Proceedings of the
ICABGEH-22

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

October 03 - 05 2022 in Samsun, TÜRKİYE

Editors:
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ISBN: 978-605-06447-2-2

Proceedings of the ICABGEH-22

Samsun, TÜRKİYE

October 03 - 05, 2022

Organized by

Proceedings of the VI. International Congress on Domestic Animal Breeding Genetics and Husbandry 2022 "ICABGEH-22"

Publisher:

Black Sea Journals

E-Book Layout, Preparation and Composition:

Hasan ÖNDER and Ugur SEN

All published articles were peer-reviewed by Scientific Referee Committee

The organizers do not have any legal liability for to contents of the presentation texts

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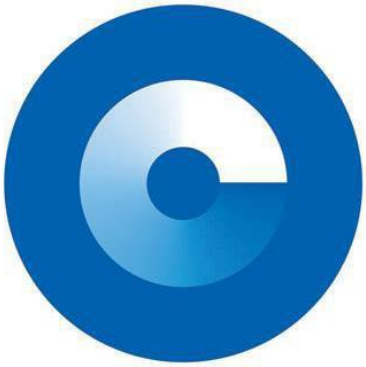


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PREFACE

This volume contains the papers presented at the VI. International Congress on Domestic Animal Breeding Genetics and Husbandry - 2022 (ICABGEH-22) was held on October 03-05, 2022, Samsun, TÜRKIYE.

The ICABGEH-22 has been organized by the Agricultural Faculty of Ondokuz Mayıs University and Animal Breeding and Biology Faculty of Bydgoszcz University of Science and Technology. ICABGEH-22 is the sixth international event of the congress series with the participation of top-rated invited speakers; Prof. Dr. Kaspar BIENEFELD (Humboldt University, Germany), Prof. Dr. Hakan SAGIRKAYA (Uludag University, Türkiye), Prof. Dr. Sergio LEDDA (University of Sassari, Italy), Prof. Dr. Dominique FRANCOIS (INRA French National Institute, France) and Prof. Dr. Edit MIKÓ (University of Szeged, Hungary). This event has been planned to bring together leading researchers, engineers, and scientists in animal science worldwide. It also provided opportunities for the delegates to exchange new ideas and application experiences, establish business or research relations, and find global partners for future collaboration. The organizing committee has done severe planning and preparation to ensure that the Turkish and international animal science scientific community meets the challenges and moves safely and successfully into the advanced information era. To this end, ICABGEH-2022 has focused on recent developments and research in animal science to protect the environment and food safety. Thus, ICABGEH-2022 has achieved its main twofold objective: Firstly, the presentation of current research works in the field of animal science, and secondly, connecting the animal science community.

Prof. Dr. Hasan ONDER,

President of ICABGEH-22

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LYOPHILIZED EXTENDER SUPPLEMENTED WITH RAINBOW TROUT (ONCORHYNCHUS MYKISS) SEMINAL PLASMA IMPROVES CRYOPRESERVATION OF RAM SPERM

Burcu USTUNER [1], Selim ALCAY [1], Elif GOKCE [2], M. Melih YILMAZ [1], Ahmet AKTAR[1], *Oguzhan HURAYDIN [1*]*, Muhammed DUMAN [3], N. Tekin ONDER[4], Eser AKAL[5], Zekariya NUR [1]

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Abstract

Ram semen cryopreservation successfully had been tried with different protocols and diluents for years. To achieve this, we approached in this study with a quite different way. The uniqueness of our approach was to investigate the effect of the Rainbow trout seminal plasma (RTSP) supplemented (control, 10% or 15%) lyophilized extender on freezability of ram semen. Collected semen was pooled and split into two aliquots, and each of the ejaculates was diluted with fresh or lyophilized extenders with RTSP (0%, FC and LC; 10% F10 and L10 or 15% F15 and L15) using two-step dilution method. Semen was frozen using the programmable freezing machine. Semen samples were examined for sperm motility, defective acrosomes, plasma membrane integrity and DNA fragmentation at native and post-thaw stage. The highest percentages of post-thaw motility and plasma integrity were observed in the F10 (52.67±1.28%, 75.6±1.36%), F15 (54.23±1.59%, 76.00±4.32%), and L15 (46.15±1.29%, 68.00±3.90%) (P<0.05). There was no significant difference in the rate of post-thaw defective acrosomes when the other extender groups were compared with the control. The highest percentage of post-thaw DNA fragmentation values were observed in the FC group (13.0±0.71%), while the lowest DNA fragmentation was obtained in the F15 (8.80±0.38%) but only significant different from FC and LC groups. In conclusion, the findings of this study show that the lyophilized extender with 15% RTSP added can be used successfully for freezing ram semen. It is an advantage that the lyophilization of this extender is more practical than the extender that is prepared fresh daily. Hereby, frozen semen will have the opportunity to be used more widely in the field.

