatura	Brown Swiss			Simmental		
Genotype	n	%	n	%		
AA	55	88,7	47	78,3		
AB	6	9,7	10	16,7		
BB	1	1,6	3	5,0		
	А	В	А	В		
Allel Gene Frequence (%)	94	6	87	13		

Genotypic and allele gene frequencies of breeds are presented in Table 1, and Hardy-Weinberg genetic equilibrium test results are presented in Table 2. When the population was examined in terms of allele frequencies, it was determined that the A allele was at the frequency of 0.87 and the B allele was at the frequency of 0.13 in Simmental cattle, while the A allele was at the frequency of 0.94 and the B allele at the frequency of 0.06 in Browns cattle (Table 2). The leptin gene AA, AB and BB genotype frequencies were found to be 78.3%, 16.7% and 5.0% in Simmental, 88.7%, 9.7% and 1.6% in Brown Swiss,

respectively. In both breeds, AA genotype frequencies were observed to be the highest in the population, while BB genotypes had the lowest frequency (Table 2). Considering other similar studies; Pomp et al., (1997), Rasor et al., (2002), Javanmard et al., 2004, Leifers et al., (2002), Leifers et al., (2003), Javanmard et al., (2005) ), Javanmard et al., 2010, Öztabak et al. 2010, Kulig et al., (2010), Aytekin, (2011) the allele frequencies stated in their study and the allele frequencies in the study are in harmony.

Table 2. LEP genotype frequencies and Ha	ard-Weinberg genetic equilibrium test results
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Breed	Ν	Observed	Estimated	X <sup>2</sup> test	Р
Бгеец		AA/AB/BB	AA/AB/BB		
Brown Swiss	62	55/6/1	54,26/7,48/0,26	2.44	ns
Simmental	60	47/10/3	45,07/13,87/1,07	4.67	*

ns: non-significant (P>0,05), \*: P<0,05)

According to the Hardy-Weinberg genetic balance test performed for both breeds, LEP/Sau3AI gene polymorphism in Brown Swiss breed was in equilibrium ( $X^2$ =2.44; P>0.05), while in Simmental breed ( $X^2$ =4.67; P<0.05) was not found to be in equilibrium. This situation in Simmental cattle; It is thought that the increase

in certain genotypes in the population is prevented or caused by sampling error due to the absence of chance mating in breeding studies. The least squares means and standard errors of the Leptin gene polymorphic structure in terms of some yield characteristics in Brown Swiss and Simmental cattle are given in Table 3.

					305-day	305-day Milk		Daily Milk		Lactation	
Breed	Genotype	Ν			Yield (kg)		Yield (kg)		period (days)		
			Mean ± SE		Mean ± SE		Mean ± SE		Mean :	Mean ± SE	
	AA	113	3997,8	1625,69	4667,8	982,67	16,2	3,81	256,5	101,52	
Brown Swiss	AB	11	3076,5	943,68	4144,5	949,47	14,4	4,10	234,8	99,21	
	BB	4	3973,2	1338,56	4395,4	1229,2	14,8	2,71	263,8	55,35	
Total		128	3917,8	1584,38	4614,3	982,62	16,0	3,82	254,9	99,88	
	AA	70	5455,7	1915,36	5665,9	1462,78	18,6	4,80	298,8	85,51	
Simmental	AB	12	5563,7	2005,61	5739,8	1583,03	18,9	5,19	295,3	83,15	
	BB	3	4079,4	585,72	4258,3	969,13	14,0	3,18	308,3	107,22	
Total		85	5422,4	1901,74	5626,6	1475,85	18,5	4,84	298,7	84,80	

When the averages of daily milk yield, actual milk yield and 305-day milk yields of LEP/Sau3AI genotype were examined, it was determined that the highest average was the AA genotype in the Brown Swiss and the AB genotype in the Simmental. The effect of LEP/Sau3AI genotype on daily milk yield, actual milk yield and 305-day milk yield was found to be insignificant (P>0.05) in both breeds. In the study, it was determined that cows with BB genotype had a longer lactation period for both breeds, while the effect of LEP/Sau3AI genotype on lactation period was found to be insignificant (P<0.05) in both breeds. That have been reported similar results for milk yields by Madeja et al., (2004), on 117 Polish Black and White bulls, by Gürses (2010), on Jersey, Brown Swiss, Holstein, Eastern Anatolian Red and Native Black breeds, by Alashawkany et al., (2008) on Holstein breeds. However, Moussavi et al., (2006) found that the effect of LEP/Sau3AI gene on 305-day milk yield was significant in their study on 238 Iranian Holsteins. Also, It was reported that in study on 595 Holstein cows (Liefers et al., 2002), AB genotype cows had higher daily milk yield than AA genotype cows and, in study on Brown Swiss breed (Ghazanfari et al., 2006), cows with AA genotype had higher yield and lactation period than BB genotype.

## CONCLUSION

As a result of the research, in order to determine the LEP gene Sau3AI polymorphism, the genotype and allele frequencies of each cattle breed were determined by using the PCR-RFLP method from genomic DNAs obtained from Simmental and Brown Swiss cattle. It was seen that the results obtained were sufficient to reveal the genotype and allele frequencies of the populations and as a result of the correlation analysis, the effect of the LEP/Sau3AI polymorphism on the performance characteristics related to milk yield was insignificant (P>0.05) in both breeds.

It is thought that demonstrating the usability of such studies in the breeding of cattle and applying similar studies in different breeds, different regions and larger populations will provide great contributions and new opportunities to the development of animal husbandry.

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# FACTORS RESPONSIBLE FOR HIGH DAILY MILK YIELD IN AN AUTOMATIC MILKING SYSTEM

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## Abstract

Optimizing daily milk yield in automatic milking systems (AMS) became one of the basic issues raised by many farmers. The aim of this study was to determine the optimal level of factors for high daily milk yield per AMS in dairy cattle herds. The study involved 4854 Polish Holstein-Friesian cows milked in AMS. Statistical analysis was performed using two methods: multifactorial analysis of variance and classification tree technique. The average daily milk yield was 1634.56 kg. Milk yield, number of cows, free robot time, milking speed and cow treatment time were the major determinants of total milk yield for all cows per milking robot and were most frequently used to construct the decision tree. Highest total milk yield per robot was observed on days which were characterized by high milk yield and milk speed, and short free robot time. In AMS dairy herds, efforts should be made to select animals that transmit high milk yield and high milking speed while shortening free robot time, which will increase daily milk yield per robot. Also, attention should be given to the robot preparation time because prolongation of this variable has a negative effect on daily milk yield per AMS.

Key words: Automatic milking system, Dairy cattle, Decision trees, Milk yield

## DETERMINATION OF ANTI-METHANOGENIC AND ANTI- PROTEOLYTIC POTENTIAL OF MIMOSA TANNIN USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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#### Abstract

Understanding the selection criteria of farmers would provide information to deciding how to approach farmers' herds in order to design community-based breeding programmes for genetic improvement. The study was conducted to investigate the selection criteria used by goat farmers in Madiga village. The study was conducted at Madiga village, Polokwane Local Municipality, in Limpopo Province, South Africa. The village is situated about 12.2 km west of the University of Limpopo. The village was purposively selected, out of 54 farmers, 26 (13 males and 13 females) goat farmers were randomly selected and given well-structured questionnaires to identify selection criteria of breeding stock. Chi-square test was used for data analysis, for comparison between males and females. The results indicated that there is no significant (P> 0.05) variation between selection criteria (growth rate, coat color, conformation and others) amongst the male and female farmers with P-value of 0.446, and the selection criteria values: 2 (15.4%), 5 (38.5%), 4 (30.8%), 2 (15.4%) and 3 (23.1%), 5 (38.5%), 1 (7.7%), 4 (30.8%), respectively. The present study suggests the importance of growth rate, skin color, conformation and others in the selection of goat breeding in Madiga village. However, the selection of traits for breeding were not different amongst the male and female farmers. Developing a communitybased breeding programme will benefit both male and female farmers since, there is no difference in selection criteria.

Key words: Growth rate, Coat color, Conformation, Chi-square test

#### INTRODUCTION

Fermentation of carbohydrate and protein in the rumen results in the formation of methane and NH3 which represent not only an environmental hazard but also a loss of dietary energy and nitrogen that could potentially be redirected to milk and meat production (FAO, 2006, Eckard et al., 2010). Archaea produces methane using the metabolic H<sub>2</sub> produced by rumen microbioata (Demeyer and Van Nevel, 1975, McAllister and Newbold, 2008). Hyper ammonia producing bacteria is responsible for degradation of feed protein and deamination of amino acids. Extensive degradation of protein and amino acid in the rumen results in large amount of NH3 and urea which might be excreted with urine which contributes to ground water pollution and greenhouse gases by nitrous oxide emission (Weimer, 1998, Teleshop et al., 2003)

Several studies have suggested that tannin in diets have a potential in decreasing of enteric methane production (Kamra et al., 2006; Waghorn et al., 2002, Woodward et al., 2001, Kaplan et al., 2014, Karabulut et al., 2006 and 2007). Tannin exerts their anti-methanogenic effect either by direct inhibition of methanogens or indirectly through inhibition of protozoa (Animut et al 2008; Bhatta et al., 2009; Jayanegara et al. 2009). The use of tannins at low levels could maximize microbial protein synthesis so that the efficiency of microbial protein synthesis increases and protein degradation in the rumen decreases (Makkar, 2000).

Therefore the aim of the current experiment was to determine anti-methanogenic and antiproteolytic potential of mimosa tannin using in vitro gas production technique.

## **RESULTS AND DISCUSSION**

The effects of mimosa tannin on the gas, methane and ammonia production of total mixed rations is given in Table 1. Supplementation of mimosa tannin had a significant effect on gas, methane and ammonia productions of total mixed ration. Gas and methane productions of total mixed rations ranged from 47.50 to 51.50 ml and 8.12 to 9.43 ml respectively. These results obtained in the current study are consistent with findings of Huyen et al. (2016) and Kamalak et al. (2018) who found that condensed tannin decreased the gas and methane production.

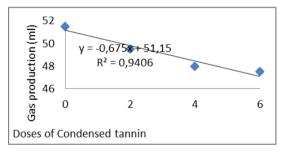
	<i>.</i>				
Table 1. Effect	of incubation	<i>in vitro</i> gas	production a	and methane	production

	Mimosa Tannin									
	0	2	4	6	SEM	Sig.				
Gas	51.50ª	49.50 <sup>b</sup>	48.0 <sup>bc</sup>	47.50 <sup>c</sup>	0.612	***				
CH4(ml)	9.43ª	8.44 <sup>b</sup>	8.18 <sup>b</sup>	8.12 <sup>b</sup>	0.264	***				
CH4 (%)	18.13ª	17.05 <sup>b</sup>	16.92 <sup>b</sup>	17.25 <sup>ab</sup>	0.422	***				
NH <sub>3</sub> -N	20.54ª	19.09 <sup>b</sup>	18.39 <sup>bc</sup>	17.39 <sup>c</sup>	0.410	***				

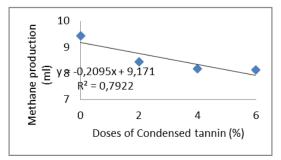
<sup>ab</sup>Row means with common superscripts do not differ (p>0.05), sem: Standard error mean, Sig:Significancy level, \*\*\*, P<0.001.

Mimosa tannin supplementation also significantly decreased the ammonia production of total mixed ration. Ammonia concentration of mixed rations ranged from 17.39 to 20.54 mg/100 ml. This result obtained in the current study is consistent with findings of Pinski et al. (2015) and Ozkan et al. (2018) who found that condensed tannin decreased ammonia production.

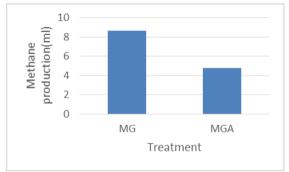
The relationships between condensed tannin doses and gas production or ammonia production are given in Figures 1-3. Gas, methane and ammonia production decreased with increasing level of condensed tannin.



**Figure 1.** The relationship between condensed tannin and gas production



**Figure 2.** The relationship between condensed tannin and methane production



**Figure 3.** The relationship between condensed tannin and methane production

#### CONCLUSIONS

This preliminary investigation clearly showed that supplementation of mimosa tannin significantly reduced the methane and ammonia production. Therefore mimosa tannin had an antimethanogenic and anti-proteolytic potential for ruminant animals. However before large implication, mimosa tannin should be further investigated in vivo experiment.

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## LIQUID STORAGE OF RAM SEMEN: ASSOCIATED DAMAGES AND IMPROVEMENT

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#### Abstract

The successful application of assisted reproductive techniques (ARTs) in ovine as in other mammal species relies on many factors among which the quality of the semen used. After collection, semen samples are generally processed for storage (liquid storage or cryoconservation) before being used for insemination or in vitro embryo production. During the liquid storage process, sperm cells are exposed to artificial conditions which lead to oxidative stress—the imbalance between pro-oxidants and antioxidants (AO), following overproduction of reactive oxygen species (ROS)—resulting in ultrastructural, biochemical and functional damages of spermatozoa. Especially, viability, motility, mitochondrial activity, membrane integrity, and acrosome integrity are reduced while morphological abnormalities, DNA fragmentation, and lipid peroxidation (LPO) are increased, affecting the fertilizing ability and subsequent early embryonic development when using standard extenders. Indeed, an optimal semen extender must not only regulate and sup- port an environment of adequate pH and buffering capacity to protect sper- matozoa from osmotic and cooling stresses, but, also prevent the generation and/or scavenge excess ROS. To improve ram semen liquid storage, several methods have been developed with the supplementation of extenders with antioxidants or antioxidant likecompounds (enzymes, amino-acids, vitamins, plant extracts), seminal plasma, sugars, fatty acids, and nanoparticles being a relevant approach. Promising results have been registered with the supplementation of extenders with these compounds, confirming they can be used to preserve ram semen quality and fertility. Therefore, the present review provides an updated overview of the damages and associated mechanisms that ram spermatozoa undergo during liquid storage. Moreover, the supplementation of extenders with different compounds as a tool to improve semen storage is also discussed as well as their efficiency to reduce and/or prevent sperm damages during storage.

**Key words:** Antioxidants, Lipid peroxidation, Liquid storage, Ovine, Oxidative stress, Reactive oxygen species

## THE RELATIONSHIPS BETWEEN PIT-1 GENE POLYMORPHISM AND SOME PERFORMANCE TRAITS IN SIMMENTAL AND BROWN SWISS BREEDS

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## Abstract

It was aimed to investigate the genotypic structures of the Pit-1 gene from genomic DNA samples taken from Simmental and Brown Swiss breeds and determine the distribution of genotype and allele frequencies of bovine breeds and to determine the relationships between the detected genotypes and some performance traits. Pit-1/Hinfl gene polymorphisms were determined using the PCR-RFLP method from genomic DNA obtained from Brown Swiss and Simmental breed cattle used in the study. According to the Hardy-Weinberg genetic test, the distribution of genotype frequencies of the studied population was found to be in equilibrium (p>0.05) in both breeds. Genotype frequencies of AA, AB, and BB alleles of the Pit-1 gene were determined as 5.73%, 36.42%, and 57.85%, respectively, for Simmental breed, while the genotype frequencies for Brown Swiss breed were determined as 9.43%, 42.86%, and 48.01%, respectively. The actual milk yield for Simmental breed was determined as 5953 ± 364.2, 5212 ± 993.4, 5507 ± 889.5 kg, the 305-day milk yield as 5953 ± 276.8, 5642 ± 782.7, 5427 ± 246.7 kg, the lactation period as 307  $\pm$  47, 293  $\pm$  29, 303  $\pm$  22 days, the daily milk yield as 19.53  $\pm$  6, 18.49 ± 8, 17.81 ± 2 kg for AA, AB, and BB genotypes, respectively. The actual milk yield for the Brown Swiss breed was determined as 3606 ± 253, 3558 ± 530, 3999 ± 099 kg, the 305-day milk yield as 4920 ± 217, 4462 ± 900, 4635 ± 870 kg, the lactation period as 222 ± 36, 240 ± 04, 258 ± 37 days, the daily milk yield as 17.62 ± 2, 15.49 ± 2, 16.04 ± 3 kg for AA, AB, and BB genotypes, respectively. In the statistical analyses, the relationships between the performance traits studied and Pit-1 genotypes in Simmental and Brown Swiss cattle were insignificant.

Key words: Pit-1, Polymorphism, Simmental, Brown Swiss, Performance traits, PCR-RFLP

## INTRODUCTION

Agriculture and animal husbandry play a vital role in meeting the nutritional needs of people. The main purpose of animal husbandry is profitable breeding with more meat and milk yield per animal. In today's world, there are dairy cattle breeds with high capacity in terms of milk yield and raised widely. In line with the breeding activities carried out in different countries according to ecological environmental conditions, important cattle breeds such as Brown Swiss, Jersey, Guernsey, Ayrshire, Holstein, and Simmental come to the fore (Özek, 2015). Brown Swiss and Simmental breeds are among the most common cattle breeds in Turkey. They are considered robust breeds with high capability to adapt to different conditions and with combined yield traits (Özhan et al., 2012).

While nutrition, genetic factors, breed, and epigenetic factors play a role in mammary gland productivity, the synthesis and secretion of milk are also associated with the lactation process (Qiang et al., 2014). The lactation process varies according to the effects of care, nutrition, and molecular mechanisms. These differences at the molecular level appear as a result of genetic and epigenetic changes. Since it is known that the epigenetic effect plays a significant role in the development of the mammary gland, studies have shown that the Pit-1 gene will be involved in this process (Dincel, 2018). The inhibition of Pit-1 synthesis leads to a significant reduction in the proliferation of PRL, GH-producing cell lines, resulting in a prominent decrease in PRL and GH expression (Beigi et al., 2010; Selvaggi and Dario 2011; Heidari et al., 2012). It has been suggested that the expression of the Pit-1 gene is superior to the growth hormone and prolactin genes and the expression of hormone-specific activators (Scully and Michael, 2000). The Pit-1 gene in cattle, which has a 129-amino acid protein, is a member of the DNA-binding POU family of the homeodomain transcription factor and is sublocalized to the centromeric region of bovine chromosome 1 located between the TGLA57 and RM95 loci (Moddy et al., 1995; Moddy et al., 1996). With QTL detection, 1q21-q22 chromosome region has also been claimed to contribute positively to animal production (Woollard et al., 2000). The Pit-1 gene plays an important role in regulating the growth and development of living creatures (Sobrier, 2016). The Pit-1 gene, which is responsible for the growth hormone gene found in the organisms of mammals, has been reported to be polymorphic using the *Hinfl* enzyme in cattle (Moddy et al., 1996). While it is stated that the use of polymorphic markers in breeding programs can make selection more accurate and efficient (Özdemir, 2012), it has been shown that PRL and GH are affected by the inhibition of Pit-1, causing a decrease in milk production, and the Pit-1 gene has an important potential as a genetic marker to evaluate the traits of milk production (Trakovicka et al., 2014).

Previous studies reported that the B allele of the Hinfi polymorphism in exon 6 of the Pit-1 gene was observed at higher frequencies, the related polymorphism was strongly associated with the protein percentage, and the AB genotype had higher milk yield and milk protein percentage than the AA and BB genotypes in cattle (Zwierzchowski et al., 2002; Vlaic et al., 2007; Yang et al., 2010; Heidari et al., 2012; Hoseinzadeh et al., 2015). Some other studies revealed that the Pit-1 AA genotype and the A allele were associated with high milk yield and protein content (Groza et al., 2005; Viorica, 2006; Lin jia et al., 2009; Hussain, 2016; Bayram et al., 2017). They also showed that polymorphisms in intron 5 of Pit-1 were significantly associated with body weight, average daily profit, and 6, 12, 18, and 24-month chest circumferences, and the A allele could be advantageous in terms of growth traits in Chinese cattle (Tang, 2012). The BB genotype in the Slovak Simmental cow population was reported to have a significant effect on milk performance (Trakovicka et al., 2015). In some studies investigating the relationships between Pit-1-Hinfl polymorphism and milk production traits in cattle, no relationship was observed (Dybus et al., 2004; Aytekin and Boztepe, 2013; Arnim et al., 2017; Özdemir et al., 2018; Zabeel et al., 2018; Pozovnikovaa et al., 2020).