Key words: *Cryopreservation, Lyophilized extender, Rainbow trout seminal plasma, Ram semen*

DETERMINATION OF GENE EXPRESSION LEVELS OF MYOGENIC FACTOR 5 IN HAIR, ANGORA, HONAMLI AND KILIS GOAT BREEDS

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Abstract

The myogenic factor 5 (Myf5) gene, one of the myogenic regulatory factors, acts as the primary regulator of skeletal muscle myogenesis (formation of muscle tissue) and is among the genes that regulate the development of skeletal muscle cells and subsequent muscle fiber differentiation. The main aim of this study was, therefore, to determine expression levels of the Myf5 gene in the Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles from 90-day weaning-aged male kids born to Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) Turkish indigenous goat breeds. RNA of muscle samples was isolated by a commercial RNA purification kit as suggested by the manufacturer. The purity and quantity of isolated RNA were measured using NanoDrop™ 2000/2000c spectrophotometer, and 1 % w/v agarose gel was used to check the RNA quality. Isolated RNA was converted to cDNA using a commercial cDNA kit in the Thermal Cycler device. Myf5 gene expression level in LD and ST skeletal muscles was determined by real-time quantitative polymerase chain reaction. GAPDH was selected as a housekeeping gene to normalize the expression of the Myf5 gene. Honamli kids had a more remarkable ($p<0.05$) Myf5 gene expression level (159.8 ± 14.6-fold change), while Hair kids had a lower ($p<0.05$) Myf5 gene expression level (42.0 ± 14.9-fold change) in LD muscle. Similarly, Hair kids had a lower ($p<0.05$) Myf5 gene expression level (57.2 ± 6.6-fold change) than those of Honamli (98.4 ± 9.2-fold change) and Kilis (80.7 ± 6.8-fold change) in ST muscle. The results of the present study indicated that Honamli kids breeds might have higher muscle growth due to their higher Myf5 gene expression level, and this breed may be suggested for a more efficient fattening practice. In conclusion, Turkish indigenous goat breeds have Myf5 gene expression levels, and these differences may be used to select more efficient breeds for fattening practices.

Key words: *Native breeds, MDF gene family, Skeletal muscle, Meat production, Kid*

INTRODUCTION

People need to consume food to meet their biological needs and maintain their vital activities. Therefore, food consumption is one of the most basic needs of people. The diversity and required consumption amount is also extremely important as well as adequacy of consumption of foods consumed by individuals (Aydoğdu et. al. 2018). Depending on the growing population in the world and Turkey, food consumption is also increasing. This creates pressure in terms of quantity, quality and adequacy on foods. Today, due to the increase in the world population, the nutritional and food problem is also increasing. The meat in the healthy nutrition of the society, the location of red meat in the total meat consumption, especially for individuals who are in the age of development is very important. Proteins are nutrients that cannot be stored in the body and must be taken from outside. For healthy and balanced nutrition, at least half or 2/3 of the proteins required to be taken daily should be from animal origin foods. Nutrition is therefore a matter of concern to all actors of society (Aydoğdu et. al. 2018).

One of the important red meat production sources in Turkey is goat and kid meat. Turkey ranks first among European countries and 22nd in the world in terms of goat population with approximately 11 million goats (TUIK, 2020; FAOSTAT, 2020). Turkey accounts for 46% of the goats in European countries (FAOSTAT, 2020). While the native gene sources of Hair, Angora, Honamlı and Kilis constitute approximately 92% of Turkey's current goat population, the share of other indigenous and cultural breeds is very low (Yılmaz et al., 2012; Akbaş et al., 2016; Şen et al., 2019). Therefore, Hair, Angora, Honamlı and Kilis domestic goat breeds are the most important and most widely grown goat breeds in our country.

In Turkey, goat breeding is carried out completely according to the extensive breeding system based on pasture conditions, and when additional feeding grazing areas cannot be used, very little (usually wheat straw) is made, and concentrated feed is almost never given (Yılmaz

et al., 2012; Daskiran and Koluman, 2014; Şen). et al., 2019). In addition, in our country, no breeding practices specific to meat production (feeding material selection and indoor kid fattening) are carried out or remain at a limited level in goat breeding. Therefore, there is very limited information about the indoor fattening (intensive breeding) potential of the animals to be used in meat production of our domestic goat breeds.

Myogenic regulatory factors (MDF) act as master regulators of skeletal muscle myogenesis, regulating the development of skeletal muscle precursor cells and subsequent muscle fiber differentiation and development by the expression of their encoded genes (Zhong et al., 2013). In addition, MDFs play an important role in determining the expression of many different genes (growth hormone and its receptor, IGF-I, myostatin; MSTN, myosin heavy chain; MyHC isoforms) associated with economic characteristics such as growth, development, meat yield, etc. in the postnatal period (Huang et al., 2016; Polin et al., 2016).

It is known that MDFs regulate myogenesis from stages in the formation, development and proliferation of muscle fibers to postnatal muscle maturation, differentiation and function (Zhong et al., 2013; Patel et al., 2014; Siqin et al., 2017). Among MDF family members, Myf5 is considered the first expressed MDF and is regulated by a 140 kb enhancer complex in its regulatory region (Carvajal et al., 2008). Braun and Arnold (1995) reported that the Myf5 gene from the MDF family is responsible for the development and proliferation phase of the muscle fibers in the early formation process.

As a result, studies have reported that the MDF gene family has significant effects on skeletal muscle development in the fetal and postnatal periods and even on the development and growth process in farm animals, indicating that the MDF gene family may have an effect on meat yield. Although studies on the determination of growth, meat yield and fattening performance of Hair, Angora, Honamlı and Kilis goat breeds show that these breeds have different phenotypic values, the basic mechanism of this difference has not been revealed, it is based on racial characteristics or environmental factors.

Therefore, determining the expression levels of the MDF gene family, which is effective on skeletal muscle development in our domestic goat breeds, which are an important source of red meat in our country, can provide important information about more descriptive and clear determination of the meat production potential of our domestic goat breeds and increasing meat yield. In addition, the fact that the expression levels of gene families responsible for muscle fiber development in our domestic goat breeds will be determined for the first time, paving the way for the elimination of a deficiency in this area, and it will also contribute to the competition with the countries where animal husbandry is developed in terms of determining the meat production potential of domestic breeds.

Due to all these facts, the current study aims to determine the expression levels of Myf5 and Myf6 genes, which are myogenic regulatory factors in the LD and ST skeletal muscles of 90-day-old weaning age boys belonging to Kıl, Tiftik, Honamlı and Kilis breeds, which are among the domestic goat gene sources of Turkey and constitute a large part of the goat existence.

MATERIAL AND METHOD

In the study, samples of Longissimus dorsi (LD) skeletal muscle - 80 °C stored after slaughter from Hair (n=6), Angora (n=6), Honamlı (n=6) and Kilis (n=6) male aged 3 months purchased for the muscle fiber characterization of our domestic goat breeds (Hair, Angora, Honamlı and Kilis) will be used as study material.

RNA Extraction and Reverse Transcription

Total RNA was isolated from muscle samples using Trizol reagent according to Chomczynski and Sacchi [1987]. The contamination of genomic DNA was removed by treating total RNA with RNase-free DNase , according to the manufacturer's recommendations. The quantity and quality of total RNA preparations were evaluated spectrophotometrically at 260 nm. The purity of total RNA was determined by the A260/280 and A260/230 ratio and its integrity was checked electrophoretically using 1% formaldehyde denaturing gel (Huang et al., 2016).

Obtained band images will be examined and quality control will be made according to the gel image of 28s - 18s RNA bands. The isolated total RNA samples will be diluted to 1µg/µl and cDNA synthesis from RNA will be performed with the cDNA synthesis kit with the help of reverse transcriptase and designed primers. Obtained cDNA samples will be stored at 20 °C until quantitative real-time polymerase chain reaction (qRT-PCR).

RT-qPCR Analyses

Mfy5 and Mfy6 gene expression levels will be examined by quantitative real-time polymerase chain reaction (RT-qPCR) method. Synthetic oligonucleotide primers designed considering the conserved sequences of the Mfy5 and Mfy6 genes will be used. Primers to be used for amplification of target genes with the RT-qPCR

method were either designed using online tools based on the relevant gene sequences of goats (http://simgene.com/Primer3 and http://www.ncbi.nlm.nih.gov/tools/primer- blast/) or from related studies (Table 1).

GAPDH primers were chosen as the housekeeping gene to normalize the expression of target genes (Table 1). RT-qPCR was performed using the SYBR Green master mix. During the process, SYBR Green mix dye binds to double-stranded DNA and as the number of double-stranded DNA in the medium increases, that is, as the amount of amplified cDNA increases, the amount of fluorescence obtained also increases. PCR protocol used in RT-qPCR application; initial DNA denaturation 2 min. (96 °C), denaturation 35 cycles (96 °C), ligation 1.5 min, extension 45 sec. (72°C), fluorescence analysis (72°C). All procedures were repeated at least 3 times in terms of the reliability of the results in RT-qPCR applications.

Standards at different concentrations (1, 1/10, 1/100 and 1/1000) were used to calculate the RT-qPCR efficiency. Values between E=1.60 and 2.10 for RT-qPCR efficiency were used in the study. Expression levels of Mfy5 and Mfy6 genes were compared with the expression level of the reference gene, and their expression levels were determined by making them suitable for normal distribution.

The 2-ΔΔCt method was used to analyze RNA expression levels (Huang et al., 2016).

Table 1. Primers used in qPCR analysis

Genler	Primer Sekansları		Ürün Boyutu	Gen Bankası
	Forward	Reverse		
Mfy5	CACGACCAACCCTAACCAGAG	TCTCCACCTGTTCCCTTAGCA	101 bp	JF829004 (Zhong vd.,2013)
Mfy6 (MRF4)	CGGAGCGCCATTAACTACAT	AAATCCGCACCCTCAAGATT	101 bp	NM_001285602 (Huang vd., 2016)
GAPDH	GCA AGT TCC ACG GCA CAG	TCA GCA CCA GCA TCA CCC	118 bp	AF035421 (Cheng vd., 2012)

Figure 1. Sequence Palindrome with no rotational symmetry, with a mirror-like palindrome on the left, and a double-stranded palindrome on the right.

However, in palindromes with rotational symmetry, the second half of the complementary DNA double helix is the mirror image of base sequence in the first half of another strand (see Figure 5). That is, the nucleic acid sequences in both strands of the DNA helix are the same when read from either 5′ or 3′ end of both strands in DNA duplex.

RESULTS AND DISCUSSION

Birth and corrected 90-day weaning weights of Honamlı, Kıl, Kilis and Angora kids are presented in Table 2. In the study, there was a statistically significant difference between breeds in terms of kid's birth weight, and it was determined that the birth weight of the kids belonging to the Honamlı breed was higher than the kids of the Hair, Kilis and Angora breeds (P < 0.05). Similarly, 90-day corrected weaning weight and total body weight gain up to weaning age of Honamlı kids were found to be higher (P < 0.05) than Kilis and Angora kids, excluding kids from Kilis breed.

Table 2. Birth and corrected 90-day weaning weights of Honamlı, Kıl, Kilis and Angora kids.