The aim of this study is to examine the genetic structures of the genomic DNA samples taken from Brown Swiss and Simmental cattle raised in two separate private enterprises in Erzurum in terms of the Pit-1 gene locus by the PCR-RFLP method, determine the distribution of genotype and allele frequencies of cattle breeds in terms of the relevant gene, and investigate the relationships between genotypic structures and some performance traits.

#### MATERIALS AND METHODS

The research material consists of blood samples taken from 70 Brown Swiss cattle and 71 Simmental cows raised in private enterprises in Erzurum and genomic DNA obtained from each sample. Primary sequence of the Pit-1 gene (F: 5'-ACT CGC TAT TAC ACA ATA GGA GCC T-3', R: 5'-TCC TGC CAA CTC ACC TCC C-3') designed by Ozdemir (2012), were used to amplify a 260 bp Fragment in cattle. In our study, amplification reactions were performed in a final volume 25 µl containing as seen in the Table 1. PCR amplification was performed at different times under the same conditions (Table 1).

To determine whether amplification took place after the PCR process, the PCR product was run on 1.2% agarose gel at 80 Watt for 25 min. The gel was prepared by adding 0.60 g of Agarose, 50 TBE 1X, and 8  $\mu$ l of ethidium bromide. It was kept in the microwave for 2 minutes and then poured into the cuvette to be loaded with agarose gel. Then, the combs were placed in the cuvette, and 20-25 minutes were waited for freezing and polymerizing. When they were frozen, 8  $\mu$ l of the prepared mixture was loaded into the wells of each comb. During this addition, bromophenol was used for dyeing and placed in the combs. After visualization under UV light, amplified PCR products were stored at -20 °C.

Approximately 10  $\mu$ l of each amplified sample was taken and placed in 0.2 ml sterile Eppendorf tubes. 6-8 units of *Hinfl*, a restriction enzyme for the relevant region (5'-3' recognition region: GA^TC), 6-8  $\mu$ l of RE buffer (buffer R and buffer

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Tango), and 6  $\mu$ l of distilled water were added, and 5  $\mu$ l of Mineral oil was poured. Incubation was carried out at 37 °C for 10-12 hours. Afterward, 3  $\mu$ l of bromophenol dye, a loading buffer, was added to each of the restricted samples. All products were moved around on the parafilm with the help of a micropipette to remove the mineral oil. Of the restricted PCR products, 8 µl was run on 3% agarose gel prepared earlier and placed in combs after its treatment with bromophenol. It was then visualized under UV light after 150 minutes of electrophoresis at 45 Volts. Genotyping processes were completed with the help of standard markers according to the band sizes of the products.

PCR com	ponent		PCR program	
Material	Volume	Temperature	Cycle	Stage
dNTP	1 µl	5 min at 95°C	1 cycle	Start
Таq	0.5-1.0 U		_ 0) 0.0	
MgCl2 PCR buffer	25 μM	50 sec at 60°C	35 cycles	Denaturing
PCR buller Primer F	5 μL(10x)	50 sec at 61°C	35 cycles	Annealing
Primer R	1 μM 1 μM	45 sec at 60°C	35 cycles	Extension
Genomic DNA distilled water	100ng 10 µl	5 min at 72°C	1 cycle	Final extension

Table 1. PCR components of the Pit-1 gene and the PCR program applied

Pit-1 allele frequencies were determined by gene counting. The Chi-square  $(X^2)$  test was carried out to check whether the populations were in Hardy-Weinberg equilibrium or not. Relationships between some yield traits and genotypic structures of Brown Swiss and Simmental cattle raised in private enterprises were investigated. In the study, performance traits such as actual milk yield, 305-day milk yield, daily milk yield, and lactation period were examined. In the analysis of the obtained data, SPSS statistical software was used on the basis of the general linear model (Harvey, 1990). In the yield traits addressed for Brown Swiss and Simmental cattle breeds, the focus was set on external intermittent environmental factors such as genotype, lactation number, and calving season.

The following statistical model was used according to the yield traits in the study.

 $Y_{ijkl}: \mu + a_i + b_j + c_k + e_{ijkl}$ 

Y<sub>ijkl:</sub> The value of any cow's performance traits (actual milk yield, 305-day milk yield, lactation period, and daily milk yield)

μ: population mean

ai: ith genotype effect (i:1-3; AA:1, AB:2, BB:3)

b<sub>j</sub>: effect of j<sup>th</sup> lactation order (j:1-3; 1st lactation:1, 2nd lactation:2, 3rd lactation:3)

 $c_k\!\!:$  Effect of  $k^{th}$  calving season (I:1-2; 1: winterspring, 2: summer-autumn)

eijkl: Marginal error

In the model used, all the factors except the error were considered constant, and the error was accepted to depend randomize.

#### **RESULTS AND DISCUSSION**

PCR was conducted on each DNA sample isolated from the bloods of Simmental and Brown Swiss cattle, and they were run on 1.2% agarose gel to obtain DNA bands. Figure 1 shows the image of PCR products on agarose gel under UV light.

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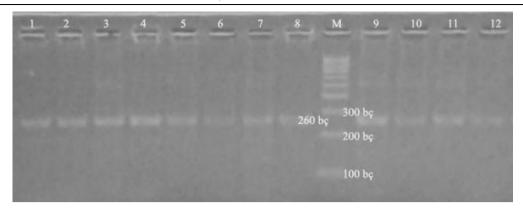


Figure 1: Agarose gel image of PCR products under UV light (M:1000-100 bp, Pit-1:260 bp)

DNA samples obtained from Simmental and Brown Swiss cattle were amplified in the PCR device and restricted with the *Hinfi* restriction endonuclease enzyme. Polymorphic regions of the Pit-1 gene were identified under UV light as a result of the electrophoresis process of 3% agarose gel prepared. Theoretically, bands are produced at the lengths of AA; 260 bp, BB; 190 bp and 70 bp, AB: 260 bp, 190 bp and 70 bp. Figure 2 shows the image of an exemplary agarose gel belonging to the PCR-RFLP result under UV light.

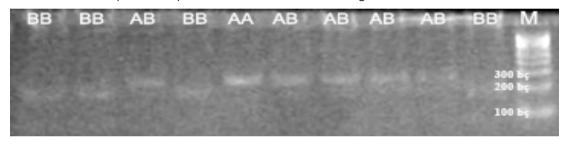


Figure 2: PCR-RFLP gel image of the Pit-1 gene (AA; 260 bp, BB; 190 bp and 70 bp, AB: 260 bp, 190 bp and 70 bp, M: Marker)

In our study, depending on the *Hinfl* restriction site polymorphism of the Pit-1 gene, AA, AB, and BB phenotypes were identified. The identified phenotypes and gene frequencies calculated in % distributions are given in Table 2, and genetic equilibrium control in Table 3. The BB phenotype of the Pit-1 gene constituted the phenotype group observed at a high rate in the Brown Swiss breed by 51.4% and in the Simmental breed by 62.0% (Table 2). The order of the phenotypes was BB>AB>AA and did not change according to the breeds. Gene frequency of the B allele of the Pit-1 gene was determined as 69.29% in the Brown Swiss breed and 76.06% in the Simmental breed. Concerning the previous research on the Pit-1

gene in different cattle breeds, the BB>AB>AA

test analysis results reported in the studies conducted by Rennaville et al. (1997) on Italian Holstein-Friesian cattle breeds, by Dybus et al. (2004) on Polish black and white cows, by Viorica et al. (2007) on the Simmental breed, by Mukesh et al. (2008) on Indian native cattle breeds, by Zhang et al. (2009) on Qinchuan, Limousin x Qinchuan, Angus x Qinchuan, Germany yellow x Qinchuan breeds, by Beigi Nassiri et al. (2010) on the Najdi cattle breed, and by Özdemir (2012) on the Holstein cattle breed were consistent with the results we obtained. The gene frequencies of the phenotype distributions of the Pit-1/Hinfl polymorphism were also consistent with our results.

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Dhanatura	Browr	n Swiss	Simmental		
Phenotype -	n	%	n	%	
AA	9	12.9	7	9.9	
AB	25	35.7	20	28.1	
BB	36	51.4	44	62.0	
Allele Gene Frequency (%)	А	В	А	В	
	30.71	69.29	23.94	76.06	

 Table 2: Genotype Distributions of the Pit-1/Hinfl polymorphism and Allele Gene Frequencies in

 Breeds

The distributions of genotype frequencies of Brown Swiss and Simmental cattle breeds were found to be in equilibrium (p>0.05) according to

the Hardy-Weinberg genetic equilibrium test (Table 3).

**Table 3:** Pit-1 genotype frequencies of Brown Swiss and Simmental cattle and the results of the Hardy-Weinberg genetic equilibrium test

Breeds	Observed		Expected	— X <sup>2</sup> test		
	IN A	AA/AB/BB	AA/AB/BB	- X- lest	F	
Brown Swiss	70	9/25/36	6.60/29.79/33.60	0.1783	NS	
Simmental	71	7/20/44	4.07/25.86/41.07	0.05620	NS	
NC: Noncignificant						

NS: Nonsignificant

For the Pit-1 gene, in the studies conducted by Rennaville et al. (1997) on Italian Friesian cattle, by Dybus et al. (2004) on Black and White Holstein cattle breeds, by Özdemir (2012) on East Anatolian Red and Holstein cattle breeds, by Hussain (2016) on Iraqi crossbred cattle, and by Aytekin and Boztepe (2013) on the Brown Swiss cattle breed, the results of the Hardy-Weinberg genetic equilibrium test were found to be consistent with this study.

Relationships between some performance traits of Pit-1 genotypes, such as actual milk yield, 305day milk yield, lactation period, and daily milk yield, were investigated. The least squares means and standard errors of the Pit-1 gene genotypes in terms of some yield traits are presented in Table 4.

**Table 4:** The least squares means and standard errors of Pit-1 Gene genotypes in terms of some yield traits.

Breed	Genotype	N	Actual Yield			ay Milk d(kg)	Daily Yield			ation d (days)
		_	x 4	⊦ S⊼	x :	± Sxī	x ±	: Sxī	x	± Srī
	AA	15	5488	364.2	5953	276.8	19.53	6.11	307	47
Simmental	AB	23	5212	993.4	5642	782.7	18.49	8.31	293	29
	BB	52	5507	889.5	5427	246.7	17.81	2.70	303	22
Tota	al	90	5464	192.2	5639	450.7	18.65	5.00	304	73
Brown Swiss	AA	18	3606	253	4920	217	17.62	2.91	222	36
DIOMII 20055	AB	62	3583	530	4462	900	15.49	2.27	240	04
	BB	70	3999	99	4635	870	16.04	3.00	258	37
Tota	al	150	160	627	4672	996	16.38	6.25	240	26

The genotypes of the Pit-1 gene were determined as AA, AB, and BB, and the percentage frequencies were calculated as 5.73%,

36.42%, and 57.85% for the Simmental breed and 12.9%, 35.7%, and 51.4% for the Brown Swiss breed, respectively. The frequency of the A allele

was 0.24 and the frequency of the B allele was 0.76 for the Simmental breed. The frequency of the A allele was 0.31 and the frequency of the B allele was 0.69 for the Brown Swiss breed. According to the Hardy-Weinberg genetic equilibrium test, it was observed that the populations examined for both breeds were in equilibrium (p>0.05).

In both breeds, the effect of Pit-1 genotypes on the examined performance traits was not found significant (p>0.05). In the analyses, the AA genotype of the Pit-1 gene was observed to have a higher mean than the AB and BB genotypes in 305-day milk yield. Likewise, the AA genotype was superior to the AB and BB genotypes in daily milk yield. However, the effect of the difference in these genotype means was found to be statistically insignificant (p>0.05)

Upon reviewing the previous similar studies on this subject, Rennaville et al. (1997) found the effect of the A allele on milk yield to be superior to the B allele in their study on Italian Friesian cattle. In their study on Polish Black and White cattle, Zwierzchowski et al. (2002) reported that the A allele had higher milk yield than the B allele. Contrary to these studies, Dybus et al. (2004) stated that they could not detect any relationship between Pit-1 genotype and milk yield traits in Black and White cattle. Similar to our study, Aytekin and Boztepe (2013) reported that there was no relationship between Pit-1 genotypes and 305-day milk yield in the Brown Swiss cattle breed, whereas Dybus et al. (2004) found this relationship to be significant in Polish Black and White cows. Among the studies investigating the relationship between milk yield and the Pit-1 gene in cattle, this relationship was not revealed to be significant in the study of Avtekin and Boztepe, (2013) on the Brown Swiss cattle breed, in the study of Hussain (2016) on cattle breeds in the Iraq region, and finally in the study of Arnim et al. (2017) on Pesisir cattle breeds. Zwierzchowski et al. (2002) and Dybus et al. (2004) reported that the effect of lactation period and Pit-1/Hinfl polymorphism was significant in their studies on black and white cows.

## CONCLUSION

Genotypic structures of the Pit-1 gene polymorphism of each cattle were identified using the PCR-RFLP method on genomic DNA samples obtained from Simmental and Brown Swiss cattle breeds. Both populations were observed to be in genetic equilibrium in terms of the investigated Pit-1 gene polymorphic region. It was revealed that the effects of the detected Pit-1 genotypic structures on all yield traits (actual milk yield, daily milk yield, 305-day milk yield, and lactation period) were not statistically significant. This necessitates such intensive studies on more different breeds and populations.

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# DETERMINATION OF SOME BEHAVIOUR CHARACTERISTICS OF MALE ANATOLIAN BLACK CATTLE

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## Abstract

In this study, it was aimed to examine some behavioural parameters in the four animals of Anatolian Black raised under the conditions of the Institute. In the study, a chip sensor (CowManager) was attached to the ears of the animals. In the study, the ear temperature of the animals was found to be 20.23 °C on average, while it was found to be higher in 2 elder than 1 elderly (P<0.001). High active, active, not active, eating and rumination data in the study were found to be 8.01%, 6.59%, 30.14%, 28.78% and 26.67%, respectively. From these data, high active, active and eating values were found to be higher in the elderly 1, but not active and rumination values were found to be higher in the elderly 2. The differences were found to be not significant in the high active and active values, while was found to be significant (P<0.05) in the not active, eating and rumination values were found to be significant in not active and to be higher in October, but not active, eating and rumination values were found to be not significant in not active and eating values, while was found to be significant (P<0.001) high active, active and rumination values (P<0.001) high active, active and rumination values. As a result, the significant correlation of behavioural data with temperature and THI values (P<0.001) may be an indication that animal behaviour is affected by meteorological events.

Key words: Anatolian Black cattle, Behaviour, Sensor, Active

## BEHAVIOURAL AND PHYSIOLOGICAL DETERMINATION OF TEMPERATURE STRESS IN CATTLE

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#### Abstract

Livestock plays an important role in the global economy. Climate change directly affects animal production. Therefore, animal food security is under threat in many parts of the world. In addition, more than one stressor affects animals directly or indirectly. Among these stresses, heat stress seems to be the most important factor that negatively affects livestock production. Among the environmental variables affecting animals, heat stress is one of the factors that complicates animal production in many parts of the world. Genotypic and phenotypic characteristics are used to determine heat stress in cattle. The response of livestock to heat stress varies according to species, genetic potential, life stage, management or production system and nutritional status. Identifying region-specific, climate- and heat-tolerant animals is critical for sustainable animal production. For this reason, animals must have the genetic ability to resist heat stress in order to sustain animal production under extreme climatic conditions. It is important to determine the responses of animals to stress in order to identify animals with superior genetic characteristics that can be economically beneficial. Adaptation mechanisms of animals against stress may vary according to their morphological, behavioural and genetic capacities. In this study, behavioural and physiological responses of cattle at extreme temperatures were evaluated. In order to determine these responses to stress in animals, factors such as feed-water consumption, lying-standing times, sweating rate, respiration rate, and rectal temperature were investigated.

Key words: Cattle, Stress, Behavioural response, Physiological response

## INTRODUCTION

The placenta is Heat stress is defined as an event that affects the homeostasis and health of the animal due to physiologically harmful heat load (Gaughan et al., 2012). Heat stress causes great economic losses in meat, milk and fertility in cattle. Adverse environmental conditions resulting from heat stress are the main cause of production losses in the global dairy industry, especially in high-yielding cows (-8.9 kg/cow/day milk under heat stress) (Biby, 2010). In the dairy industry, annual economic losses are estimated to be 897-1,500 million dollars (St-Pierre et al., 2003). Another report reported that impact of heat stress on global livestock production is more than 1.2 billion dollars (Baumgard and Rhoads, 2012).

Among the environmental variables affecting animals, heat stress is one of the factors that make animal production difficult in many parts of the world (El-Tarabany et al., 2017). Although it is claimed that cattle raising can be done in any environment, the production must be done in a suitable environment since the aim is an economical and profitable production. Although animals can adapt to climatic stress factors, survival response mechanisms harm their production performance (Pragna et al., 2018). Heat stress affects the growth performance of animals (Baumgard et al., 2012), milk production (Das et al., 2016), reproductive performance (Rhoads et al., 2009), meat production (Archana et al., 2018) and disease occurrence (Rojas-Downing et al., 2017) negatively. The negative effects of heat stress on these functions depend on species and breed differences. The magnitude of this effect determines the adaptation potential of animals. The vulnerability of farm animals to heat stress varies according to species, genetic potential, life stage, management or production system, and nutritional status (Das et al., 2016).

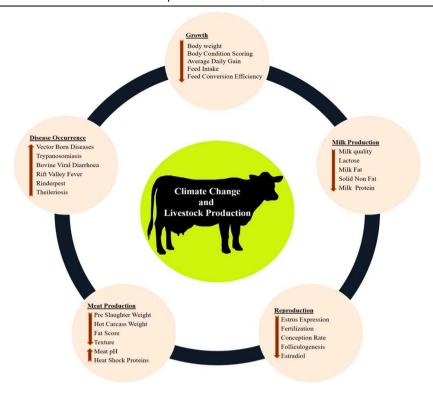


Figure 1. Various impacts of climate change on livestock production

It is thought that knowing the methods to be used in the determination of the effects of heat stress in cattle will contribute to the development of applications that will reduce these effects and mechanisms that will provide permanent endurance (Dincel et al., 2013). Various biomarkers that can be used to measure heat stress responses in animals could cause the way for breed identification. Identified markers can then be incorporated into breeding programs to develop region-specific heattolerant breeds. The various effects of climate change on dairy cattle are presented in Figure 1.

## **ANIMAL REACTIONS**

There are certain phenotypic and genotypic traits that give an animal the potential to adapt, thereby allowing it to cope with harsh conditions. These mechanisms help animals survive in a particular environment (Figure 2). Basically, adaptation can vary in animal according to their morphological, behavioural and genetic capacity. As animals adapt to extreme conditions, they evolve their adaptation mechanisms over generations. This adaptation process is morphological, behavioural, physiological, neuro-endocrine, biochemical, metabolic, molecular and cellular functions in order to survive and provide efficiency in a certain environment (Sejian et al., 2018). In this study, behavioural and physiological responses of cows to the effects of heat stress will be examined.