	Breeds			
	Honamlı	Kıl	Kilis	Angora
BW	3,55±0,30[a]	2.90±0,29[b]	2.66±0,16[b]	2.95±0,11[b]
WW	20,40±1,03[a]	18,24±1,68[a]	14,20±0,21[b]	13,03±0,26[b]
Differnt	16,85±0,93[a]	15,34±0,87[a]	11,54±0,18[b]	10,08±0,23[b]

BW = kid's birth weight, WW = 90 days adjusted weaning weight.

Total DNA and RNA amounts and RNA-DNA ratio in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı, Kıl, Kilis and Angora kids are presented in Table 3. In the study, when the total DNA amount of LD muscle was compared, it was determined that Honamlı kids had the highest (P < 0.05) DNA amount and Angora kids had the lowest (P < 0.05) DNA amount. Similarly, total DNA amount of ST muscle was found to be highest (P < 0.05) in Honamlı kids and lowest (P < 0.05) in Angora and Kilis kids.

In the study, when the total RNA amount of LD muscle was compared, it was determined that the kids of the Hair and Honamlı breeds had the highest (P < 0.05) RNA amount, while the kids of the Angora and Kilis breeds had the lowest (P < 0.05) RNA amount. Interestingly, the total amount of RNA in ST muscle was highest (P < 0.05) in Honamli kids when the breeds were compared. In the study, it was determined that the Angora breed kids had the highest (P < 0.05) RNA/DNA ratio in the LD muscle, while the Honamlı and Kilis kids had the lowest (P < 0.05)

RNA/DNA ratio. Similarly, it was determined that Angora kids had a higher (P < 0.05) RNA/DNA ratio in ST muscle than Kilis and Honamlı kids, except Kilis kids.

Table 3. Total DNA and RNA amounts and RNA-DNA ratio in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı, Kıl, Kilis and Angora kids

		Irk			
		Angora	Kıl	Honamlı	Kilis
DNA (mg/g)	LD	0,44±0,08^{c}	0,94±0,22^{b}	2,26±0,955^{a}	1,25±0,26^{b}
	ST	0,41±0,09^{c}	1,08±0,35^{b}	1,80±0,30^{a}	0,59±0,13^{c}
RNA (mg/g)	LD	0,76±0,08^{b}	0,96±0,16^{a}	0,99±0,07^{a}	0,39±0,02^{b}
	ST	0,57±0,09^{b}	0,59±0,08^{b}	0,94±0,12^{a}	0,51±0,04^{b}
RNA/DNA	LD	1,73±0,28^{a}	1,02±0,21^{b}	0,44±0,10^{c}	0,31±0,09^{c}
	ST	1,39±0,29^{a}	0,55±0,13^{b}	0,52±0,15^{b}	0,86±0,20^{ab}

^{a,b,c} The difference between the means shown with different letters in the same row is significant (P < 0.05).

The expression levels of the myogenic factor 5 (Myf 5) gene in the Longissimus-dorsi (LD) skeletal muscle of Honamlı, Kılis, Kilis and Angora are presented in Figure 4.1. In the study, there were statistically significant differences between breeds in terms of the level of expression of the Myf 5 gene in the LD muscle, and the highest (P < 0.05) Myf 5 gene expression level was determined in the Honam breed and the lowest (P < 0.05) gene expression level was detected in the Hair. The Honamlı race expressed the Myf 5 gene in the LD muscle about 62.6, 67.0 and 117.8 times more (P < 0.05) than the Hair, Kilis and Angora kids, respectively. As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of the Myf 5 gene in the LD muscle of Honamlı, Kılis, Kilis and Angora r were calculated as 159±8.14.6, 97.2±18.6, 92.8±18.9 and 42.0±14.9 times change, respectively.

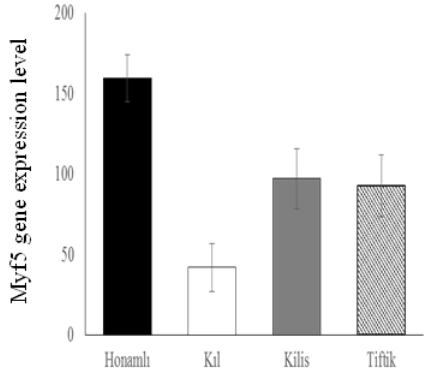

Figure 2. Expression levels of myogenic factor 5 (Myf 5) gene in Longissimus-dorsi skeletal muscle of Honamlı, Hair, Kilis and Angora kids. The bars above the bars represent the standard error of the means and the difference between bars with different letters is significant (P < 0.05).

The expression levels of the myogenic factor 5 (Myf 5) gene in the Semitendinosus (ST) skeletal muscle of Honamlı, Kılis, Kilis and Angora are presented in Figure 2. In the study, there were statistically significant differences between the breeds in terms of the expression level of the Myf 5 gene in the ST muscle, and it was found that the kids belonging to the Hair race had lower (P < 0.05) gene expression levels than the kids belonging to the Honamlı and Kilis breeds, except for the Angora. As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of the Myf 5 gene in the ST muscle of Honamlı, Kılis, Kilis and Angora were calculated as 95.4±9.2, 57.2±6.6, 80.7±6.8 and 73.7±13.1 fold changes, respectively. According to these results, the kids belonging to the Hair race expressed the Myf 5 gene in the ST muscle approximately 38.2, 23.5 and 16.6 times less (P < 0.05) than the Honamlı, Kilis and Tiftik, respectively.

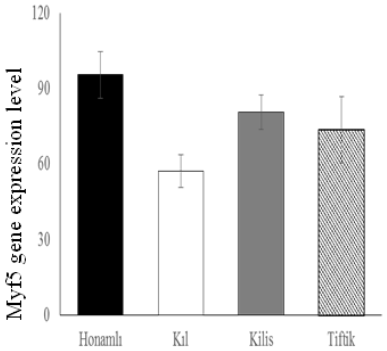

Figure 3. Expression levels of myogenic factor 5 (Myf 5) gene in Semitendinosus skeletal muscle of Honamlı, Hair, Kilis and Angora kids. The bars above the bars represent the standard error of the means and the difference between bars with different letters is significant (P < 0.05).

The expression analysis of MYF6 showed that its polymorphism may be important for the myotube fusion, maturation and maintenance of the skeletal muscle weight [Wyszynska-Koko et al. 2006]. They identified significant correlation between the MYF6 polymorphism identified in the promoter region, exon 1 and carcass weight. However, several studies on MYF5, MYF6 showed that their polymorphism does not affect the expression of MRF genes [Ernst et al. 1994, Stratil and Cepica 1999, Te Pas et al. 1999a, Cieslak et al. 2002, Vykoukalova et al. 2003, Urbanski and

Kuryl 2004, Wyszynska-Koko and Kuryl 2004, Urbanski et al. 2006].

However, these results suggest that the effects of age and breed-specific expression of MYF5 and MYF6 observed in this study could also represent indirect effects of another miogenic regulatory mechanism expressed in goat skeletal muscle. Nevertheless, the existence of numerous regulatory elements at large distances to MYF5 and MYF6 pointed to a very complex pattern of these genes regulation, which show also significant differences between species [Maak et al. 2006]. Furthermore, several investigations suggest that MYF5 and MYF6 genes may be expressed at a very low level in myofibres (Te Pas et al. 2005a, Te Pas et al. 2005b, Wyszynska-Koko et al. 2006]. Therefore, the observed variation of age/breed-dependent expression of MYF5 and MYF6 genes in goat skeletal muscles may represent a mix of transcriptional activity of satellite cells and myofibres.

Pierzchała et al. ın 2011, general postnatal expression of MYF5 in porcine skeletal muscle did not differ significantly between pig breeds and ages. In contrast, MYF6 expressed significant differences at the transcriptional level of the investigated muscles. Recently, significant differences between MYF6 expression levels in pig skeletal muscles (semimembranosus, biceps femoris, and gracilis) have been reported by RopkaMolik et al. They found it in 2010. Among the three ham muscles, they found the highest mRNA level of MYF6 gene in the gracilis muscle of all breeds, but the significant ($P<0.01$) difference was found only in Pietrains.

Pierzchała et al. reported in 2011 that the expression of the MYF6 gene in the skeletal muscle of PIE was higher than in the growth of other breeds and that their findings in the skeletal muscles of pigs were consistent with those indicated in his 2010 study, as they found that there was no significant relationship between age and the level of expression of the MYF5 and MYF6 genes. Pierzchała et al. Results of their study in 2011 also suggested that, as in the PIE breed, higher MYF6 gene expression may be associated with higher muscularity of carcasses.

This study The significantly different expression was presented of MYF5 and MYF6 in goat skeletal muscles. To our knowledge, this study is one of the first analyses of age- and breed-dependent relations of goat MYF5 and MYF6 expressed in postnatal period of skeletal muscle growth.

Significant differences between MYF5 and MYF6 expressions allowed us to select both candidate genes for further trait-associated studies. The further identification of causal polymorphism and determination of functional role are even more challenging, since there are many different molecular mechanisms through which expression activity of specific genes in myogenic cells can be regulated.

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DETERMINATION OF GENE EXPRESSION LEVELS OF MYOGENİC FACTOR 6 IN SOME TURKISH NATIVE GOAT BREEDS

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Abstract

The myogenic factor 6 (Myf6) gene, which is one of the myogenic regulatory factors, acts as the main regulator of skeletal muscle myogenesis (formation of muscle tissue) and is among the genes that regulate the development of skeletal muscle cells and subsequent muscle fiber differentiation. The main aim of this study was, therefore, to determine expression levels of the Myf6 gene in the Longissimus-dorsi (LD) and Semitendinosus (ST) skeletal muscles from 90-day weaning-aged male kids born to Angora (n=6), Hair (n=6), Honamli (n=6) and Kilis (n=6) Turkish indigenous goat breeds. RNA of muscle samples was isolated by a commercial RNA purification kit as suggested by the manufacturer. The purity and quantity of isolated RNA were measured by using NanoDrop™ 2000/2000c spectrophotometer, and 1 % w/v agarose gel was used to check the RNA quality. Isolated RNA was converted to cDNA using a commercial cDNA kit in the Thermal Cycler device. Myf6 gene expression level in LD and ST skeletal muscles was determined by real-time quantitative polymerase chain reaction. GAPDH was selected as a housekeeping gene to normalize the expression of the Myf6 gene. Kilis kids had a lower ($p<0.05$) Myf6 gene expression level (12.0 ± 2.9-fold change) compared to Angora (39.8 ± 10.1-fold change), Hair (34.2 ± 7.5-fold change), and Honamli (44.5 ± 11.3-fold change) kids in LD muscle. Similarly, Kilis kids had a lower ($p<0.05$) Myf6 gene expression level (57.2 ± 6.6-fold change) than those of Angora (59.2 ± 16.0-fold change), Hair (28.3 ± 7.9-fold change) and Honamli (60.1 ± 16.4-fold change) kids in ST muscle. Additionally, the Myf6 gene expression level in ST muscle was higher ($p<0.05$) in Angora and Honamli kids. The results of the present study indicated that Kilis kids breeds might have lower muscle growth due to their lower Myf6 gene expression level. This breed may not be recommended as a fattening material. In conclusion, Turkish indigenous goat breeds have Myf6 gene expression levels, and these differences may be used to select more efficient breeds for fattening practices.