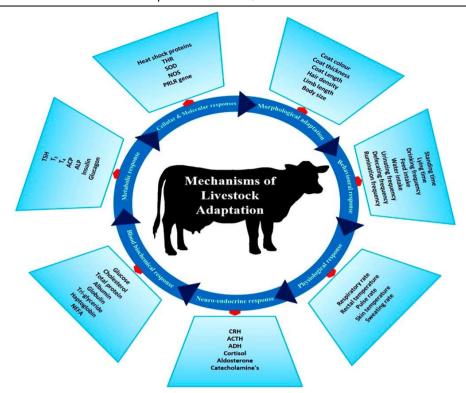


Figure 2. Different adaptive mechanisms of livestock to cope to the harsh climatic condition

## **Behavioural Responses**

Animals exhibit a variety of behavioural responses to adapt to changing environmental conditions (Bayssa et al., 2021). Behavioural changes are considered to be the first and most important response adopted by animals to reduce heat load (Shilja et al., 2016). The most important behavioural responses in cattle are shadow seeking, standing time, feed intake, defecation and urination frequency, water intake and frequency (Ratnakaparan et al., 2017).

**1. Shade seeking:** One of the most rapid and profound behavioural changes seen in heatstressed animals is shade seeking. Stressed animals try to alleviate the negative effects of direct heat load by using shade whenever they can reach it. Studies clearly show that dairy cattle use shade in hot environments and the frequency of this behaviour increases with higher air temperature and solar radiation (Curtis et al., 2017).

**2. Feed and water consumption:** Another behavioural response to stress in ruminants is a decrease in feed intake and use as an adaptive response to regulate metabolic heat production in a warm environment, and a decrease in water excretion by concentrating urine (Valente et al., 2015; Shilja et al., 2016). Shilja et al. (2016) and

Valente et al. (2015) reported reduced feed intake in cattle compared to their counterparts kept under normal conditions. In addition, higher drinking frequency and increased water intake have been reported in various animal species during the summer months (Valente et al., 2015; Shilja et al., 2016). Breeds adapted to desert regions compensate for higher water loss during periods of high heat load by concentrating urine (Chedid et al., 2014). Lower feed intake in warm conditions is defined as an adaptive response to regulate metabolic heat production in heatstressed animals. In order to protect animals from possible heat stress, should be provided warm and easily accessible water in winter, cool water in summer in accordance with body temperature.

**3. Lying/standing time:** It is one of the main behavioural responses of animals to cope with heat stress. In many studies, it has been reported that increased standing and decreased bedtime in animals are associated with higher ambient temperatures (Silanikove, 2000; Darcan et al., 2008; Hienicke et al., 2020). Cows tend to stand more at noon as the temperature increases during the day (Allen et al. 2015; Hienicke et al. 2020). This shows that noon hours should be a very important target to improve cow comfort. In

general, animals under heat stress tend to spend more time standing as they can orient themselves in different directions to avoid direct solar radiation and ground radiation (Sejian et al., 2018; Bayssa et al., 2021). In addition, it has been determined that cows mostly lie down at night to alleviate the effects of heat stress experienced during the daytime. Measuring of laving behaviour can be done visually by observing cows, but this method is laborintensive and therefore expensive. With the technology, development of measuring instruments that can record animal behaviour have begun to be used instead of visual observations. These systems include (CowManager Sensor, Agis, Harmelen, The Netherlands), HOBO Data Logger (HOBO Pendant G Acceleration Data Logger, Onset Computer Corp., Pocasset, MA), CowAlert IceQube (IceRobotics Ltd., Edinburgh, Scotland), The Smartbow (Smartbow GmbH, Jutogasse, Austria) and Track A Cow (ENGS, Rosh Pina, Israel). These systems can also calculate the feedeating and rumination times of animals.

## **Physiological Responses**

1. Sweating or sweating rate: Among the various physiological and behavioural responses, it is one of the first responses to high ambient temperatures. Cows use perspiration as an effective cooling system through evaporative heat transfer (Ji et al., 2020). Sweating rate is highly dependent on the blood flow rate and sweat glands per unit skin area (Blazquez et al., 1994). Measuring skin moisture loss is technically difficult, with highly variable results in the range of 77 to 279 g moisture/m<sup>2</sup>/hr (Blazquez et al., 1994). Rungruang et al., (2014) used devices such as the evapometer (Delfin Technologies Ltd., Kuopio, Finland) to measure the sweating rate of animals. This practice was applied by measuring the rate of perspiration in the shoulder areas of the dairy cow skin and the upper part of the hair that was clipped and shaved with a razor.

**2. Respiratory rate (RR):** It is an early warning indicator of the onset of heat stress and the animal's body can increase the respiratory rate to dissipate excess heat (Ji et al., 2020). Silanikove (2000) reported that RR is a good way to cool the brain under heat stress. Valente et al. (2015) reported an increase in RR when cattle were exposed to high temperature stress in their study in Angus, Nellore and Sahival breeds. Lanham et

al. (1986) reported that RR can drop significantly after drinking water, which in turn can reduce body temperature through conductive cooling of water. The breathing rate is usually given as breaths per minute (Demirören et al., 2002). Monitoring of RR frequency can be accomplished through human observation or by scanning video recordings at fixed time intervals (Scharf et al., 2012). Eigenberg et al. (2005) developed measuring automatic devices (Automatic dataloggers) that fix the data recording system to the animal body. These devices have been applied in research to model the relationship between RR and heat stress (Eigenberg et al., 2005; Brown-Brandl et al., 2005).

3. Heart rate (HR): As the amount of energy used to maintain the body temperature of animals increases, the energy held for productive activities will decrease. Therefore, as heat stress increases in animals, the heart rate or pulse rate will increase. Therefore, heart rate is considered an indirect indicator of heat stress (Demirören et al., 2002; Darcan et al., 2008). The pulse rate can be determined by direct intervention on the animals, by resting the thorax of the animals with a stethoscope, and is calculated by counting the pulse rate in thirty seconds or per minute (Demirören et al., 2002). With the development of technology, devices that can measure these data based on electrocardiograph analysis without disturbing the animals under stress conditions have been developed. Brosh (2007), Kumar and Hancke (2015), and Alphonce et al. (2017) developed commercial systems that can measure the heart rate of animals.

4. Rectal temperature (RT): It is an indicator of body temperature, which is closely related to production performance (Zimbelman et al., 2009). RT remains nearly constant at thermal neutral temperature but tends to increase with increasing ambient temperature (Zimbelman et al., 2009). Church et al. (2014) reported a strong relationship between positive cow eve temperature and rectal temperature. This positive correlation indicated that although eye temperatures are consistently lower than rectal temperatures, heat stress can be reliably estimated by detecting eye temperature. Rectal temperature can be measured with digital thermometers (GLA M500/525/550/700) inserted into the rectum of animals (Demirören et al., 2002; Zimbelman, 2009; Darcan et al., 2008; Ji et al. 2020;). Brown-Brandl et al. (2003) developed a device (1700 Brannum Lane, Yellow Springs, OH., USA) that is inserted approximately 20 cm deep into the rectum of animals and can record at one-minute intervals.

**5. Skin temperature (ST):** A reliable indicator of temperature stress and thermal balance in animals (Sejian et al., 2018). However, animals must be transported to facilities specifically designed to measure body temperature. To overcome all these challenges, advanced temperature measurement techniques such as infrared thermometers are used in place of conventional contact temperature measuring devices. Infrared thermal devices that can measure the skin temperature of animals have been developed by some researchers (Scharf et

al., 2012; Banhazi et al., 2009). With these devices, temperatures can be measured from different parts of the animals such as shoulders, waist, rump and breast.

6. Vaginal temperature (VT): It is another method of measuring neutral core body temperature of animals. Technological devices capable of measuring have also been developed, as measurable by contact to the animal. Nabenishi et al. (2011) developed a thermal sensor (Thermochron, SL, KN Laboratories, Osaka, Japan) that can measure the vaginal temperature of animals. In addition, Schütz et al. (2009) can measure temperature with an intravaginal drug delivery device (CIDRTM, InterAg, Hamilton, New Zealand).

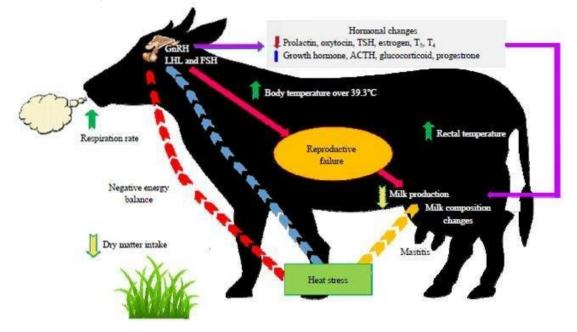


Figure 3. Impact of heat stress on milk production in dairy cattle

# CONCLUSIONS

To manually measure temperature stress in animals, it is necessary to contact animals. With manual measurement, the frequency of data collection is limited, that is, measurements can be made at certain periods and hours. Human observation can add uncertainty to the collected data, causing errors and inconsistencies. It may also put the animals under extra stress during manual detection. Considering all these negative effects, the use of technological devices instead of manual measuring devices comes to the fore in determining the responses of cows to heat stress. In the climate change scenario, increased temperature and relative humidity will create heat stress on all livestock species and adversely affect their productivity and reproductive potential, especially in dairy cattle. An urgent need for livestock researchers aiming to counter the effects of heat stress on animals is an understanding of the biology of heat stress responses. This can provide breeders with information to identify when an animal is under stress and when it needs attention.

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# GENETIC DIVERSITY AND PHYLOGENETICS STUDY OF ACTIVIN GENE IN GUINEA FOWL AND OTHER AVIAN

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#### Abstract

Activin gene has been reported to have implication in cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair, and endocrine function. Bioinformatics analysis was done to analyze the sequence of activin gene in avians (turkey, chicken, and guinea fowl). Fifteen (15) nucleotide and amino acid sequence were retrieved from Genbank at Center for Biotechnology Federal University of Agriculture Abeokuta, using software tools (Clustal W, dnaSP, MEGA and BLAST) to determine the percent identity, similarity in function and evolutionary relationship of activin gene. The result obtained showed that percent identity and similarity of activin gene in avian ranged from 65 -99% and were similar in function. Chicken has the highest haplotype number (4) indicating that activin gene sequences of avian were grouped into the same taxonomy. Chicken and quail shared most recent common ancestor and were closely related than activin gene of turkey in the phylogenetic tree showed a relative relatedness of those avian and genetic function of activin gene.

Key words: phylogenetics, genetic diversity, Activin gene, evolutionary trend, bioinformatics

#### INTRODUCTION

Phylogenetics is the study of evolutionary relationships among biological entities often species, individuals or genes. Phylogenetics is important because it enriches our understanding of how genes, genomes, species (and molecular sequences more generally) evolve (Nazie, 2013).

Genetic diversity is the variation in the amount of genetic information within and among individuals of a population, an assemblage or a community. Diversity is another word for variation: the presence of differences among whatever you consider. Related to genetics, most obvious is the genetic diversity between populations (Boniface et al., 2016).

Bioinformatics and insilico genetics is both an umbrella term for the body of biological studies that use computer programming as part of their methodology, as well as a reference to specific analysis "pipelines" that are repeatedly used, particularly in the field of genomics (Mount, 2004).

The aim of this study was to carry out an insilico analysis of activin gene in guinea fowl and in some avian species, to determine the percentage identity and percentage similarity of activin gene between guinea fowl and some avian species. In addition, the evolutionary relationship and genetic diversity of activin gene nucleotide sequence in guinea fowl and some avian were determined

#### MATERIAL AND METHOD

The study was carried out at Center for Biotechnology Federal University of Agriculture Abeokuta, Fifteen (15) nucleotide and amino acid sequences of activin gene belonging to guinea fowl and other avian were retrieved from the Genbank. Experimental procedure was done by obtaining the FAST alignment format of nucleotide and amino acid sequence of species

the National Center for from gene at Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov), with the use of Basic local alignment search tool (BLAST) to obtain their similar sequence. Multiple sequence alignment was carried out to obtain sequence using Cluster W Software incorporated in molecular evolution and genetic analysis software (MEGA). Multiple sequence alignment and genetic diversity was carried out on all the sequence using Clustal W software (Crawford, 2003) incorporated in Molecular Evolution and Genetics Analysis Software (MEGA, version 6). The genetic diversity indices such as number of polymorphic sites, number of monomorphic sites, haplotype number, haplotype diversity, nucleotide substitution per site, parsimony singleton informative site. variable and conservation of activin gene of guinea fowl and other avian was determine using dnaSP Software. The percent identity and similarity among the amino acid sequence of activin gene of guinea fowl and other avian were carried out by conducting a pair wise comparison of two or more sequences via Basic local alignment search tool (BLAST) incorporated in NCBI website. The phylogenetic relationship among the activin gene of guinea fowl and other avian was determined using the Molecular Evolution and Analysis Genetic (MEGA) software. А phylogenetic tree was constructed using the Neighbour Joining statistical method, to

determine the evolutionary relationship of activin gene sequence among avian species.

#### **RESULT AND DISCUSSION**

The length of the nucleotide sequence of the activin gene varied from 381 base pairs to 2637 base pairs while the length of the amino acid sequence varied from 315 amino acid residues to 421 amino acid residues. The gene sequence for chicken, turkey, and guinea fowl activin gene contained; 948, 2859, and 2764 base pairs respectively. The length of the amino acid sequence of chicken, turkey and guinea fowl contained 315, 352 and 421 amino acid residues respectively. The shortest activin nucleotide sequence (948 base pairs) was observed in chicken while the longest activin nucleotide sequence (2764 base pairs) was observed in guinea fowl. The shortest amino acids sequence (315 amino acids) was observe in chicken while the longest amino acids sequence (421 amino acids) was observe in guinea fowl.

The below table shows that nucleotide of activin gene of chicken is 4 whereas the nucleotide sequence length is 948 and amino sequence length 315. Across the table it can be observed that morphology is directly related with increase in nucleotide sequence length; as turkey has highest nucleotide sequence length followed by guinea fowl and quail respectively while chicken has the least.

With their t		in numbers and sequence	e tength		
Species	Ν	Genbank	Location	nucleotide	amino
-		accession number		sequence length	acid length
Chicken	4	NC_006094.5	Chromosome 7	948	315
Turkey	2	NC_015017.2	Chromosome 7	2859	352
Guinea	2	NC_034413.1	Chromosome 5	2764	421

**Table 1.** Retrieved nucleotide and amino acid sequences of the activin gene of the selected species with their accession numbers and sequence length

The percent identity of chicken, turkey and guinea fowl activin gene and other avian species are shown in table 4. From the result obtained using comparative sequence analysis, it was revealed that the activin gene of chicken, turkey and guinea fowl shared percent identity ranging from 65 to 99%. It was obtained that chicken and turkey shared 85% identity, while turkey and guinea fowl also shared 99% identity.

fowl

Percent identity refers to a quantitative measurement of the similarity between two sequences (DNA, amino acid or otherwise). Closely related species are expected to have a higher percent identity for a given sequence than would more distantly related species, and thus percent identity to a degree reflects relatedness, (Pearson, 2013). From the result, it was obtained that chicken, turkey and guinea fowl had identity of 85%, 86% and 99% respectively.

	Chicken	Turkey	Guinea fowl	Duck	Dove
Chicken	-				
Turkey	85				
Guinea fowl	86	99			
Quail	99	85	86		
Duck	75	65	71		
Dove	75	65	70	99	-

**Table 2.** Percent identity among turkey, chicken and quail activin nucleotide sequence and the activin nucleotide sequences of other avian species

Chicken and turkey shared 85% identity of activin gene; an indication that the evolutionary relationship between chicken and turkey is moderately high. However, chicken and guinea fowl shared 86% identity which is 1% higher than that of chicken: turkey relationship and is therefore higher in evolutionary relationship than the former and can perform same biological function (promoting follicular dominance) by recruiting folliculogenesis during each ovarian cycle (Hiller et al., 2000). This further buttress that they come from same ancestral line. Chicken and quail are highly related in evolutionary line stemming from same most common recent ancestor. Chicken share same percent identity of 75% with both dove and duck.

Nevertheless turkey and guinea fowl is high in percent identity of activin gene which implies that these two species are closely related in the evolutionary tree and the gene can perform same biological function (increasing fertility potential) in both species by breaking down germ cell nest (Bristol et al., 2006). Turkey and quail share percent identity of activin gene 86% although closely related in ancestral line but the relatedness can't be compared with that of turkey: guinea fowl relationship in both biological function and evolutionary relationship. Guinea fowl share percent identity of 86% with quail, closely related in ancestral line more than duck and dove which shares identity of 71% and 70% respectively. They all function similarly in reducing the sensitivity of ageing animals to the effect of dietary restriction. From the result above it was observed and stated that chicken shared most common recent ancestor (99%) with quail than other avian species, turkey shared most common recent ancestor with guinea fowl than other species and guinea fowl shared most common recent ancestor with quail than other species.

The result in table 3, revealed that chicken, turkey and guinea fowl values of percentage similarities in function of the activin gene falls within the range of 70-100%, meaning that the activin protein within which each avian evolved is as a result of divergence. Percent similarity is homology among proteins or DNA which is typically inferred from their sequence similarity (Welt et al., 2002). From the above result chicken has percent similarity of 95% with turkey and guinea fowl which means they are more closely related in evolutionary role and biological function (promoting oligodendrocyte development and CNS myelination) of activin gene in both species (Welt et al., 2002). Whereas chicken and quail has similarity of 100% which is certainly higher than that of turkey and guinea fowl, this implies that chicken and quail are from most common recent ancestor closely related in function and specificity (regulating both morphogenesis of branching organ such as lungs and most especially kidney). Chicken shares similarity of 78% with both duck and dove; which is certainly lower in ancestral relationship than that of chicken with turkey and guinea fowl.

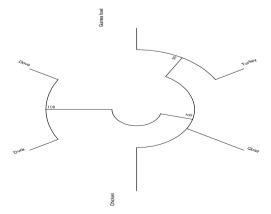
Turkey and guinea fowl has similarity of 100% which is certainly higher than that of quail duck and dove, this implies that turkey and guinea fowl are from most common recent ancestor closely related in both function and specificity than other species (regulating proliferation and adhesion of hemocytes) NCBI links: precomputed BLAST. Guinea fowl shared similarity of 85% with quail higher than that of duck and dove. Summarily, chicken shared 100% similarity with guinea fowl while guinea fowl shared 85 % similarity with quail.

The evolutionary relationship among chicken, turkey and guinea fowl activin gene and activin of other avian are shown in figure 4. The result showed an optimal tree (figure 1) with the sum of branch length = 1.10475583. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The above shows the evolutionary relationship of avian activin gene as they shared various percent identity and similarity showing the ancestral relatedness and similarity in biological functions respectively. This equally gives the breeder an insight in the various functions and how diversely activin gene can be used to improve each species.

**Table 3.** Percent similarity among turkey, chicken and guinea fowl activin nucleotide sequence and the activin nucleotide sequences of other species

	Chicken	Turkey	Guinea fowl	Duck	Dove
Chicken	-				
Turkey	95				
Guinea fowl	95	100			
Quail	100	95	85		
Duck	78	70	77		
Dove	78	72	71	100	-

Phylogenetic analysis can be used to discover gene preservation events which are conservation between modern and classic methods. Modern phylogeny (shows genetic material which is DNA and protein sequence) that frequently cause them to group together rather than classic method that deals with physical or morphological features (Hogde and Cope, 2000). The phylogenetic tree in figure 1 showed the evolutional relationship between guinea fowl, turkey, chicken and quail. The avian species had a common ancestor because they were on the same phylogenic tree branch which confirms the morphological classification of chicken, turkey and guinea fowl as aves (non-ruminant) animals and agrees with the taxonomy of National center for biotechnology information (NCBI) even though some are more closely related than others.



**Figure 1.** A phylogram showing the evolutionary relationship of avian activin gene

Chicken and quail are classified under the same clade (group of animals or organisms derived from a common ancestor species), they share most recent common ancestor, more closely related and highly similar than others because the distance between the length of the clade are more close compare to other avian in the phylogenetic tree. Although chicken and turkey are in the same clade but they did not share most recent common ancestor because the distance between the lengths of the clade is high compare to that of the chicken and quail; this can be as a result of changes that might occur in evolution or little changes in the coding sequence of activin gene. Kang et al., (2008) in his work showed that chicken and turkey showed similarities but did not share most recent common ancestor. Guinea fowl is more closely related to turkey than chicken and quail but closely related to chicken than quail because they have the highest clade distance in the phylogenetic tree. Evolutionary tree shows how two or more organisms are derived or comes to existences and not how similar they are in function (Egom et al., 2019). This result shows that avian activin gene can adapt to similar environmental conditions and also can have close external resemblance. Evolutionary relationship analysis helps the breeders to understand how species of activin gene in avians are related in order to use the knowledge to identify species that can give the same phenotype .Since their genes are closely related,

it also enable farmers to identify individuals or population of animals that can adapt to similar environmental condition.

Genetic diversity is useful especially for selecting animals with high reproductive efficiency and fertility potential which are most preferred by ranchers. From the result of genetic diversity study in table 4, it was obtained that chicken had the highest haplotype diversity of 0.1903, followed by guinea fowl (0.0671), quail (0.0500) and turkey (0.0241). Hence, the results suggest that genetic diversity of activin gene will be more in chicken and least in turkey, with that of guinea fowl and quail recording a relatively higher level of diversity than turkey. Diversity in activin gene may lead to variation in the expression of function with chicken having more of such variation.

	Н	Hd	ND	$\pi_s$	$\pi_n$	S
Chicken	4	0.1903	0.59142	0.6543	0.5472	295
Turkey	2	0.0241	0.47527	0.5317	0.3950	57
Guinea fowl	2	0.0671	0.55085	0.4099	0.4831	35

h- Number of haplotypes, Hd- haplotype diversity, ND- Nucleotide diversity, πs- Synonymous nucleotide diversity, πn- Non synonymous nucleotide diversity, S- Number of polymorphic sites.

Egom et al., (2019) reported that diversity in avian IGF-1 (insulin growth factor-1) gene gave rise to variation in the gene, which is a useful tool in evolution and natural selection. Therefore, it can be said that chicken activin gene has high adaptability and can survive the changing environmental condition since diversity helps ensure survival of species. Thus, although these species are closely related from their phylogeny, activin gene will perform more biological function in chicken than in other avian species studied.

The highest nucleotide diversity value (0.59142) seen in chicken shows that there is more genetic variation in the activin nucleotide sequence of chicken than in turkey and guinea fowl. Synonymously nucleotide diversity of activin in chicken, turkey and guinea fowl which are 0.6543, 0.5317 and 0.4099 shows that at natural selection deleterious and lethal alleles are eliminated because synonymous mutation is strong in the gene. Out of the 948, 2859 and 2764 nucleotide sequence of chicken, turkey and guinea fowl, only 295, 57 and 35 polymorphic sites were identified for chicken, turkey and guinea fowl respectively. With chicken having the highest number of polymorphic sites compared to the nucleotide sequence length, this shows that nucleotide polymorphism occurs at the highest number of sites in chicken activin. The higher the base pair alignment the higher the polymorphism and the higher the gene mutation. In breeding and selection of activin gene for ovarian folliculogenesis and increased

fertility potential, the mutant gene is preferable, hence chicken activin gene will probably have higher level of mutant genes compared to turkey and guinea fowl.

# CONCLUSION

From the result obtained, percent identity and similarity in function of ACTIVIN gene of guinea fowl, turkey, and chicken were in the range of 70-100 percent, indicating that activin gene of avian possesses similar functions, well conserved and are very effective in performing functions like increasing FSH binding, FSH-induced aromatization, improves wound healing and scar formation, regulates enhances morphogenesis of branching organs, enhances ovarian folliculogenesis. The phylogenetic relationship of activin gene in the three of species studied supports taxonomy classification of NCBI. The study also showed that activin gene of chicken, turkey, and guinea fowl also originated from a common ancestor with turkey and guinea fowl sharing a most recent common ancestor. From this study, it can be recommended that since the effect of activin gene in avian promotes ovarian folliculogenesis that enhances reproduction; farmers can select and breed for activin gene in order to promote reproductive efficiency thereby barricading species extinction. Activin gene sequences available in the Genbank were from exotic breeds. It is necessary to carry out research on local breeds of chicken, guinea fowl and turkey so that their sequence can also be available in the Genbank for comparative analysis of local and exotic breeds of the three avian.

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# EFFECT OF INCUBATION TYPE OF FEED SAMPLES ON *IN VITRO* GAS PRODUCTION AND METHANE PRODUCTION

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## Abstract

The aim of the current experiment was to determine the effect of incubation type of feed samples on in vitro gas production and methane production. Alfalfa hay and maize grain were incubated within filter bags or directly dispersedly in buffered rumen fluid for 24 h incubation. The type of incubation of samples had a significant effect on the in vitro gas and methane production. The incubating of feed samples within filter bags reduced not only gas production but also methane production. Therefore, incubating of feed samples within filter bags is not appropriate when measuring methane emission during 24 h of incubation.

Key words: Gas Production, Ancom, In vitrio, Alfaalfa

# INTRODUCTION

In vitro gas production method is widely used to determine gas production kinetics, methane production, metabolisable energy and organic matter digestibility (Kamalak and Canbolat (2010), Canbolat, (2012), Ramin et al., (2013), Kaplan et al., (2014a, b). Generally, feed samples were incubated directly dispersedly in buffered rumen fluid. On the other hand, recently samples have been started to be incubated in F57 filter bags to determine true dry matter digestibility and gas production. However, it was suggested that incubation of samples within filter bags may reduce the gas and methane production due to restriction of microbial digestion (Noziere and Michalet-Doreau, 2000). Therefore, the aim of the current experiment was to determine the effect of incubation type of feed samples on in vitro gas production and methane production.

# MATERIALS AND METHODS

Alfalfa hay and maize grain samples of 200 mg milled through a 1 mm sievewas weighed directly or F57 filter bags (ANKOM Technology Corp, Macedon NY) in triplicate into calibrated glass syringes of 100 mL following the procedures of Menke et al., (1979). The syringes were prewarmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60 %) and concentrate (40%). Gas production was recorded at 24 h after incubation and corrected for blank incubation. Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methaneanalyzer (Sensor Europe GmbH, Erkrath, Germany) (12). Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) X Percentage of methane (%)

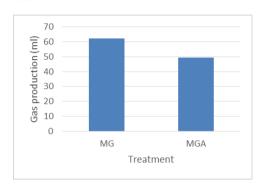
#### **RESULTS AND DISCUSSION**

The type of incubation of samples on gas and methane production was given in Table 1 and Figures 1-6. Type of incubation of samples had a significant effect on the *in vitro* gas and methane production. The gas and methane productions of maize grain and alfalfa hay were significantly lower when they are incubated in filter bags. The reduction in gas and methane production is possibly associated with the restriction for microbial digestion. The filter bag can act as a physical barrier for micro-organism or end products (Noziere and Michalet-Doreau, 2000). These results are consistent with findings of Ramin et al., (2013) who found that gas and methane production decreased when samples were incubated in filter bags for 48 h incubation.

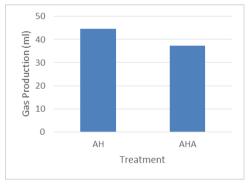
	Gas	(ml)		Metha	ne (ml)		Metha	ne (%)	
	D	WB		D	WB		D	WB	
MG	62.17ª	49.48 <sup>b</sup>	***	8.64ª	4.80 <sup>b</sup>	***	13.87ª	9.74 <sup>b</sup>	***
AH	44.76ª	33.30 <sup>b</sup>	***	7.60ª	5.21 <sup>b</sup>	***	16.97ª	13.97 <sup>b</sup>	***

**Table 1.** Effect of incubation type on *in vitro* gas production and methane production

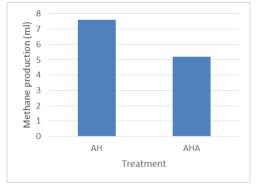
<sup>ab</sup>Row means with common superscripts do not differ (P<0.05); AH –Alfalfa hay, MG- Maize grain, D- Dispersed, WB- Within bag \*\*\*P<0.001



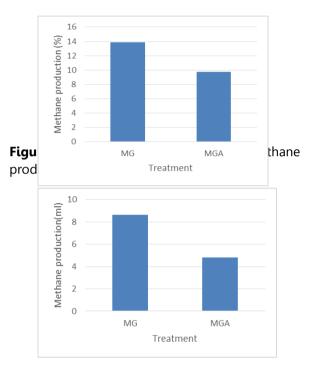
**Figure 1.** Effect of incubation type on *in vitro* gas production of maize grain

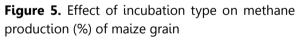


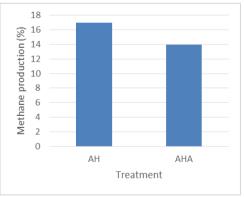
**Figure 2.** Effect of incubation type on *in vitro* gas production of alfalfa hay



**Figure 3.** Effect of incubation type on methane production (ml) of maize grain







**Figure 6.** Effect of incubation type on methane production (%) of alfalfa hay

# CONCLUSIONS

The incubating of feed samples within filter bags reduced not only gas production but also methane production.Therefore, incubating of feed samples within filter bags is not appropriate when measuring methane emission during 24 h of incubation.

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# METRIC CHARACTERISTICS OF THE BANYO GUDALI ZEBU (BOS INDICUS) CATTLE IN THE HIGH GUINEAN SAVANNAH ZONE OF CAMEROON

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## Abstract

This study aimed at evaluating the genetic diversity of local cattle breeds in Cameroon. To this end, biometric data were collected randomly in seven (7) localities of the Mayo-Banyo Division, on a sample of 321 adult Banyo Gudali zebu cattle (234 cows and 87 bulls) aged between 6 and 16 years and having a body condition score varying from 2 to 4. The following mean body measurements (in cm) were obtained: height at withers (129.34  $\pm$  0.50), height at sacrum (134.56  $\pm$  0.37), chest depth (67.37) ± 0.33), head length (47.62 ± 0.42), forehead length (20.33 ± 0.20), horn length (34.45 ± 1.56), ear length (22.66 ± 0.26), body length (187.74 ± 2.45), trunk length (130.5 ± 1.27), scapulo-ischial length (137.88 ± 1.33), pelvic length (42.87 ± 0.31), tail length (100.52 ± 0.88), pelvis width (38.31 ± 0.32), face width (17.61 ± 0.27), muzzle circumference (44.60 ± 0.44), chest circumference (167.81 ± 1, 46), barrel circumference (18.60 ± 0.16), hock circumference (38.72 ± 0.35), hump circumference (73.37 ± 1.92) and live weight (350.24 ± 8.70). The main biometric indices are: the massiveness (2.69 ± 0.06 kg/cm), proportionality (94.42 ± 1.06), cephalic (37.13 ± 0.55), body profile (0.69 ± 0.01), surface area (1.79 ± 0.02m 2), format (1.44 ± 0.01), scapulo-ischial (1.06 ± 0.01), dactylo-thoracic (0.11 ± 0.00), of thoracic development (0.77 ± 0.00) and ossature (0.14 ± 0.00) indices. Bulls and animals reared in controlled systems showed a large size. The discriminant factor analysis identified three morphometric types identifiable with two phenotypes.

Key words: Gudali, Biometry, Biometric index, Cameroon

# MULTIDIMENSIONAL SCALING ANALYSIS AND AN APPLICATION

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## Abstract

The aim of this study is to determine the factors affecting student's failure. For this, a survey study was carried out. In the questionnaire applied to people of all ages and occupations living in Turkey between 2013 and 2015, inferences were made on the answers of 4183 people, 2928 of whom were men and 1255 were women, regarding the relationship between 26 variables that are closely related to the success factor. In this study, non-metric multidimensional scaling technique was used considering the Euclidean distance. This is because the data is not quantitative and smaller size solutions can be obtained. As a result of the analysis, Kruskal's Stress Value was observed as .00 and no significant difference was observed between the variables. Therefore, the observation of stress values in the range of (0-0.025) clearly reveals that there is a "complete" agreement between the variables. Another one words, the measure of the actual figure compatibility chart obtained S =0.00 and shows full compliance. In this case, we can say that the results obtained adequately reflect the data set we have.

**Key words:** Multidimensional scaling analysis, Metric multidimensional scaling, Non-metric multidimensional scaling, Stress value

# EFFECT OF MATURITY ON CHEMICAL COMPOSITION, NUTRITIVE VALUE AND METHANE PRODUCTION OF LEAVES OF *LAURUS NOBILIS SHRUB*

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## Abstract

The aim of the current study was to determine the effect of maturity on the chemical composition, metabolisable energy (ME) and organic matter digestibility (OMD), gas and methane (CH4) production of Laurus nobilis leaves. There are significant variations among Laurus nobilis leaves at different maturity in terms of the chemical composition, gas production, CH4 production ME and OMD values. The CP contents of Laurus nobilis leaves ranged from 6.40 to 16.25%. The CP contents of young and old leaves of Laurus nobilis are sufficient to meet minimum level of CP requirement although senescence leaves are not sufficient to meet minimum level of CP requirement. Therefore, protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of senescence leaves were included into ruminant diets. The ME energy and OMD values of Laurus nobilis leaves ranged from 13.17 to 13.92 %. Laurus nobilis leaves had a low antimethanogenic potential. Laurus nobilis leaves may be used as a feed additive in ruminant diets to decrease the enteric methane production. However, it should be tested in vivo before large implication.

Key words: Laurus nobilis, chemical composition, gas production, methane, nutritive value

#### INTRODUCTION

Shrub and tree leaves are very important feed sources to provide energy, protein and mineral requirements for small ruminant animals in the most parts of world (Kamalak et al., 2010; Kaya and Kamalak 2012). Laurus nobilis is an evergreen shrub and native to the Mediterranean region of Turkey. Leaves of Laurus nobilis are not only used for human nutrition but also freshly used to meet sheep and goats requirement during the critical periods of the year in the Mediterranean part of Turkey in a cut and carry system. Although there are several researches carried out on the chemical composition and nutritive value of Laurus nobilis leaves (Ulger 2017), the information on the effect of maturity on the chemical composition, in vitro gas production, metabolisable energy and organic matter digestibility and antimethanogenic potential of leaves of Laurus nobilis is limited. Chemical composition in combination with in vitro gas production technique has been used to evaluate the potential nutritive value and anti-methanogenic potential of previously uninvestigated alternative feed sources used in ruminant diets. (Kamalak et al., 2011; Canbolat 2012; Guven 2012; Kaya and Kamalak 2012). Therefore, the aim of the current study was to determine the effect of maturity on the chemical composition, metabolisable energy (ME) and organic matter digestibility (OMD), gas and methane (CH4) production of Laurus nobilis leaves.

#### MATERIALS AND METHODS

Young, old and senescent leaves from *Laurus nobilis* were collected in, May and June 2020 by hand from at least 10 shrubs in Kahramanmaras,

Turkey. Leave samples were pooled and dried at 65 °C using a forced air oven. Leaves were ground using a laboratory mill with 1 mm screen size for subsequent analysis. Dry matter (DM) of leave samples was determined by drying the samples at 105 °C overnight and ash by igniting the samples in muffle furnace at 525 °C for 8 h. Nitrogen (N) content of leaves was measured by the Kieldahl method (AOAC, 1990). Crude protein content of leaves was calculated as N X 6.25. Ether extract contents of leaves were determined according to AOAC (1990). Cell wall contents (NDF, and ADF) were determined by the method of Van Soest et al., (1991). Condensed tannin contents of samples were determined by butanol-HCl method as described by Makkar et al., (1995). All chemical analyses of samples were carried out in triplicate.

## In vitro gas and methane production

Approximately 0.500 gram leave samples of *Laurus nobilis* were incubated with buffered rumen fluid for 24 h in a water bath at 39 °C in triplicate using the methods of Menke et al., (1979). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60 %) and concentrate (40 %). Net gas productions of samples were determined at 24 h after incubation and corrected for blank and hay standard (University of Hohenheim, Germany).

Methane contents (%) of total gas produced at 24 h fermentation of leave samples were measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). After measuring gas produced at 24 h incubation, gas samples were transferred into inlet of the infrared methane analyzer with the plastics syringe. The infrared methane analyzer displays methane as percent of total gas. Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) X Percentage of Methane (%)

The metabolisable energy (MJ/kg DM) and organic matter digestibility (OMD) of leave samples was calculated using equations of Menke and Steingass (1988) as follows:

 $ME (MJ/kg DM) = 2.20 + 0.1357 GP + 0.057 CP + 0.002859EE^{2}$ 

OMD (%) = 15.38 + 0.843GP + 0.595 CP + 0.675CA Where, GP is 24 h net gas production (ml/200 mg), CP = Crude protein (%) EE: Ether extract (%) CA: Crude ash (%) One-way analysis of variance (ANOVA) was used to determine the effect of maturity on the chemical composition, metabolisable energy, organic matter digestibility, gas and methane production of Laurus nobilis leaves. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at p<0.05.

## **RESULTS AND DISCUSSION**

Effect of maturity on the chemical composition of *Laurus nobilis* leaves is given in Table 1. Maturity had a significant effect on the chemical composition of *Laurus nobilis* leaves. The CP contents of *Laurus nobilis* leaves ranged from 6.40 to 16.25. The young leaf of *Laurus nobilis* shrub is significantly higher than those of old and senescent leaves. Van Soest (1994) suggested that CP content browse species should be higher than the minimum level of 7-8% of DM for optimum rumen function and feed intake in ruminant animals.

The CP contents of old and young leaves were higher than minimum level of CP requirement of ruminant animal whereas the CP content of senescent leaves is not sufficient to meet minimum level of CP requirement. Therefore, protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of *senescent* leaves were included into ruminant diets. The cell wall contents (NDF and ADF) of Laurus nobilis leaves ranged from 32.91 to 45.70% and 23.83 to 30.50% respectively. The EE contents ranged from 3.9 to 5.7 %. The senescent leaves have a significantly higher EE content than those of young and old leaves The CT content of Laurus nobilis leaves ranged from 11.23 to 17.34 %. The CT content of young leaves significantly higher than those of old and senescent leaves.

Parameters	Young	Old	Senescent	SEM	p
DM (% of fresh)	22.78 <sup>b</sup>	54.68ª	53.44ª	0.518	0.001
CA (% of DM)	4.97°	5.37 <sup>b</sup>	6.71ª	0.633	0.001
CP (% of DM)	16.25ª	9.68 <sup>b</sup>	6.40 <sup>c</sup>	0.495	0.001
NDF (% of DM)	32.91 <sup>c</sup>	48.47ª	45.70 <sup>b</sup>	0.402	0.001
ADF (% of DM)	23.83 <sup>c</sup>	30.50ª	30.02ª	0.522	0.001
EE (% of DM)	2.67 <sup>c</sup>	5.11 <sup>b</sup>	11.11ª	0.775	0.001
CT (% of DM)	17.34ª	11.23ª	12.56 <sup>b</sup>	0.172	0.001

<b>Table 1</b> . Effect of maturity on chemical composition of <i>Laurus nobilis</i> leaves	Table 1. Effect of maturity	on chemical composition	of <i>Laurus nobilis</i> leaves
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abc Row means with common superscripts do not differ (p>0.05); SEM: Standard error mean

(%), CA: Crude ash (%), CP: Crude protein (%), NDF: neutral detergent fiber (%), ADF: Acid detergent fiber (%), EE: Ether extract (%), CT: Condensed tannin (%)

# Effect of maturity on *in vitro* gas production, methane production, metabolizable energy and organic matter digestibility of *Laurus nobilis leaves*

The effect of maturity on *in vitro* gas production, methane production, ME and OMD of *Laurus nobilis* leaves is given in Table 2. Maturity had a significant effect on gas production, methane production, ME and OMD whereas maturity had no effect on the percentage of methane in gas of *Laurus nobilis* leaves.