Key words: *Native breeds, MDF gene family, Skeletal muscle, Meat production, Kid*

INTRODUCTION

People need to consume food to meet their biological needs and maintain their vital activities. Therefore, food consumption is one of the most basic needs of people. The diversity and required consumption amount is also extremely important as well as adequacy of consumption of foods consumed by individuals (Aydoğdu et. al. 2018). Depending on the growing population in the world and Turkey, food consumption is also increasing. This creates pressure in terms of quantity, quality and adequacy on foods. Today, due to the increase in the world population, the nutritional and food problem is also increasing. The meat in the healthy nutrition of the society, the location of red meat in the total meat consumption, especially for individuals who are in the age of development is very important. Proteins are nutrients that cannot be stored in the body and must be taken from outside. For healthy and balanced nutrition, at least half or 2/3 of the proteins required to be taken daily should be from animal origin foods. Nutrition is therefore a matter of concern to all actors of society (Aydoğdu et. al. 2018).

One of the important red meat production sources in Turkey is goat and kid meat. Turkey ranks first among European countries and 22nd in the world in terms of goat population with approximately 11 million goats (TUIK, 2020; FAOSTAT, 2020). Turkey accounts for 46% of the goats in European countries (FAOSTAT, 2020). While the native gene sources of Hair, Angora, Honamlı and Kilis constitute approximately 92% of Turkey's current goat population, the share of other indigenous and cultural breeds is very low (Yılmaz et al., 2012; Akbaş et al., 2016; Şen et al., 2019). Therefore, Hair, Angora, Honamlı and Kilis domestic goat breeds are the most important and most widely grown goat breeds in our country.

In Turkey, goat breeding is carried out completely according to the extensive breeding

system based on pasture conditions, and when additional feeding grazing areas cannot be used, very little (usually wheat straw) is made, and concentrated feed is almost never given (Yılmaz et al., 2012; Daskiran and Koluman, 2014; Şen). et al., 2019). In addition, in our country, no breeding practices specific to meat production (feeding material selection and indoor kid fattening) are carried out or remain at a limited level in goat breeding. Therefore, there is very limited information about the indoor fattening (intensive breeding) potential of the animals to be used in meat production of our domestic goat breeds.

Myogenic regulatory factors (MDF) act as master regulators of skeletal muscle myogenesis, regulating the development of skeletal muscle precursor cells and subsequent muscle fiber differentiation and development by the expression of their encoded genes (Zhong et al., 2013). In addition, MDFs play an important role in determining the expression of many different genes (growth hormone and its receptor, IGF-I, myostatin; MSTN, myosin heavy chain; MyHC isoforms) associated with economic characteristics such as growth, development, meat yield, etc. in the postnatal period (Huang et al., 2016; Polin et al., 2016).

It is known that MDFs regulate myogenesis from stages in the formation, development and proliferation of muscle fibers to postnatal muscle maturation, differentiation and function (Zhong et al., 2013; Patel et al., 2014; Siqin et al., 2017). Among MDF family members, Myf5 is considered the first expressed MDF and is regulated by a 140 kb enhancer complex in its regulatory region (Carvajal et al., 2008). Braun and Arnold (1995) reported that the Myf5 gene from the MDF family is responsible for the development and proliferation phase of the muscle fibers in the early formation process.

As a result, studies have reported that the MDF gene family has significant effects on skeletal muscle development in the fetal and postnatal periods and even on the development and growth process in farm animals, indicating that the MDF gene family may have an effect on meat yield. Although studies on the determination of growth, meat yield and fattening performance of Hair, Angora, Honamlı and Kilis goat breeds show that these breeds have different phenotypic values, the basic mechanism of this difference has not been revealed, it is based on racial characteristics or environmental factors.

Therefore, determining the expression levels of the MDF gene family, which is effective on skeletal muscle development in our domestic goat breeds, which are an important source of red meat in our country, can provide important information about more descriptive and clear determination of the meat production potential of our domestic goat breeds and increasing meat yield. In addition, the fact that the expression levels of gene families responsible for muscle fiber development in our domestic goat breeds will be determined for the first time, paving the way for the elimination of a deficiency in this area, and it will also contribute to the competition with the countries where animal husbandry is developed in terms of determining the meat production potential of domestic breeds.

Due to all these facts, the current study aims to determine the expression levels of Myf5 and Myf6 genes, which are myogenic regulatory factors in the LD and ST skeletal muscles of 90-day-old weaning age boys belonging to Kıl, Tiftik, Honamlı and Kilis breeds, which are among the domestic goat gene sources of Turkey and constitute a large part of the goat existence.

MATERIAL AND METHOD

In the study, samples of Longissimus dorsi (LD) skeletal muscle - 80 °C stored after slaughter from Hair (n=6), Angora (n=6), Honamlı (n=6) and Kilis (n=6) male kids aged 3 months purchased for the muscle fiber characterization of our domestic goat breeds (Hair, Angora, Honamlı and Kilis) will be used as study material.