The gas and methane production ranged from 64.88 to 72.2 ml and 8.58 to 10.05 ml. respectively. The gas and methane production of the old leaves was significantly higher than those of young and senescence leaves. The ME and OMD ranged from 6.44 to 6.81 MJ/kg and 45.59 to 51.20% respectively. The ME and OMD of the senescence leaves are significantly lower than those of young and old leaves of *Laurus nobilis* shrub. The similar observation has been obtained *by* Sagocak (2011) who showed that the ME and OMD of *Arbutus adrachne* leaves decreased with increasing maturity due to increase in cell wall contents and CT contents with increasing

maturity. It is well known that chemical composition of leaves had a significant effect on the gas, methane, OMD and ME values (Kamalak 2006; Karabulut et al., 2007; Kamalak et al., 2010). Especially tannin contents of tree leaves have a significant effect on these parameters. Tannin in leaves can exert their negative effects in digestibility through combining with dietary protein or microbial enzymes (Singleton 1981; Silanikove et al., 1996).

Lopez et al., (2010) suggested that CH<sub>4</sub> percentage of total gas produced after 24-hour fermentation can be used to determine the CH<sub>4</sub> reduction potential of any feedstuffs and the feedstuffs can be classified in three groups, low potential (% methane in gas between >11% and  $\leq$ 14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore, it seems to be likely that *Laurus nobilis* leaves obtained at three maturity stages had low methane reduction potential since the percentages of methane for *Laurus nobilis* leaves are between >11% and  $\leq$ 14%.

**Table 2**. The effect of maturity on *in vitro* gas production, methane production, metabolisable energy and organic matter digestibility of *Laurus nobilis* leaves (n=4)

		Maturity			
Parameters	I		III	SEM	p
Gas(ml)	67.62 <sup>b</sup>	72.28ª	64.88 <sup>b</sup>	1.454	0.002
CH4 (ml)	8.90 <sup>b</sup>	10.05ª	8.58 <sup>b</sup>	0.392	0.011
CH <sub>4</sub> (%)	13.17	13.92	13.24	0.650	0.470
ME (MJ/kg DM)	6.81ª	6.75ª	6.44 <sup>b</sup>	0.033	0.001
OMD (%)	51.20ª	49.13 <sup>b</sup>	45.59 <sup>c</sup>	0.261	0.001

abc Row means with common superscripts do not differ (P>0.05); SEM: Standard error mean

ME (MJ/kg DM): Metabolisable energy, OMD (%): organic matter digestibility

# CONCLUSIONS

There are significant variations among Laurus nobilis leaves at different maturity in terms of the chemical composition, gas production, CH<sub>4</sub> production ME and OMD values. The CP contents of young and old leaves of Laurus nobilis are sufficient to meet minimum level of CP requirement although senescence leaves are not sufficient to meet minimum level of CP requirement. Therefore, protein supplementation is required for optimum rumen function and feed intake in ruminant animals when large amount of senescence leaves were included into ruminant diets. Laurus nobilis leaves had a low anti-methanogenic potential. Therefore, Laurus nobilis leaves may be used as a feed additive in ruminant diets to decrease the enteric methane production. However, it should be tested in vivo before large implication.

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# MODELING OF EGG PRODUCTION AND WEIGHTS IN JAPANESE QUAIL

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## Abstract

In this study, some models commonly used in modeling egg production and egg weights in Cukurova and commercial lines were comparatively examined. For this purpose, the average of 9-week egg yields and egg weights in three groups (chosen by chance, selection and control group) in both lines. In the modeling study, cubic, Gompertz, logistic, gamma, Richard, Schunute, quadratic spline and McNeally equations were used to model the curves of average egg yields.Cubic, Gompertz, logistic, Richard, Schunute and quadratic spline models were used to model the average egg weight curves. In the comparison of the models, mean squares of error, coefficient of determination, Akaike information criterion and Durbin-Watson autocorrelation values were taken into account. As a result of the study, the best results were obtained from the two node cubic piece regression model in both lines and all groups in average egg production. It was determined that Richard and gamma model had the worst results. In average egg weights, Gompertz, logistic and Richard models gave the best results in both lines and in all groups, while the worst results were obtained from the Schunute model in all groups. Values very close to each other were obtained in the Gompertz, logistics and Richard models.

Key words: Japanese quail, Egg yield, Egg weight, Curve, Modeling

## MEASUREMENT OF GAS AND METHANE PRODUCTION FROM ALFALFA HAY IN NYLONBAG OR DISPERSED IN THE MEDIUM IN AN *IN VITRO* GAS PRODUCTION

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#### Abstract

The aim of the current experiment was to determine the effect of nylon bag type of alfalfa hay on in vitro gas production and methane production. Alfalfa hay was incubated within nylon bags with or directly dispersedly in buffered rumen fluid for 24 h incubation. The type of incubation of samples had a significant effect on the in vitro gas and methane production. The incubating of feed samples within nylon bags reduced not only gas production but also methane production. However the pore size has no significant effect on the gas and methane production. Therefore incubating of feed samples within nylon bags is not appropriate when measuring methane emission during 24 h of incubation.

Key Word: Gas Production, In vitro, Alfaalfa

#### INTRODUCTION

In vitro gas production technique is used to evaluate the feedstuffs used ruminant animals. Recently in vitro gas production has been used to determine methane emission of feedstuffs (Pellikaan et al. 2011b Pellikaan et al. 2011b). Although feed samples were generally incubated dispersedly in buffered rumen fluid in in vitro gas production techniques. Recently it was suggested that samples can be incubated in F57 filter bags to determine both gas production and true dry matter digestibility. However incubation of samples within filter bags may reduce the gas and methane production due to restriction of microbial digestion (Noziere and Michalet-Doreau, 2000 Ramin et al. 2013). Nylon bag can be used as alternative to filter bag since the price of filter bag is very high. Therefore the aim of the current experiment was to evaluate the possibility of the use of nylon bag as a filter on in vitro gas production and methane production.

#### **MATERIAL AND METHODS**

Approximately 0.200 g of alfalfa hay samples milled through a 1 mm sieve was weighed directly or nylon bags with 23 and 45 micron pore size in triplicate into calibrated glass syringes of 100 mL following the procedures of Menke et al. (1979). The syringes were prewarmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60 %) and concentrate (40%). Gas production was recorded at 24 h after incubation and corrected for blank incubation. Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al. 2008). Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) X Percentage of methane (%)

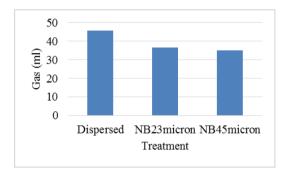
#### **RESULTS AND DISCUSSION**

Gas and methane production of alfalfa hay determined for 24 h incubationwas given in Table 1. There is significant difference among the treatments. The gas production of alfalfa hay incubated dispersedly in medium was significantly higher than those incubated in nylon bags with 23 and 45 micron pore size. However there are no significant differences between bags with 23 and 45 micron pore size. The decrease in gas and methane production is possibly related to the restriction of nylon bag for microbial digestion. The nylon bag can act as a physical barrier for micro-organism or end products as in filter bags (Noziere and MichaletDoreau, 2000). These results are in agreement with findings of Ramin et al (2013) who clearly showed that gas and methane production decreased when samples were incubated in filter bags for 48 h incubation.

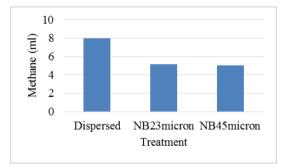
Table 1. Effect of incubation ty	/pe of alfalfa hay on	i <i>in vitro</i> gas production an	d methane production
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	21	,	5 1		
	Dispersed	NB <sub>23m</sub>	NB <sub>45m</sub>	SEM	Sig.
Gas	45.80ª	36.73 <sup>b</sup>	34.98 <sup>b</sup>	1.950	***
Methane (ml)	7.95ª	51.15 <sup>b</sup>	5.05 <sup>b</sup>	0.561	***
Methane (%)	17.37ª	14.70 <sup>b</sup>	13.65 <sup>b</sup>	0.877	***

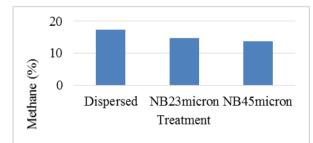
<sup>ab</sup>Row means with common superscripts do not differ (P<0.05); NB23- Bag with 23 micron NB45- Bag with 45 micron, \*\*\*P<0.001



**Figure 1.** Effect of incubation type on *in vitro* gas production of alfalfa hay



**Figure 2.** Effect of incubation type on *in vitro* methane production (ml) of alfalfa hay



**Figure 3.** Effect of incubation type on *in vitro* methane production (%) of alfalfa hay

# CONCLUSION

The incubating of feed samples within nylon bags reduced not only gas production but also methane production. Therefore incubating of feed samples within nylon bags is not appropriate when measuring gas and methane emission during 24 h of incubation. Further investigation is required to determine the effect of incubation type on other fermentation parameters such as ammonia and volatile fatty acids.

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# PATH ANALYSIS FOR PLACENTAL TRAITS ON LAMB BIRTH WEIGHT IN BAFRA SHEEP BREED

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#### Abstract

Path analysis is used to describe the directed dependencies among a set of variables and application of path analysis in animal breeding improve breeding practices. The aim of this study was to examine the direct and indirect effect of some placental traits on lamb birth weight born to Bafra sheep breed using path analysis. For this aim, relationship between lamb birth weight and seven traits (placental weight; PW, total cotyledon numbers; TCN, total cotyledon surface area; TCSA, cotyledon efficiency; CE, total cotyledon volume; TCV, volumetric cotyledon efficiency; VCE, plancenta efficiency; PE) were studied in 40 singleton - bearing sheep. Birth weight, lamb sex and placental measurements were recorded within 12 h after parturition. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, the indirect effects of PW on birth weight were found statistically significant (p<0.05). While PE was found with highest direct effect (0.602) on BW, the highest total indirect effect (-0.468) on BW was found in PW variable. The direct effect (-0.046) of TCN was lowest on BW. Also, the indirect effect (0.093) of PE was lowest on BW. The results showed that PW and PE were the most explanatory variable for birth weight of Bafra lambs.

Key words: Path analysis, Lamb birth weight, Placental traits, Direct effect, Indirect effect, Bafra

## INTRODUCTION

Placental characteristics are important indicators of the postnatal mortality of offspring in small ruminants (Dwyer et al., 2005). Mellor and Stafford (2004) reported that the postnatal viability of newborns is associated with placental growth and development during gestation. The exchange capacity of ovine placenta between maternal and fetal systems depends on placental size and number of placentomes (Ocak et al., 2013; Sen and Onder, 2016). Therefore, placental size, which is related to the nutrient transfer capacity of the placenta, plays a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013). Placental growth and development support fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that there are significant relationships between placental weight and birth weight of the newborn

(Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016).

In general, the aim of animal breeding is to genetically improve populations of livestock so that they produce more efficiently under the expected future production circumstances (Önder and Abacı, 2015). Genetic improvement for economic traits is achieved by selecting the best individuals of the current generation and by using them as parents of the next generation (Dekkers et al., 2004). In many cases, the offspring with higher birth weight are selected as future breeding material or these criteria are used to valorize the animals. Placental traits have important effect on birth weight of the newborn (Osgerby et al., 2003; Dwyer et al., 2005; Sen et al., 2013; Sen and Onder, 2016). Generally, to evaluate relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the

newborn is selected as response variable and placental characteristics are selected as explanatory variables. Therefore, it is aimed to explain the response variable from explanatory variables. However, indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013). Path analysis is used to describe the directed dependencies among a set of variables (Önder and Abacı, 2015). Therefore, application of path analysis in animal breeding practices began to increase (Önder and Abacı, 2015). In addition, there is lack of studies that examined the direct and indirect effects of placental characteristics on birth weight of the newborn. The aim of the current study was therefore to investigate direct, indirect and total effects of placental characteristics on lamb birth weight in Bafra ewes.

#### **MATERIALS AND METHODS**

Experimental animals were of the Bafra breed, 3-5 years of age, and maintained at the Sheep Farm of Ondokuz Mayis University, Samsun, Turkey. The study was conducted on 40 singleton-bearing Bafra ewes in the normal breeding season. All Bafra ewes were housed and cared for under the same conditions in the stockyard and were allowed to graze for 5 h daily during gestation. Birth weight (BW) and the sex of lambs were recorded within 12 h after parturition. Each Bafra ewe was left to deliver the placenta naturally and placentas were collected immediately after delivery. Placental weight (PW) was measured and recorded after removing placental fluid. The total cotyledon numbers (TCN) and total cotyledon weights (TCW) of placental cotyledons dissected from the chorioallantois were also counted and determined. Cotyledon length (CL), depth (CDe), and width (CWi) were measured with a digital compass and 30 cotyledons of the same size were selected (small, <10 mm diameter; medium, 10-30 mm diameter; large, >30 mm diameter). Placental efficiency (PE) was calculated for each Bafra ewe, as the ratio of lamb birth weight to placental weight (PW). Cotyledon density (CD) was calculated as the number of cotyledons per gram PW. Cotyledon efficiency (CE) was defined as the ratio of kid BW in grams to the total cotyledon surface area (TCSA). TCSA was calculated after the measurements of all the cotyledons in individual placenta as cm<sup>2</sup> with the

following formula: radius squared of cotyledon [((CWi + CL) / 4)<sup>2</sup>] × 3.14 ( $\pi$ ) × TCN. PE was calculated as the ratio of kid BW to PW for each Bafra ewe.

SPSS (2004) statistical software was used to analyze the data with the license of Ondokuz Mayıs University. Every linear model has a direct effect and amount of indirect effect which is number of explanatory variables minus one. The general expression of multiple regression model formed for the measurements (one response and p explanatory variables) is given in Equation 1.

$$y_{k} = \beta_{0} x_{k_{1}}^{\beta_{1}} x_{k_{2}}^{\beta_{2}} x_{k_{3}}^{\beta_{3}} \dots x_{k_{p}}^{\beta_{p}} e_{i}; \quad i = 1, 2, ..., n$$
(1)

The multiple linear regression model adopted was

$$\hat{\mathbf{y}}_{k} = b_0 + b_1 \mathbf{x}_{k1} + b_2 \mathbf{x}_{k2} + b_3 \mathbf{x}_{k3}$$
(2)

where:

 $\hat{y}_{k}$  = response variable (BW),

 $b_0 =$ intercept,

 $b_{i}$  = standardized regression coefficients,

 $x_{kp}$  = explanatory variables (CG, BL, HS)

A path coefficient (P) is a standardized regression coefficient (b) showing the direct effect of an independent variable on a dependent variable in the path model (Garson, 2008; Önder and Abacı, 2015). Path coefficient, which indicates the effect of one standard deviation change of any explanatory variable X versus on response variable Y, can be calculated as (Mendes et al., 2005).

$$Pyx_k = b\frac{Sx_k}{S_y}$$
(3)

Here; Pyx is the path coefficient which indicates the direct effect of X explanatory variable on response variable Y, Sx indicates the standard deviation of X, Sy indicates the standard deviation of Y and b indicates the partial regression coefficient. Path coefficients can be shown with path diagrams. One way and two way arrows are used in path diagrams. One way arrows which named as direct effects are drawn from explanatory variable to response variable and two way arrows which showed correlations 133 are drawn between explanatory variables (Tahtali et al., 2011).

To obtain the path coefficients should be replaced in linear equation system as given in Equation 4.

$$\begin{bmatrix} P_{YX_1} \\ P_{YX_2} \\ P_{YX_3} \end{bmatrix} = \begin{bmatrix} 1 & r_{X_1X_2} & r_{X_1X_3} \\ r_{X_2X_1} & 1 & r_{X_2X_3} \\ r_{X_3X_1} & r_{X_3X_2} & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{YX_1} \\ r_{YX_2} \\ r_{YX_3} \end{bmatrix}$$
(4)

In the Equation 4, coefficients given by  $P_{YXi}$  were path coefficients (direct effects) between explanatory variable and response variable and  $r_{xixy}P_{YXi}$  represented indirect effects of explanatory variable i<sup>th</sup> on response variable via explanatory variable j<sup>th</sup>,  $r_{xixj}$  represented pearson correlation coefficients between i<sup>th</sup> an j<sup>th</sup> traits (Topal et al., 2008).

## **RESULTS AND DISCUSSION**

Direct and indirect effects of explanatory variables on birth weight in Bafra ewes are present Table 1. The direct effects of PE on birth weight were found statistically significant (p<0.05). Additionally, direct effects of PW, TCN, TCSA, CE, TCV and VCE on birth weight were not found statistically significant. The indirect effects of PW on birth weight were found statistically significant (p<0.05). Moreover, direct effects of TCN, TCSA, CE, TCV, VCE and PE on birth weight were not found statistically significant in the present study. While PE was found with highest direct effect (0.602) on BW, the highest total indirect effect (-0,468) on BW was found PW variable. The direct effect (-0.046) of TCN was lowest on BW. Also, the indirect effect (0.093) of ΡE was lowest on BW

Table 1. Direct and indirect effects of explanatory variables on birth weight in Bafra ewes.

Trait	Direct effects	Indirect effect
PW	0.371	-0.468*
TCN	0.046	0.303
TCSA	0.115	0.178
CE	0.068	0.197
TCV	-0.088	0.188
VCE	-0.089	0.428
PE	0.602*	0.093

PW= placental weight, TCN= total cotyledon numbers, TCSA= total cotyledon surface area, CE= cotyledon efficiency, TCV= total cotyledon volume, VCE= volumetric cotyledon efficiency, PE= plancenta efficiency. \* p<0.05

Placental traits, such as placental weight, cotyledon cotyledon numbers, efficiency, plancenta efficiency etc., are important indicators of the postnatal survive of lambs in sheep (Dwyer et al., 2005; Sen et al., 2013). Therefore, some factors affecting lambs birth weight should be determined. Owing to this, the path analysis is very important for determining factors affecting lamb birth weight (Önder and Abacı, 2015). In this study, which aimed to investigate the direct, indirect and total effects of placental characteristics on birth weight in Bafra lambs. Mostly, evaluation of relationship between placental characteristics and birth-related traits data relational statistics such as regression and correlation are used (Ocak et al., 2013; Sen et al., 2013; Sen and Onder, 2016). Birth weight of the lambs were selected as response variable and placental characteristics were selected as explanatory variables in the present study. Therefore, path analysis was used to describe the

directed dependencies among a set of variables in the present study. Onder et al. (2017) direct and total effects on lamb birth weight were supported by studies in Akkaraman lambs. Some direct and total effects of placental traits on lamb birth weight were similar with results of Onder et al. (2017).

#### CONCLUSIONS

As a result, it was concluded that PW and PW could be used for management decisions and as indirect selection criteria for selection on lamb birth weight due to PW had the highest direct and PE the lowest indirect effect lamb birth weight in Bafra sheep breed.

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# DETERMINATION OF ENERGY AND PROTEIN NEEDS OF PARTRIDGES PRODUCED IN KAPICAM RED PARTRIDGE PRODUCTION CENTER

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#### Abstract

The first production of Alectoris Chukar by the state in Turkey was made by the Ministry of Agriculture and Forestry of the Republic of Turkey in Kahramanmaras Kapıcam Partridge Production Center. While many poultry have been produced before and there is sufficient information about the needs of these poultry animals, there is no local information about partridges. For this reason, the existing knowledge of other poultry is used for this need. In this case, it negatively affects both the development and yield of thyme. With this study, information will be given about the protein and energy needs of the ration used in hennaed partridges produced in the Kapıcam Red Partridge Production Center.

#### Key words: Energy, Protein, Partridge

#### INTRODUCTION

The red partridge (Alectoris chukar) is a bird belonging to the pheasant (Phasianidae) family, and are birds that live in flocks. They have a light brown back, gray breasts and brownish yellow belly feathers. It has a prominent black choker on the face. It has a mixture of brown-orange-red color with striped flanks and red legs. (Durak et al, 2016, Anonymous 2021a, Anonymous 2021b, Anonymous, 2008).

The partridge is an animal with a plump body structure, short tail, round wings and a red beak, legs and feet. The characteristic black bandshaped line that starts on the forehead of red partridges goes down to the eyes and from there to the lower part of the neck. (Kasım, 2001, Anonymous 2021b).

Live weights of partridges vary between 450-650 grams. Males have a mature weight of 550-650 g, while mature females are 10% lighter than males. While males and females have the same physical characteristics, when they reach 32 weeks of age, they are distinguished by the spurs formed in the two legs of the males. The spurs of males are larger than those of females, and females are found only in animals older than one foot. Gender determination can be made by looking at the spurs in the partridge, or the exact gender distinction can be made by looking at the cloaca.

In addition, the head part of the male partridges is a little bigger (Kasım, 2001).

Partridges are suitable animals to be raised in the cage system, and they can be raised in double, triple and larger colonies in cages. The fertility rate decreases when henna partridge is grown in large colonies. The fertility rate reaches up to 84%, especially when it is cultivated as 1: 1 or 2: 1. (Çetin et al., 1997, Alkan et al., 2008. Özkan et al, 2020. Özkan, 2020).

The Kapicam Red Partridge Production Center, which is in the Kapicam nature park, is located at latitudes and longitudes at 37 S 0325270 UTM 4151496 (Özkan 2020, Özkan and et all, 2020).

Kapicam Red Partridge Production Center was established in 2002 and started production under The partridge intensive conditions. red established production station in Kahramanmaras is established on a 7.2 hectare forest area, has 1 administration building, 1 breeding house, 1 hatchery and 6 nature adaptation voliers. When we look at the instrument equipment status; There are 2 predevelopment machines, 1 hatcher, 13 main machines, 1 egg holding cabinet, 1 fumigation machine, 19 breeder cages, 1 pressure washing device, 1 generator and 35 transport cages. The annual production capacity of the station is V. International Congress on Domestic Animal Breeding, Genetics and Husbandry - 2021 (ICABGEH-21) September 27 - 30, 2021

15,000 pieces, and the annual average production varies between 15,000-20,000 pieces. Partridge production is a tough job, and the production season should be kept very short and released to nature as soon as possible. For this reason, the ration used should adjust the energy and protein well and meet the needs of the animals. While preparing partridge rations, the needs of the ornamental birds were taken into consideration and were prepared especially on the basis of the nutrient needs of the quail. (Beer 1995, Cufadar and Bahtiyarca, 2006).

The purpose of breeding poultry is to produce eggs at the maximum level per female animal and subsequently produce chicks with high quality and high growth performance. Therefore, breeding eggs must be the right size and robust. Because there is a close relationship between egg weight and chick weight (Larbier and Lerlercq 1994). Feed expenses constitute approximately 60-70% of production costs in partridge farming under intensive conditions as in other poultry (Vohra 1993).

The animals that will be released to the nature in the production stations need to grow as soon as possible, therefore, the nutrient needs of the developing partridges change on a weekly basis. In addition, the needs of the breeding animals change periodically. They need a balanced ration in terms of energy and protein for both body conditions and efficiency.

## MATERIALS AND METHODS

In this study, henna partridges in Kapıçam Henna Partridge Production Station were used. In addition, the feeds were produced in the Gürdal feed factory.

#### **RESULTS AND DISCUSSION**

When the Kapipam Red Partridge Production Station was established, there was no determined ration for partridges. Generally, feeds prepared for laying hens were used. Of course, although this ration meets the basic needs, it was not necessary to create a basic requirement table periodically due to the different structures of the red partridges.

For this reason, dialogues started with the Gürdal Feed Factory in the province. In 2007, the registration of feed for partridge was made by the relevant company.

The energy and protein amount of the rations prepared was tried to be calculated by NRC (1994) considering the needs of breeding pheasants and quails.

In addition, taking into account the previously made resources in table 1, a registration certificate has been obtained for the rations with energy protein content.

According to the results of the study given so far, it will be useful to present the HP and energy needs reported by different organizations and researchers in a table 1.

Resources	Metabolic Energy (kcal/kg )	Crude Protein (%)
NRC, 1994 (Pheasant)	2800	15
NRC: National Research Council		

**Table 1.** Energy and protein needs for partridges obtained from some scientific studies

55 1	I	5
Forage name	Metabolic Energy (kcal/kg )	Crude Protein (%)
Partridge Chick (0-5 weeks)	28	3000
Partridge Growing (6-9 weeks)		3100
	24	
Partridge Enhancement Feed (10-20 Weeks)	18	2900
Partridge Egg Feed (1st Term)	20	2750
Partridge Egg Feed (2nd Term)	17	2850

 Table 2. Energy and protein contents of the rations used in Kapıcam Red Partridge Production Center

## CONCLUSIONS

Red partridges, which were started to be produced by the state in Turkey, are also produced by many private sectors today and have an important commercial potential. Red partridges produced are hunted especially in special hunting grounds. For this reason, it is important that red partridges complete their physical and morphological development.

With this study, energy and protein requirements of red partridges, which are widely produced, are given in table 2, and it will help henna oregano to reach their maximum performance.

In addition, it would be appropriate to use animal protein sources (grasshoppers, mealworms, etc.) for the red partridges that will be released into the nature.

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# USE OF FLOUR WORM (TENEBRIO MOLITOR. L.) IN PARTRIDGE PRODUCTION

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#### Abstract

Possibility of using flour worm as a protein source in partridge production. Due to the climate zone in which Turkey is located, it offers the opportunity to shelter and raise many wild animals. One of them is partridges. It can be cultivated both intensively and semi-intensively. The partridges produced by the state in Turkey are used for the protection of nature and for hunting materials of hunters. The main purpose in the production of poultry wild animals is to ensure the continuity of the natural population by releasing it to the nature. Many studies are carried out to ensure the adaptation of animals to nature. The use of animal-derived proteins is one of them. In partridges, the use of locusts and mealworms is both to supplement the ration and to facilitate the adaptation of animals to natural life. In terms of nutrient content, there are % 34.92 dry matter and 64.83% crude protein. The high content of fat and protein in terms of nutrient content of insects has recently brought its use as both human and animal food. In this study, the potential of insects to be used as animal feed for partridge grown under intensive conditions is discussed.

Key words: Insects, Wild Animal Feed, Protein

#### INTRODUCTION

Hunting and wildlife activities in Turkey are carried out by the Republic of Turkey Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks. Due to the decrease in the red partridge population, the relevant ministry established Kahramanmaras Kapıcam Red Partridge Production Center in 2002. In the production center, production activities were started under intensive conditions. Development studies have been started to raise animals in accordance with welfare and nature. (Ozkan et al., 2013).

One of them is feeding with animal-derived proteins. First of all, it bought grasshoppers from companies that produced grasshoppers and offered them to the consumption of animals. Then he continued with the flour worm.

Flour worm is preferred because it is easy and fast to produce and it is easier to supply. In addition, insects are a new protein source and a natural food source with a value close to fishmeal in terms of protein and amino acid content. In nature, they take place in the food chain of poultry. It is even used as human food in

some societies. Examples include Africa, Korea, Japan and China (Ozkan et al., 2013, Van H A., 2013., Veldkamp T and Bosch G 2015., Ozkan et al, 2020, Aniebo A O et al., 2009 and Ozkan ., 2020).

The availability of animal protein sources in rations is gradually decreasing due to legal regulations. As an alternative to these, insects have taken their place in the rations.

In a study, the % crude protein ratios of fish meal, meat bone meal and poultry meal were reported as 70.04, 49.76 and 52.93, respectively (Yang et al., 2018). In our study with flour worm, it was found to be 64.83%.

#### **MATERIALS AND METHODS**

Flour worms given to partridges were obtained from Kahramanmaras Sütcü imam University, Faculty of Agriculture, and Department of Plant Protection. The weight, length, dry matter and crude protein analyzes of the mealworms were carried out in the feeds and animal nutrition laboratory of the Faculty of Agriculture, Department of Animal Science (AOAC, 1990). **RESULTS AND DISCUSSION**  Flour worms (Tenebrio molitor. L.) used in feeding were obtained from an insect farm in Antalya that produces live bait. The results of the analyzes of mealworms are given in Table 1.

**Table 1.** Results of the flour worm used in the experiment

ANALYSIS VALUES	%
Weight (gr)	0,07gr
Length (cm)	1,93
Dry Matter (DM)	34,92
Crude Protein (CP)	64,83

The partridges, which are produced and released into the nature by the relevant ministry, are required to grow rapidly and to have the highest level of adaptation to nature. For this reason, it is desired that the animals reach the desired weight for release into the nature as soon as possible. Therefore, proteins of animal origin are important.

Red partridges consume nutrients of plant and animal origin in nature. Especially the vegetative and generative evenings of plants and insects are the main sources of nutrients. Insects are important in the first 10 days for chicks hatching in nature. Considering these, the introduction of these insects in production centers is very important for both rapid development and adaptation to nature.

# CONCLUSIONS

The importance of insect feeding has been understood in terms of ensuring the easiest adaptation of wild animals produced for a sustainable environment and wildlife.

In addition, the fact that mealworms are rich in nutrient content accelerated their physical development. Leaving them as fodder in the nature during intense winter conditions will increase the chances of survival of the animals.

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# THE MEANING OF THE OVERSEAS OF ALECTORIS CHUKAR

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## Abstract

The aim of in this study, the meaning content of the sounds used by the red partridge (Alectoris chukar), one of the winged game animals, in their life stages and their daily lives. Especially the sound used by the animals in general, the sound at the time of danger, the sound at the time of finding feed, the sound at the time of cheering, the sound they use to find and mate during mating, the sound at the time they fight for herd preference and habitat boundary determination and herd leadership, research was conducted on the sounds that they used to call other friends or off spring when they found feed and the sounds they made when they saw their predators. Material of the study, T.C. The Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks, Wild Animal Production Stations and hunting associations in Kahramanmaras center and districts established a survey conducted hunters registered. When the chukar partridges find bait, the age groups of the sound they make, the gender of the partridge, the educational status of the subjects and the hunting certificate, the fact that they have the knowledge of arid prey, whether they feed the partridge and the sound they start to sing are mainly.

Key words: Alectoris chukar, Wild animal, Sound, Hunter, Arid hunt

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## THE EFFECTS OF OZONE CLARIFICATION OF DRINKING WATER ON SOME HEMATOLOGICAL PARAMETERS AND BODY WEIGHT IN LAMB

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## Abstract

All ruminants need water to produce high yields. The water requirements of the ruminants are basically obtained from three sources: 1) drinking water, 2) water present in feed and 3) metabolic water formed by oxidation of food and body tissues. It is important to remember that animals with high production need plenty of quality, and clean water to achieve the best yields. Drinking water is the main source of microbial pathogens; poor sanitation and food sources are part of an enteric pathogen exposure. Chlorine, chlorine dioxide, ozone and monochloramine; the main substances used in water disinfection. Ozone, a gas discovered in the middle of the 19th century, is a molecule consisting of three oxygen atoms in a dynamically unstable structure due to the presence of mesomeric conditions. Gas is colorless, odorless and explosive in liquid or solid form. It has a half-life of 40 minutes at 20 °C and about 140 minutes at 0 °C. Its main function is to protect people from the harmful effects of ultra-violet radiation. In this study, the effects of using water clarified with ozone gas as drinking water on some hematological parameters and body weight were investigated in lambs. At the beginning of the experiment, 20 male Kıvırcık × Akkaraman AG1 crossbred lambs, weaned at the beginning of the experiment, were taken with an average weight of 19.18 in terms of kilograms, and the experimental group was formed. Ozone-clarified water was drunk for 48 days and at the end of the experiment, they have reached. The average weight of 19.64 in the control group was measured as 24.30 kg at the end of 48 days. Although there was no statistically significant difference in the body weight gain and total body weight gain parameters between the groups where ozone and non-ozone water (spring water) and drinking water were supplied as a result of the treatment of drinking water with ozone in the control and experimental groups fed the same feeding method and the same feeds. Experimental group erythrocyte distribution width is considered to be high. In conclusion to, when drinking water containing 5 ppm ozone is rested for 24 hours; it suggests that it has negative effects on intra-rumen microbiota, which may cause macrocytic anemia.

Key words: Ozone, Animal drinking water, Water clarification

## SEROPREVALENCE AND ASSOCIATED RISK FACTORS FOR SALMONELLA DUBLIN INFECTION IN CATTLE IN KHENCHELA, ALGERIA

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## Abstract

Salmonella Dublin is a causative agent of a gastrointestinal bacterial infection that is widespread in many cattle herds around the world. The objective of this study is to estimate the seroprevalence of S. Dublin infection in healthy cows in the Khenchela region and to identify potential risk factors that may be associated with the presence of S. Dublin antibodies. 256 cow's milk from 38 farms was tested using an enzyme-linked indirect immunosorbent assay (ELISA) and immuno results showed a prevalence of 36.33% (95% CI 30.44 - 42, 22) for S. Dublin. The final multivariate regression models showed that breed, region and introduction of cattle purchased were associated with the presence of S. Dublin antibodies. We concluded that this study is the first to report the seroprevalence and risk factors associated with S. Dublin infection in Algeria, as well as S. Dublin circulating in cattle farms in the Khenchela region in Algeria and could be considered as a point of comparison for other studies in Algeria. In addition, we recommend the implementation of hygiene practices and biosecurity measures on farms to reduce the spread of infection and the use of vaccination in animals and people at risk.

Key words: Cows, Salmonella Dublin, Milk, Seroprevalence, Risk factors

## MORTALITY OF SWAMP BUFFALO CALVES DURING THE LACTATION PERIOD

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#### Abstract

This study was conducted to analyze mortality of swamp calves under semi-intensive rearing conditions at Shtiha government Station in Al-Ghab District, Syria. 501 buffalo calves born throughout period 2008-2018 were analyzed for suckling calf mortality rate study. SAS software (2012) was used to compare four mathematical models (basic and full linear fixed models, logistic, and logarithmic models) to estimate calf mortality during nursing period from birth to 90 days of age. Overall mean mortality rate ranged from 14.5±3.3 to 19.2±2.7%. Sex effect of a calf was significant in the basic model. Calving period and calf weight at birth were significant according to the basic and full models. Interaction between the calving periods and birth weight was highly significant by full linear fixed and logarithmic models, confirming that birth weights during period, and birth weight were significant in the linear logarithmic model. Effect of birth weight was significant in all models, which confirms its importance in calf mortality. The study concluded that management of buffalo calves and improvement of buffalo calves.

Key words: Bubalus bubalis, Buffalo Calves, Mortality rate, Models

#### INTRODUCTION

In Syria, a total number of buffaloes is about 8000 heads (FAO, 2018) which supply more than 620 and 6195 tons of meat and dairy products, respectively (MOAAR., 2011). Environmental factors greatly affect buffalo production. Among these factors, the death of calves before weaning is a major problem for breeders. Each calf surviving from a dam is an advantage in increasing productivity of farm because it saves more weight at weaning and more milk. It is accepted that calf deaths occur during nursing period. Many studies have reported that environmental factors affect calf survival before weaning. Unfavorable calving seasons lead to the mortality of calves, so health management and proper nutrition are key factors in calves' survival. Calf mortality control is vital, not only to improve calf welfare but to increase productivity. Higher calf mortality rates can be associated with higher numbers of calves in herd, breeder performance, severe weather, and nursing period covering first two months of life. Diseases appear in calves when breeders fail to transfer immunoglobulins to them, resulting in increased neonatal mortality (Katsuji, 2019). Maximum mortality has been found in young calves due to diseases as infections so special care is needed for such pathological problems (Dinesh et al., 2014). To control the mortality of calves, the effects of environmental factors should not be ignored to improve the care of calves. Therefore, the objectives of this research are to estimate the mortality rate of calves, the effect of calf sex, year of calving, parity, and weight of calves at birth during the nursing period under the conditions of semi-intensive Syrian rearing.

#### MATERIAL AND METHODS

This study was conducted at Shatiha station for buffaloes; which is located about 73 km northwest of the Al-Ghab area in Syria. Buffaloes are raised freely in the pastures located on the sides of water channels. Where they go out to

pasture in the morning and return in the evening and spend the daytime napping in waters of canals from eleven in the morning until four in the afternoon to escape from the high summer heat and periods of decrease Napping in canal waters in autumn and spring, while almost nonexistent in winter. At night, buffaloes take haven in open pens. The calving takes place in the winter and the newborns are weaned in the spring to go out with their dams to the pastures. Newborn calves are left with their dams for a week to be fed with colostrum milk after the calves get milk twice daily in the morning and evening for a month. Calves gradually get used to green grass, concentrated feed, and hay during the first month of age. Then the amount of milk that the newborn takes from his dam gradually absorbs what remains after milking and continues until the dam rejects it.

501 records of buffalo calves were analyzed during the period 2008-2018. Each record included the identity of a calf, the sex, parity, year of birth, birth weight, and the fate of the calf whether it was weaned or dead during the lactation period. The data were analyzed using SAS (2012) software according to four mathematical models to study the non-genetic factors that influence the mortality rate of buffalo calves;

 $Y_{ijklm} = \mu + X_i + PR_j + PE_k + BW_l + e_{ijklm}$ 

 $Y_{ijklm} = \mu + X_i + PR_j + PE_k + BW_l + XPR_{ij} + XPE_{ik}$  $+ XBW_{il} + PRPE_{jk} + PRBW_{jl} + PEBW_{il}$  $+ e_{ijklm}$ 

Where,  $Y_{ijklm}$  = calf mortality during the suckling period of the  $ijklm^{th}$  records, µ=grand mean.  $X_i$  = effect of  $i^{th}$  sex of calf,  $PR_j$  = effect of  $j^{th}$ parity,  $PE_k$  = effect of  $k^{th}$  period of calving, and  $BW_l$  = effect of  $l^{th}$  birth weight of calf [first=BW <= 30, second=BW >= 31/kg).  $e_{ijklm}$  = random error term associated with the  $Y_{ijklm}$  observations with zero mean and variance  $I \sigma^2 e$ .

 $XPR_{ij} + XPE_{ik} + XBW_{il}$  = the interaction of the calf sex with parity, a period of year and birth weight, respectively.  $PRPE_{jk} + PRBW_{jl}$  = the interaction of the parity with a period of year and birth weight, respectively.  $PEBW_{kl}$  = the

interaction of the period of years with birth weight.

$$\begin{split} RES_{ijklm} &= A + X_i + PR_j & (\mbox{Logistic model}) \\ &+ PE_k \\ &+ BW_l \\ &+ LR_{ijklm} \\ RES_{ijklm} &= X_i \times PR_j \times PE_k & (\mbox{Log linear} \\ &\times BW_l & \mbox{model}) \\ &\times RES_{ijklm} \\ &+ LR_{ijklm} \end{split}$$

Where, RES=Response (Mortality, Survival) of the  $ijklm^{th}$  records that were 16 samples for Logistic and 29 Responses for Log-linear models. A=Intercept, LR=Likelihood Ratio, Other symbols are explained in the previous models.