RNA Extraction and Reverse Transcription

Total RNA was isolated from muscle samples using Trizol reagent according to Chomczynski and Sacchi [1987]. The contamination of genomic DNA was removed by treating total RNA with RNase-free DNase , according to the manufacturer's recommendations. The quantity and quality of total RNA preparations were evaluated spectrophotometrically at 260 nm. The purity of total RNA was determined by the A260/280 and A260/230 ratio and its integrity was checked electrophoretically using 1% formaldehyde denaturing gel (Huang et al., 2016).

Obtained band images will be examined and quality control will be made according to the gel image of 28s - 18s RNA bands. The isolated total RNA samples will be diluted to 1µg/µl and cDNA synthesis from RNA will be performed with the cDNA synthesis kit with the help of reverse transcriptase and designed primers. Obtained cDNA samples will be stored at 20 °C until quantitative real-time polymerase chain reaction (qRT-PCR).

RT-qPCR Analyses

Mfy5 and Mfy6 gene expression levels will be examined by quantitative real-time polymerase chain reaction (RT-qPCR) method. Synthetic

oligonucleotide primers designed considering the conserved sequences of the Mfy5 and Mfy6 genes will be used. Primers to be used for amplification of target genes with the RT-qPCR method were either designed using online tools based on the relevant gene sequences of goats (http://simgene.com/Primer3 and http://www.ncbi.nlm.nih.gov/tools/primer- blast/) or from related studies (Table 1).

GAPDH primers were chosen as the housekeeping gene to normalize the expression of target genes (Table 1). RT-qPCR was performed using the SYBR Green master mix. During the process, SYBR Green mix dye binds to double-stranded DNA and as the number of double-stranded DNA in the medium increases, that is, as the amount of amplified cDNA increases, the amount of fluorescence obtained also increases. PCR protocol used in RT-qPCR application; initial DNA denaturation 2 min. (96 °C), denaturation 35 cycles (96 °C), ligation 1.5 min, extension 45 sec. (72°C), fluorescence analysis (72°C). All procedures were repeated at least 3 times in terms of the reliability of the results in RT-qPCR applications.

Standards at different concentrations (1, 1/10, 1/100 and 1/1000) were used to calculate the RT-qPCR efficiency. Values between E=1.60 and 2.10 for RT-qPCR efficiency were used in the study. Expression levels of Mfy5 and Mfy6 genes were compared with the expression level of the reference gene, and their expression levels were determined by making them suitable for normal distribution.

The 2-ΔΔCt method was used to analyze RNA expression levels (Huang et al., 2016).

Table 1. Primers used in qPCR analysis

Genler	Primer Sekansları		Ürün Boyutu	Gen Bankası
	Forward	Reverse		
Mfy5	CACGACCAACCCTAACCAGAG	TCTCCACCTGTTCCCTTAGCA	101 bp	JF829004 (Zhong vd.,2013)
Mfy6 (MRF4)	CGGAGCGCCATTAACTACAT	AAATCCGCACCCTCAAGATT	101 bp	NM_001285602 (Huang vd., 2016)
GAPDH	GCA AGT TCC ACG GCA CAG	TCA GCA CCA GCA TCA CCC	118 bp	AF035421 (Cheng vd., 2012)

Figure 1. Sequence Palindrome with no rotational symmetry, with a mirror-like palindrome on the left, and a double-stranded palindrome on the right.

However, in palindromes with rotational symmetry, the second half of the complementary DNA double helix is the mirror image of base sequence in the first half of another strand (see Figure 5). That is, the nucleic acid sequences in both strands of the DNA helix are the same when read from either 5′ or 3′ end of both strands in DNA duplex.

RESULTS AND DISCUSSION

Birth and corrected 90-day weaning weights of Honamlı, Kıl, Kilis and Angora kids are presented in Table 2. In the study, there was a statistically significant difference between breeds in terms of kid's birth weight, and it was determined that the birth weight of the kids belonging to the Honamlı breed was higher than the kids of the Hair, Kilis and Angora breeds (P < 0.05). Similarly, 90-day corrected weaning weight and total body weight gain up to weaning age of Honamlı kids were found to be higher (P < 0.05) than Kilis and Angora kids, excluding kids from Kilis breed.

Table 2. Birth and corrected 90-day weaning weights of Honamlı, Kıl, Kilis and Angora kids.

	Irk			
	Honamlı	Kıl	Kilis	Tiftik
DA	3,55±0,30^{a}	2.90±0,29^{b}	2.66±0,16^{b}	2.95±0,11^{b}
DSKA	20,40±1,03^{a}	18,24±1,68^{a}	14,20±0,21^{b}	13,03±0,26^{b}
Fark	16,85±0,93^{a}	15,34±0,87^{a}	11,54±0,18^{b}	10,08±0,23^{b}

DA = kid's birth weight, DSKA = 90 days adjusted weaning weight.

Total DNA and RNA amounts and RNA-DNA ratio in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı, Kıl, Kilis and Angora kids are presented in Table 3. In the study, when the total DNA amount of LD muscle was compared, it was determined that Honamlı kids had the highest (P < 0.05) DNA amount and Angora kids had the lowest (P < 0.05) DNA amount. Similarly, total DNA amount of ST muscle was found to be highest (P < 0.05) in Honamlı kids and lowest (P < 0.05) in Angora and Kilis kids.