## **RESULTS AND DISCUSSION**

The overall calf mortality rate ranged from 14.5±3.3 to 19.2±2.7% according to models (Table 1). These values are relatively high due to poor management and unfavorable environmental conditions during the lactation period as 15-25% before weaning calf mortality is seen as an indicator of poor calf management (Moran, 2011). The overall mortality rates for buffalo calves were found at 9.4 and 11.05 in Pakistan and Egypt, respectively, and these values display accepted management of calves (fixed basic.model) during the suckling period (Zaman et al., 2006; El-Regalaty and Aboul-Ela, 2014). On the other hand, there were total mortality rates for buffalo Calves of 17.98%, 18.78%, and 15.89% in Peshawar, Punjab, and Nagpur (Zaib Ullah, 2007; Bilal et al., 2019; Kharkar et al., 2019), respectively; These values indicate poor management during the suckling period. Some studies reported high mortality values of 31%, 38.8%, and 42.11% before weaning (Oswin 1999; Thiruvenkadan and Devendran 2014; Amit et al., 2017), respectively, and these values may point poor management and unfavorable to environmental conditions for the care of buffalo calves. In Pakistan, the mortality rate of buffalo calves was 60% and 79.51% due to worms, lack of preventive measures, and male neglect due to low expected return (Ahmad et al., 2009: Bilal et al., 2009) respectively. Therefore, the calf needs to improve various aspects of calf breeding such as nutrition, housing, and weaning (Bilal et al.,

2019). Archana et al., (2020) noted that parasites killed 34.21% of buffalo calves, of which 58.33%

were males, 23.07% were females, and 75% of dead calves had diarrhea.

<b>Table 1.</b> Least Square Means and Standard Errors of the calf mortality rates from birth to weaning at
90 days of age, in buffaloes

Source of Variance		Obs. –	LSM±SE		
	Source of Variance		Main Fixed Model	Full Fixed Model	
Overall m	ean (µ)	501	0.192±0.027	0.145±0.033	
Sex	Male	249	0.232±0.024a	0.222±0.028	
Sex	Female	252	0.151±0.023b	0.181±0.026	
Parity	1 <sup>st</sup>	127	0.177±0.029	$0.189 \pm 0.030$	
Failty	+2 <sup>nd</sup>	374	0.207±0.021	0.213±0.024	
Calving pariod	2008-2013	228	0.086±0.022b	0.060±0.024b	
Calving period	2014-2018	273	0.297±0.025a	0.343±0.030a	
BW	BW<=30	117	0.270±0.031a	0.291±0.031a	
DVV	BW>=31	384	0.113±0.020b	0.113±0.022b	

SOV: Source of variance, LSM: Least square means, BW: Birth Weight of calf, kg.

In the basic model, the male mortality rate was found to be 8.1% higher than females. Calves' mortality in the second period (2014-2018) was 11.1% higher than the first period (2008-2013). The mortality rate of calves born with weight equal to or less than 30 kg was higher by 15.7% than that of 31 kg or more during the nursing period based. Whereas the mortality rate in the second period (2014-2018) was 28.3% higher than the first period (2008-2013) and the mortality rate of calves born with weight equal to, less than 30 kg was higher by 17.8% than that of 31 kg or more in the full model. The mortality of calves may be due to the higher sensitivity of unfavorable environmental conditions during the nursing period (Table 1).

**Table 2.** Analysis of variance for the mortality rate of calves from birth to weaning at 90 days of age, in buffaloes

			Fixed	Model	
Source of Variance	DF	Μ	Main		Full
	-	MS	Pr.>F	MS	Pr.>F
Gender	1	0.822	0.0070	0.130	0.2760
Parity	1	0.076	0.4104	0.040	0.5434
Period of calving	1	5.436	0.0001	6.072	0.0001
Birth weight	1	1.977	0.0001	2.291	0.0001
Gender×Parity	1			0.011	0.7435
Gender×Period of calving	1	Non-available		0.023	0.6474
Gender×Birth weight	1			0.222	0.1555
Parity×Period of calving	1			0.003	0.8517
Parity×Birth weight	1			0.001	0.9083
Calving period × Birth weight	1			1.339	0.0005
Residual	490	0.112		0.109	

Table (2) shows that the effect of sex was significant (P<0.01) in the basic model. The calving period and calf weight at birth were significant (P<0.01) according to the basic and full models. The interaction between a calving period and birth weight was highly significant (P<0.01), confirming that the birth weights during the calving period were heterogeneous. Calving period and birth weight effects

demonstrated the importance of environmental factors and management practices on mortality of buffalo calves from birth to weaning at 90 days of age under semi-intense buffalo rearing conditions.

An analysis of the probability of maximum mortality variation showed that the sex of the calf, the period of calving, and the birth weight were significant (P<0.01) through the logistical

model. The calving period and birth weight were significant (P<0.01) in the linear logarithmic model (Table 3). The logarithmic linear model showed that there is real differentiation in mortality response (survival and mortality). The differences between birth weights, calving periods, and calves sex within the mortality response were heterogeneous. Differences in birth weights in each period of calving, parity, and calf sex were heterogeneous. There is considerable variation in the sex of calves during calving periods (Table 3). Differences in response to mortality, and calving periods, sex calves within parity were homogeneous (Table 3).

In reviews of the literature, Hammad et al (2013) demonstrated that sex is the most important

source of difference in mortality for buffalo calves, with Amit et al (2017) finding that the male sex is significantly higher in mortality as in the current study. While Zaman et al. (2006) reported that the effect of sex was not significant on the mortality rate of Nili Ravi calves. Amit et al. (2017) found that male calves had a higher mortality rate than females at 60.47% for males compared to 23.53% for females due to male calves being neglected. While Kharkar et al. (2019) reported that female mortality was higher than male mortality, and Khatun et al. (2009) reported that female calves showed 42.85% compared to 28% for males, where females were more likely to be infected.

**Table 3.** Maximum Likelihood analysis of variance for the mortality rate of calves from birth to weaning at 90 days of age, in buffaloes

		Logistic Model		Log line	ar Model
Source of Variance	DF -	Chi-Square	Pr>ChiSq	Chi-Square	Pr>ChiSq
Gender	1	6.53 0.0106		1.91	0.1674
Parity	1	0.66	0.4151	29.43	<.0001
Period of calving	1	37.26	<.0001	0.69	0.4057
Birth weight	1	16.25	<.0001	11.77	0.0006
RES	1			90.71	<.0001
Birth weight×RES	1			16.89	<.0001
Period of calving × RES	1			29.00	<.0001
Parity×RES	1			0.57	0.4505
Gender×RES	1			8.86	0.0029
Calving Period×Birth weight	1	Non-available		8.06	0.0045
Parity×Birth weight	1			39.44	<.0001
Gender×Birth weight	1				0.0275
Parity×Period of calving	1			0.71	0.3995
Gender×Period of calving	1			6.10	0.0135
Gender×Parity	1			1.43	0.2317
Likelihood Ratio	13	14.50	0.2065	13.34	0.4219

There is no significant effect of parity (Nitin et al. 2016; Kharkar et al 2019), respectively as in the current study while El-Regalaty and Aboul-Ela (2014) determined the significant effect of parity on the mortality rate of buffalo calves. Mortality rates of calves born from dams in parities 1 to 6 were similar and ranged between 14% and 16.7%, while calves mortality rates increased significantly in parities 7 and beyond, reaching

the highest value of 27.5%. This may be due to dams getting older, which makes them less caring for the newborn during suckling (Table 1). Zaman et al (2006) reported that the mortality rate for Nili-Ravi calves was 11.3% at first parity. Calves mortality had been significantly affected over the years (Kharkar et al. 2019; Al Masalati and Abu Allah 2014) in line with the current

study, while in other studies this did not affect

the mortality of buffalo calves (Nitin et al. 2016; Hammad et al. 2013). Table 1 shows that calf mortality rates were represented by two periods of years. The first period (2008-2014) and the second period (2015-2018) spanned and the differences between them were real. These real differences between the two periods reflect the importance of calf cares to reduce mortality during breastfeeding.

The mortality rate was significantly affected by the weight of the calf (El-Regalaty and Aboul-Ela 2014) as in this study, while the effect of birth weight was not significant on the mortality of Neil Rafi calves (Zaman et al. 2006). Table (1) shows that calves weighing more than 30 kg had a 17% lower mortality rate compared to calves of equal or less birth weight and could withstand unfavorable conditions during the lactation period. Therefore, work must be done to improve birth weight. El-Sayed et al (2020) showed in a study on the same herd that the estimate of genetic change was very low and non-significant; confirming that effective selection for birth weight was not present during the study period

## CONCLUSIONS

All models gave an odds ratio for the effect of birth weight on calf mortality, taking into account other explanatory variables. Whereas the interaction between birth weight and birth periods was heterogeneous in both the full and logarithmic models. Therefore, good care and management are essential for reducing calf mortality. Moreover, genetic selection for calf weight at birth to be more than 30 kg should be applied to avoid the risk of mortality.

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## SEROEPIDEMIOLODICAL STUDY OF LEPTOSPIRA INTERROGANS INFECTION OF CATTLE IN SETIF AREA, ALGERIA

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## Abstract

Leptospirosis is an infectious, zoonotic disease with worldwide distribution. It is classified as an emerging and re-emerging disease responsible for heavy losses, both economically and to human and animal health. The main objective of this work was to make an original contribution to the knowledge of Leptospira infection in cattle and its associated risk factors in the Sétif region in Algeria. Thus, 406 bovine sera belonging to 48 different farms were, randomly, sampled during a period going from 2015 to 2019. The sera underwent serological analyzes by the MAT test, making it possible to reveal the prevalence of 8 serovars of Leptospira spp, using 50% agglutination, at a dilution  $\geq$ 1: 100 as a cut-off point. The results obtained indicated that a total of 170/408 cattle were positive by the MAT test for one or more Leptospira spp serovars, with a seroprevalence of 41.87% (95% CI 33.07-46.67%). The positivity rate at the farm level was 89.58% (95% CI 80.94-98.23%) or 43 farms +/- 48. The most prevalent Leptospira serovar was Patoc (32.75%) for the Leptospira biflexa species, followed by the Leptospira interrogans serovars: Icterohaemorrhagiae (7.14%) and Hardjo (6.89%) and Canicola (4.43%). finally, the serovar the least present was Autumnalis (0.74%). Multivariable logistic regression analysis showed that cattle drinking from rivers have a lower risk of exposure to Leptospira spp (OR 0.51, 95% CI 0.32-0.82%), whereas, cows between 3 and 6 years old were the most exposed (OR = 9.25 95% CI 1.19-71.47%). The herd size of > 20 heads (OR = 13.65 95% CI 1.58-117.7%) and the semiintensive system (OR = 0.21 95% Cl 0.06-0.82%) appeared to have a statistically significant association with seropositivity to Leptospira interrogans serovar Hardjo (P <0.05). In conclusion, the seroprevalences obtained with respect to the various Leptospira serovars, in particular the serovar Hardjo, indicate that the latter are widely distributed in the Sétif region, probably, in an enzootic unstable form.

Key words: Zoonotic, Leptospirosis, Serovars, Cattle, MAT, Sétif, Risk factors

## GENOMIC SELECTION STRATEGIES IN BREEDS WITH SMALL POPULATION SIZE

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#### Abstract

In small populations, the reference population size is the most limiting factor for implementation of genomic selection Therefore, alternative strategies must be considered to increase accuracy of genomic selection in small populations. One strategy to increase the size of reference population in small population breeds is to blend individuals of two or more breeds with similar breeding objectives and recorded traits to create a multi-breed reference population. For transboundary breeds with similar breeding objectives, international genomic evaluation could be increase reliability of predictions. Another strategy for breeds with crossbred data is to use this data in the reference population for genomic evaluation. However, when the mentioned strategies are not applicable to small population breeds, a single-step genomic method that integrates all available phenotypic, pedigree, and genomic information is recommended.

Key words: Small population, Genomic breeding value, Reference population

# THE EFFECT OF MASSAGE ON THE COW'S UDDER CONDITION AND HEALTH

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#### Abstract

The purpose of this thesis was to determine the effect of udder massage with the use of care products (gel, balm, ointment) on the health and condition of mammary glands of cows. The study was carried out in one of the individual farms located in Kujawy-Pomerania Province, selecting from the herd 2 groups (control - K and experimental - D), comprising 5 animals each. Cow milk samples from both groups were analyzed, determining the cytological quality (somatic cell count - SCC) of quarter milk (udder health index). Point-score schemes were used to determine the condition of the skin of the quarters and the teats. Systematic visual and palpate evaluation of udders was conducted, accompanied by photographic documentation. It was demonstrated that daily post-milking massage had a positive effect on the condition and health of the cows' udders. Despite the good health condition of the udders at the very start of the tests (average SCC in both groups was <150.000/ml milk), the experimental factor resulted in improved milk quality. The differences between groups K and D were statistically significant (natural logarithm of the mean number of somatic cells- LNSCC, was 11.57 and 11.47 in group K and D, respectively). It was noted that after 10 weeks of applying the massage, SCC in group D decreased around 30%. The condition of the cows' udders subject to the treatment improved significantly, by over 50% compared to the initial state. The condition of the skin of the quarters, teat bases and the teats themselves also improved, with skin dryness, irritation, all skin changes and injuries, painfulness and edema completely reduced. During the study, there was no case of mastitis (assumed preventive effect accomplishment) observed in group D.

Key words: Dairy cows, Massage, Udder, Condition, Health

## SEROEPIDEMIOLOGICAL SURVEY OF CHLAMYDIA ABORTUS INFECTIONS IN CATTLE: CASE-CONTROL STUDY IN THE REGION OF JIJEL

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#### Abstract

Chlamydia abortus is a gram-negative bacterium with a zoonotic character. The infection by this germ can be asymptomatic as can be expressed by the occurrence of abortions in animals and human beings. The objective of this work was to determine the prevalence of abortions in cattle breeding in the region of Jijel and to contribute to the knowledge of Chlamydia abortus infections in cattle by conducting a case-control study. For this purpose, 184 breeding cows from 90 farms were serologically tested by indirect ELISA technique. The study revealed an abortion prevalence of 16% and a seroprevalence of Chlamydia abortus of 1.8%. The case-control study did not reveal a causal link between seropositivity and the occurrence of abortions in cattle in Jijel.

Key words: Bacterium, Zoonotic, Abortion, Prevalence, Indirect ELISA

## DISTRIBUTION OF MONOGENEAN PARASITES IN CYPRINID FISH FROM THE MACEDONIAN AQUACULTURE

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## Abstract

The representatives of the class Monogenea are ectoparasites with a direct life cycle and most of them live mainly on the gills or the surface of freshwater or marine fish. Monogenea parasites in aquatic ecosystems are present in large numbers and manifest a clear specificity to their host - fish. They can cause enormous damage not only to the host but also to the aquatic environment. They are also considered to be a sensitive indicator of the health status of an ecosystem. This study aimed to determine the distribution of parasites of the class Monogenea in cyprinid fish from the Macedonian aquaculture. 656 fish out of 1134 examined were infected with monogenean parasites, with a mean intensity of 6.84, and a prevalence of 57.85%. In this study, the following monogenean parasite species in cyprinid fish were established: Dactylogyrus extensus, Dactylogyrus minutus, Dactylogyrus vastator, Dactylogyrus anchoratus, Dactylogyrus lamellatus, Dactylogyrus aristichthys and Eudiplozoon nipponicum. Representatives of class Monogenea are commonly occurring during the winter (in 88.89 % of examined fish), followed in spring (56.47%), autumn (45.67%), and less in summer (39.78%). Based on the total number of fish examined from cyprinid aquaculture facilities in Macedonia, the highest prevalence (16.58 %) is determined with Dactylogyrus extensus in winter while the lowest (0.44 %) with Dactylogyrus aristichthys in spring and autumn. The highest mean intensity (90.40) is determined with Dactylogyrus aristichthys in autumn, while the lowest (1.89) with Dactylogyrus minutus and Eudiplozoon nipponicum in spring.

Key words: parasites, monogenean, aquaculture, cyprinid fish

## INTRODUCTION

Representatives of the class Monogenea are of great importance in fish pathology. Most of the Monogenea are ectoparasites with a direct life cycle. According to Buchmann & Bresciani monogenean (2006), trematodes are hermaphroditic plate worms that complete their life cycle in a single host. Most of them live mainly as ectoparasites on the gills or the outer surface of the body of freshwater or marine fish. The most recognizable morphological features of this group of parasites, according to which they can be identified, are the posterior adhesive apparatus, called the descriptor, and the attachment organ called the haptor.

Abowei et al. (2011) state that only a few of these parasites are viviparous. Everyone else is oviparous. Specially adapted so-called haptor and opishaptor enable their attachment to the host. Hooks and sucks are responsible for damage to the host, which allows entry for opportunistic pathogens. It occurs only in severe infestations with these parasites species.

Monogenea parasites in aquatic ecosystems are present in large numbers and manifest a clear specificity to their host - fish. They can cause enormous damage not only to the host but also to the aquatic environment. They are also considered to be a sensitive indicator of the health status of an ecosystem. Obiakezie & Taege (1991) state that monogenean can cause enormous damage to fisheries and aquaculture and thus contribute to high mortality and large losses of fish populations. Because monogenean trematodes are ectoparasites in fish, they are more exposed to changes caused by the physicochemical properties of the environment, i.e. the water to which they must adapt.

According to Koskivaara et al. (1991), Overstreet (1997), and Dušek et al. (1998), the number of monogenean decreases in polluted waters, compared to unpolluted waters. However, in waters with different trophic levels and degrees of pollution, the extent of the infestation is approximately the same, but there are differences in the intensity of infestation, which is significantly higher in polluted waters.

## MATERIALS AND METHODS

Samples of four fish species including common carp (Cyprinus carpio), grass carp (Ctenopharingodon idella), silver carp (Hypophtalmichthys molitrix), and bighead carp (Hypophtalmichthys nobilis) from the most significant and larger cyprinid aquaculture facilities in Macedonia, including fishponds and cage farms on reservoirs, were examined for parasitological investigations. This study was carried out by seasons, in three years. The specimens were placed in plastic tanks with fishpond water and immediately transferred to

the research laboratory or were inspected on the spot.

During the dissection, gills, fins, and skin were examined using stereomicroscopes "Zeiss"-Stemi DV4 and "MBS 10", as well as microscopes "Zeiss"- PrimoVert and "Reichart", at the Laboratory for fish diseases in Hydrobiological Institute - Ohrid (Macedonia).

All parasites found in each fish were identified and enumerated. During the study period, data on parasite species were categorized according to the season, including prevalence and mean intensity. Classical epidemiological variables (prevalence and mean intensity) were calculated according to Bush et al. (1997). The parasite specimens were identified using reference keys of Bauer (1985, 1987) and Gussev (1983).

## **RESULTS AND DISCUSSION**

During the parasitological examinations of the fish from the most significant and larger cyprinid aquaculture facilities in Macedonia, a total of 1134 fish samples were examined, from which parasite infestation with class Monogenea was determined in 656 fish, with a total prevalence of 57.85 % and a mean intensity of 6.84 (Table 1).

**Table 1.** Total prevalence and mean intensity with representatives of class Monogenea in cyprinid aquaculture facilities in Macedonia

Parasites	Number of examined fish	Number of infected fish	Mean intensity	Prevalence
Monogenean species	1134	656	6.84	57.85 %

During our research, seven (7) parasite species were identified from the representatives of class Monogenea, as following: Dactylogyrus extensus, Dactylogyrus minutus, Dactylogyrus vastator, Dactylogyrus anchoratus, Dactylogyrus aristichthys, Dactylogyrus lamellatus and Eudiplozoon nipponicum. Based on the total number of fish examined (1134), the highest prevalence (32.804 %) with monogenean parasites is confirmed with Dactylogyrus extensus, followed by Eudiplozoon nipponicum (10.670 %), *Dactylogyrus minutus* (6.702 %),

Dactylogyrus vastator and Dactylogyrus anchoratus (2.381 %, each), Dactylogyrus lamellatus (2.028 %) and Dactylogyrus aristichthys (0.882 %). The highest mean intensity with monogenean parasites is determined with Dactylogyrus aristichthys (70.000), followed by Dactylogyrus lamellatus (19.000), Dactylogyrus vastator (6.850), Dactylogyrus extensus (6.212), Dactylogyrus minutus (4.750), and Dactylogyrus anchoratus (3.852) and Eudiplozoon nipponicum (2.554). (Table 2).

	Parasites species	Mean intensity	Prevalence (%)
1	Dactylogyrus extensus	6.212	32.804
2	Dactylogyrus minutus	4.750	6.702
3	Dactylogyrus vastator	6.850	2.381
4	Dactylogyrus anchoratus	3.852	2.381
5	Dactylogyrus aristichthys	70.000	0.882
6	Dactylogyrus lamellatus	19.000	2.028
7	Eudiplozoon nipponicum	2.554	10.670

Table 2. Mean intensity and prevalence with determined monogenean parasites in cyprinid aquaculture facilities in Macedonia

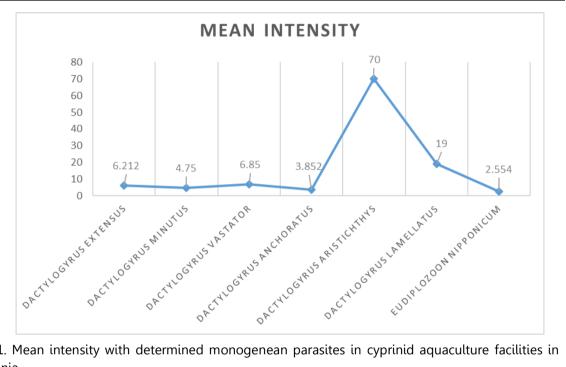


Figure 1. Mean intensity with determined monogenean parasites in cyprinid aquaculture facilities in Macedonia

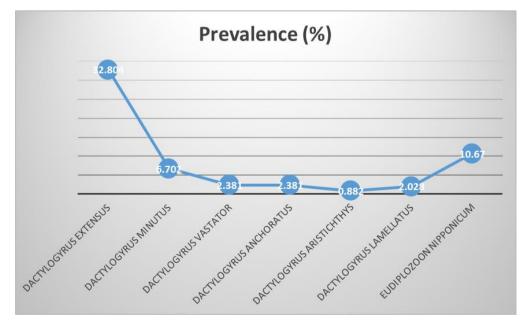


Fig. 2. Prevalence with determined monogenean parasites in cyprinid aquaculture facilities in Macedonia

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Table 5. An unesta	tion of monogenean parasites durin	y the seasons		
Season	Number of examined fish	Number of infected fish	% of infected fish	
Spring	278	157	56.475	
Summer	279	111	39.785	
Autumn	289	132	45.675	
Winter	288	256	88.889	

<b>Table 3.</b> An infestation of monogenean	parasites during the seasons

Representatives of the class Monogenea are commonly occurring during the winter (in 88.889 % of examined fish), followed in spring (56.475%), autumn (45.675%), and less in summer (39.785%). From the total number of examined fish, 656 fish are infested with parasites from the class Monogenea. By season, the number of infected fish is as follows: spring - 157; summer - 111; autumn - 132, and winter - 256. (Table 3).

**Table 4.** Prevalence (P) and mean intensity (I) with monogenean parasites in cyprinid aquaculture facilities in Macedonia, by seasons

Parasite species	Spring		Summer		Autumn		Winter	
ralasile species		P (%)	I	P (%)	I	P (%)	I	P (%)
Dactylogyrus extensus	4.063	7.055	2.562	2.822	5.500	6.349	8.021	16.578
Dactylogyrus minutus	1.889	0.794	4.792	4.233	3.400	0.596	6.929	1.235
Dactylogyrus vastator	/	/	/	/	/	/	6.850	2.381
Dactylogyrus anchoratus	/	/	/	/	/	/	3.852	2.381
Dactylogyrus aristichthys	49.600	0.441	/	/	90.400	0.441	/	/
Dactylogyrus lamellatus	7.333	0.794	/	/	26.500	1.235	/	/
Eudiplozoon nipponicum	1.889	4.762	2.387	6.526	3.694	11.728	/	/

Based on the total number of fish examined (1134), the highest prevalence (16.578 %) with parasites from the class Monogenea is determined with *Dactylogyrus extensus* in winter, while the lowest (0.441 %) with Dactylogyrus aristichthys in spring and autumn. The highest mean intensity (90.400) is determined with Dactylogyrus aristichthys in autumn, while the lowest (1.889) with Dactylogyrus minutus and Eudiplozoon nipponicum in spring. During our 5 monogenean parasites were research, identified in the spring, as follows: Dactylogyrus extensus, Dactylogyrus minutus, Dactylogyrus aristichthys, Dactylogyrus lamellatus and *Eudiplozoon nipponicum.* During the summer 3 monogenean seasons, parasites were determined: *Dactylogyrus extensus, Dactylogyrus* minutus and Eudiplozoon nipponicum. During the autumn seasons, 5 monogenean parasites Dactylogyrus were determined: extensus, Dactylogyrus minutus, Dactylogyrus aristichthys, Dactylogyrus lamellatus and Eudiplozoon nipponicum. During the winter seasons, 4 monogenean parasites were determined: Dactylogyrus extensus, Dactylogyrus minutus, Dactylogyrus and Dactylogyrus vastator anchoratus. By seasons, in spring, the highest prevalence (7.055 %) is determined with *Dactylogyrus extensus*, and the lowest (0.441 %) with Dactylogyrus aristichthys. On the other hand, the highest mean intensity (49.600) is determined with Dactylogyrus aristichthys, and the lowest (1.889) with Dactylogyrus minutus and Eudiplozoon nipponicum. In summer, the highest prevalence (6.526 %) is determined with Eudiplozoon nipponicum, and the lowest (2.822 %) with Dactylogyrus extensus. The highest mean intensity (4.792) is determined with Dactylogyrus minutus, and the lowest (2.387) with Eudiplozoon nipponicum. In autumn, the highest prevalence (11.728%) is determined with Eudiplozoon nipponicum and the lowest (0.441%) with Dactylogyrus aristichthys. The highest mean intensity (90.400) is determined with Dactylogyrus aristichthys, and the lowest (3.400) with Dactylogyrus minutus. In winter, the highest prevalence (16.578 %) is determined with *Dactylogyrus extensus*, and the lowest (1.235 %) with *Dactylogyrus minutus*. The highest mean

intensity (8.021) is determined with *Dactylogyrus extensus*, and the lowest (3.852) with *Dactylogyrus anchoratus* (Table 4).

<b>Table 5.</b> Prevalence and mean intensity with monogenean parasites in common carp ( <i>Cyprinus carpio</i> )
from cyprinid aquaculture facilities in Macedonia

	Parasite species	Number of infected fish	Mean intensity	Prevalence (%)
1	Dactylogyrus extensus	372	6.212	38.830
2	Dactylogyrus minutus	76	4.750	7.933
3	Dactylogyrus vastator	27	6.850	2.818
4	Dactylogyrus anchoratus	27	3.852	2.818
5	Eudiplozoon nipponicum	121	2.554	10.670

\* Prevalence is estimated according to the total number of examined fish of Cyprinus carpio (958)

**Table 6.** Prevalence and mean intensity with monogenean parasites in grass carp (*Ctenopharyngodon idella*) from cyprinid aquaculture facilities in Macedonia

	Parasite species	Number of infected fish	Mean intensity	Prevalence (%)
1	Dactylogyrus lamellatus	23	19.000	30.263
* Prevalence is estimated according to the total number of examined fish of <i>Ctenopharvngodon idella</i> (76)				

\* Prevalence is estimated according to the total number of examined fish of *Ctenopharyngodon idella* (76)

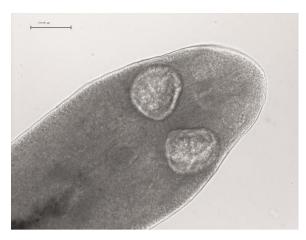
**Table 7.** Prevalence and mean intensity with monogenean parasites in bighead carp (Hypophtalmichthys nobilis) from cyprinid aquaculture facilities in Macedonia

	Parasite species	Number of infected fish	Mean intensity	Prevalence (%)
1	Dactylogyrus aristichthys	10	70.00	18.868
* Drevelance is activated according to the total graph of a consistent field of Aristichthy a schille (F2)				

\* Prevalence is estimated according to the total number of examined fish of *Aristichthys nobilis* (53)

By fish species, in common carp, the highest prevalence (38.830 %) is determined with Dactylogyrus extensus, while the lowest (2.818 %, each) with Dactylogyrus vastator and Dactylogyrus anchoratus. The highest mean intensity (6.850) is determined with *Dactylogyrus* vastator, while the lowest (2.554) with Eudiplozoon nipponicum (Table 5). Dactylogyrus lamellatus is the only monogenean parasite found in grass carp with a prevalence of 30.263% and a mean intensity of 19.000 (Table 6). aristichthys Dactylogyrus is the only monogenean parasite found in bighead carp with a prevalence of 18.868% and a mean intensity of 70.000 (Table 7). The family Dactylogyridae includes a large number of parasitic species that mainly parasitize the gills of freshwater fish. Dove & Ernst (1998) state that Dactylogyrus is one of the largest genera of parasitic helminths, 95% of which are gill parasites in fish of the family Cyprinidae. According to Woo (2006), the representatives of

the genus *Dactylogyrus* represent the largest group of fish ectoparasites and are of great importance for the pathology of fish. Young fish are more at risk of infection with these parasites, which can lead to disease and high mortality in lakes, but older fish are not excluded. Kearn (1994) states that in general, most species of the genus Dactylogyrus are strictly host-specific, or limited to one or more closely related hosts. Pietrock et al. (2001) found that the diversity of monogenean trematodes in fish decreases in polluted areas, probably because pollutants causing damage to the life of the leisureswimming stage of the parasite. On the other hand, according to Galli et al. (2001) and Lafferty & Kuris (2005) prevalence and intensity of infestation of monogenean in the fish gills increases in polluted areas due to increasing host acceptability.



**Figure 3.** *Eudiplozoon nipponicum* in common carp (glandular formations on the anterior part) (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)



**Figure 4.** *Dactylogyrus lamellatus* in grass carp - whole parasite (Blazhekovikj - Dimovska Dijana & Stojanovski Stojmir)

#### CONCLUSIONS

Seven representatives of monogenean parasites in cyprinid fish from the Macedonian aquaculture were identified in this study: Dactylogyrus extensus, Dactylogyrus minutus, Dactylogyrus vastator, Dactylogyrus anchoratus, Dactylogyrus Dactylogyrus aristichthys, lamellatus and Eudiplozoon nipponicum. A total of 1134 fish samples were examined, from which parasite infestation with representatives of the class Monogenea was determined in 656 fish, with a total prevalence of 57.85 % and a mean intensity of 6.84. Based on the total number of fish examined, the highest prevalence (32.804 %) with monogenean parasites is confirmed with Dactylogyrus extensus, while the lowest with Dactylogyrus aristichthys (0.882 %). The highest mean intensity with monogenean parasites is with Dactylogyrus determined aristichthys (70.000), while the lowest with *Eudiplozoon nipponicum* (2.554).

There is a close correlation between parasitic communities and the level of pollution of the water. In polluted environments, the degree of parasites prevalence and mean intensity can be an indicator of environmental guality. Because the level of water contamination can directly or indirectly affect the aquatic ectoparasites through the action of their intermediate host, ectoparasites that are directly exposed to water may be more sensitive to contaminants, thereby reducing their rates of survival and reproduction. The degree of activity of ectoparasites and endoparasites of the body surface and/or inside the fish body depend on the degree of water purity in combination with other environmental factors. In conditions of inadequate water quality that filled ponds, poor hygienic ponds conditions have a major contribution to the development of the parasitic disease.

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# PREDICTION OF COW'S FERTILITY BASED ON DATA RECORDED DURING THE PERIPARTURIENT PERIOD

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#### Abstract

The results of most studies show the beneficial effect of milking automation on production parameters of dairy cows, but its effect on fertility traits is debatable. Therefore, a study was undertaken to predict cow fertility – services per conception (SC) and calving interval (CI) – based on automatic milking system (AMS) data collected in the periparturient period subdivided into second and first week before calving, 1-4, 5-7, 8-14, 15-21 and 22-28 days of lactation. SC and Cl were predicted using daily indicators such as concentrate intake, number of milkings, cow box time, milking time, milking speed, colostrum and milk yield, composition, temperature and electrical conductivity. The study material was derived from the AMS management system and from the SYMLEK milk recording system. The analysis covered data for 16,329 milkings of 398 Polish Holstein-Friesian cows, which were used in 3 AMS herds. The collected numerical data were statistically analysed by decision tree technique. It has been established that the decision tree method may help breeders, already during the postcalving period, to choose the level of factors associated with AMS milking, which will ensure good fertility of cows in a herd. The most favourable number of services per conception are to be expected from cows that were milked <1.6 times per day from 1 to 4 days of lactation and electrical conductivity of their colostrum did not exceed 69 mS during that time. In turn, shortest Cl (366 days) will be characteristic of the cows whose average daily colostrum yield did not exceed 20.2 kg and their daily concentrate intake from 8 to 14 days of lactation was at least 5.0 kg.

Key words: Fertility, Dairy cattle, Correlation, Data mining, Forecasting

# THE EFFECT OF TOXIC SUBSTANCES OF ANIMAL FEED ON SEMEN QUALITY

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#### Abstract

This study prepared from comprehensively present disorders of the reproductive system in ruminants exposed to contact with toxic materials, such as toxic metals, microbiological threats, environmental substances. Heavy metal collects in liver and kidney also in muscles and fat tissue. Have a negative impact on the fertility of animals. Microbiological threats on cattle Livestock by counting and identifying the microorganisms. Balanced nutrition during pre-weaning and post-weaning stages of male calves has a significant impact on testicular steroidogenesis and gonadotropins releasing hormone which ultimately determine the bull fertility. In herbal substances, Especially, Gossypol, produced by cotton plant. Toxic metals such as lead (Pb), arsenic (As), mercury (Hg), and cadmium (Cd) can damage spermatogenesis, and cause apoptosis of sperm and oxidative reactions. Chromium (Cr), arsenic (As), and cadmium (Cd) are excreted in the urine, while lead can be detected by examining the blood of animals, while in milk, arsenic (As), cadmium (Cd), nickel (Ni), and lead (Pb) can be detected. To evaluate microbiological threats on cattle breeding premises by counting the microorganisms (via culturing and high-throughput sequencing), assessing the endotoxin concentrations (via gas chromatography-mass spectrometry [GC-MS]). Gossypol exists in two forms, (+) and (-). (±)-Gossypol is found in cottonseed and cottonseed products in two forms: free gossypol, which is readily extractable with solvents, and bound gossypol. The latter bounded with proteins, we can free gossypol with heating and extracting. Environmental toxins may exert estrogenic and/or anti-androgenic effects, which in turn alter the hypothalamic-pituitary-gonadal axis (HPGA), induce sperm DNA damage, or cause sperm epigenetic changes. It is important to monitor environmental pollution with toxic metals. Detected 172 and 210 bacterial/archaeal genera and 89 and 43 fungal genera in the dust and in the air, respectively. The dust samples were found to contain endotoxin concentrations of 98.98–178.31 nmol LPS mg-1 and a cytotoxicity of 5.66-13.99 mg mL-1. Gossypol has inhibition effects on spermatogenesis, embryo development and increased erythrocyte fragility occur at doses of 6-18 mg/kg b.w. per day in cattle and cardiomyopathy in lambs at 2-3 mg/kg b.w. per day. Toxic metals do not breakup in the environment to resolution this particle biological or chemical methods should be used. Understanding the presence and underlying mechanism of these toxins will help us to keep the integrity of the male reproduction system and formulate better regulations against their indiscriminate use. Gossypol is transferred to edible parts, muscle and offal of ruminants. Bull fertility is complicated and sensitive.

Key words: Semen, Fertility, Toxic, Feed

# OSMOLALITY EFFECTS OF EXTENDER MEDIA IN CANINE SEMEN

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## Abstract

The most important factor in Canine semen cryoconservation is the best extender used as a cryoprotectant to have a similar osmolarity and pH compared to the seminal plasma to enable sperm survival. Egg yolk is considered an excellent cryoprotectant for sperm of different animal species. The low-density lipoproteins (LDL), contained in EY, show a mobility up to 54.5% which is the best combination found. According to prior endorsement, the future of canine sperm cryopreservation is expected in liposome use and synthetic substances which mimics LDL role. Egg yolk (EY) is conventionally used to reduce sperm cryodamage. Recent investigations have designed to evaluate the dose-dependent protection of the EY against osmotic and cryogenic damage of dog sperm. In both hypo- and hyper-osmotic conditions, EY had a protective effect regardless of concentration. Nevertheless, new studies demonstrated that EY reduces osmotic and cryogenic damage when used at 5 % or 10% concentrations and these concentrations can be used to protect dog spermatozoa more effectively than the conventionally used concentration (20%). Also it is known that calcium is one of the main modulators of physiologic capacitation and acrosome reaction (AR), which influx from sperm environment and from its intracellular reservoirs. We can evaluate the effect of reducing free calcium in the cryopreservation medium by using the calcium chelator ethylene diamine tetracetic acid (EDTA) at 0.3% and 0.5% concentrations. Calcium concentration reduced in EDTA 0.3% and all the calcium ions were chelated in EDTA 0.5%. Nonetheless, the partial or complete calcium removal, through EDTA addition, is not able to prevent acrosomal damage or premature acrosomal reaction and therefore does not improve the dog sperm binding ability.

Key words: Semen, Extender, Osmolarity, Canine

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Country	n	%
Algeria	3	4.69
Belgium	2	3.12
Cameroon	2	3.12
Egypt	1	1.56
Germany	1	1.56
Greece	2	3.12
Indonesia	1	1.56
Iran	4	6.25
Jordan	2	3.12
Kyrgyzstan	1	1.56
Macedonia	1	1.56
Nigeria	1	1.56
North Macedonia	1	1.56
Pakistan	3	4.69
Poland	6	9.38
South Africa	3	4.69
Turkey	30	46.88
TOTAL	64	100

# **Nationality of Presenters**

Country	n	%
Algeria	11	8.94
Belgium	1	0.81
Cameroon	5	4.07
China	1	0.81
Egypt	2	1.63
Germany	1	0.81
Greece	6	4.88
Indonesia	4	3.25
Iran	6	4.88
Jordan	3	2.44
Kyrgyzstan	2	1.63
Macedonia	4	3.25
Nigeria	5	4.07
North Macedonia	2	1.63
Pakistan	10	8.13
Poland	5	4.07
Russia	1	0.81
Slovakia	1	0.81
South Africa	4	3.25
Turkey	46	37.4
USA	3	2.44
TOTAL	123	100

# Nationality of Authors