In the study, when the total RNA amount of LD muscle was compared, it was determined that

the kids of the Hair and Honamlı breeds had the highest (P < 0.05) RNA amount, while the kids of the Angora and Kilis breeds had the lowest (P < 0.05) RNA amount. Interestingly, the total amount of RNA in ST muscle was highest (P < 0.05) in Honamli kids when the breeds were compared. In the study, it was determined that the Angora breed kids had the highest (P < 0.05) RNA/DNA ratio in the LD muscle, while the Honamlı and Kilis kids had the lowest (P < 0.05) RNA/DNA ratio. Similarly, it was determined that Angora kids had a higher (P < 0.05) RNA/DNA ratio in ST muscle than Kilis and Honamlı kids, except Kilis kids.

Table 3. Total DNA and RNA amounts and RNA-DNA ratio in Longissimus-dorsi (LD) and Semitendinosus (ST) muscles of Honamlı, Kıl, Kilis and Angora kids

		Irk			
		Tiftik	Kıl	Honamlı	Kilis
DNA (mg/g)	LD	0,44±0,08^{c}	0,94±0,22^{b}	2,26±0,955^{a}	1,25±0,26^{b}
	ST	0,41±0,09^{c}	1,08±0,35^{b}	1,80±0,30^{a}	0,59±0,13^{c}
RNA (mg/g)	LD	0,76±0,08^{b}	0,96±0,16^{a}	0,99±0,07^{a}	0,39±0,02^{b}
	ST	0,57±0,09^{b}	0,59±0,08^{b}	0,94±0,12^{a}	0,51±0,04^{b}
RNA/DNA	LD	1,73±0,28^{a}	1,02±0,21^{b}	0,44±0,10^{c}	0,31±0,09^{c}
	ST	1,39±0,29^{a}	0,55±0,13^{b}	0,52±0,15^{b}	0,86±0,20^{ab}

^{a,b,c} The difference between the means shown with different letters in the same row is significant (P < 0.05).

The expression levels of the myogenic factor 6 (Myf 6) gene in the Longissimus-dorsi (LD) skeletal muscle of Honamlı, Kılis, Kilis and Angora are presented in Figure 2. In the study, there were statistically significant differences between the breeds in terms of the expression level of the Myf 6 gene in the LD muscle, and it was found that the belonging to the Kilis race had lower (P < 0.05) gene expression levels than the belonging to the Honamlı, Kıl and Tiftik breeds. As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of the Myf 6 gene in the LD muscle of Honamlı, Kılis, Kilis and Angora were calculated as 44.5±11.3, 34.2±7.5, 12.0±2.9 and 39.8±10.1 fold changes, respectively. According to these results, kids belonging to the Kilis race expressed the Myf 6 gene in the LD muscle approximately 32.5, 22.2 and 27.8 times less (P < 0.05) than the Honamlı, Kıl and Tiftik, respectively.

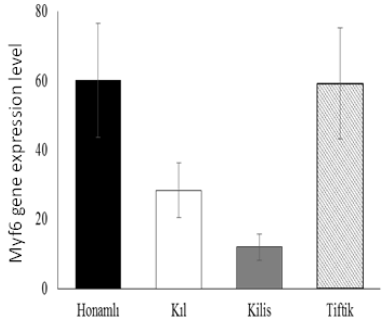

Figure 2. Expression levels of myogenic factor 6 (Myf6) gene in Longissimus-dorsi skeletal muscle of Honamlı, Hair, Kilis and Mohair kids. The bars above the bars represent the standard error of the means and the difference between bars with different letters is significant (P < 0.05).

The expression levels of the myogenic factor 6 (Myf 6) gene in the Semitendinosus (ST) skeletal muscle of Honamlı, Kılis, Kilis and Mohair are presented in Figure 3. In the study, there were statistically significant differences between the breeds in terms of the expression level of the Myf 6 gene in the ST muscle, and the highest (P < 0.05) Myf 6 gene expression level was detected in the boys belonging to the Honamlı and Angora, and the lowest (P < 0.05) gene expression level was detected in the kids belonging to the Hair and Kilis breeds. Kids belonging to the Honamli race expressed the Myf 6 gene in the ST muscle about 31.8 and 48.1 times more (P < 0.05) than the Kıl and Kilis, respectively. In addition, Angora kid expressed the Myf 6 gene in ST muscle approximately 30.9 and 47.2 times more (P < 0.05) than Hair and Kilis, respectively. As a result of quantitative real-time polymerase chain reaction (Real time-qPCR), the expression levels of the Myf 5 gene in the LD muscle of Honamlı, Kılis, Kilis and Angora were calculated as 60.1±16.4, 28.3±7.9, 12.0±3.8 and 59.2±16.0 fold changes, respectively.

The expression analysis of MYF6 showed that its polymorphism may be important for the myotube fusion, maturation and maintenance of the skeletal muscle weight [Wyszynska-Koko et al. 2006]. They identified significant correlation between the MYF6 polymorphism identified in the promoter region, exon 1 and carcass weight. However, several studies on MYF5, MYF6 showed that their polymorphism does not affect the expression of MRF genes [Ernst et al. 1994, Stratil and Cepica 1999, Te Pas et al. 1999a, Cieslak et al. 2002, Vykoukalova et al. 2003, Urbanski and Kuryl 2004, Wyszynska-Koko and Kuryl 2004, Urbanski et al. 2006].

However, these results suggest that the effects of age and breed-specific expression of MYF5 and MYF6 observed in this study could also represent indirect effects of another miogenic regulatory

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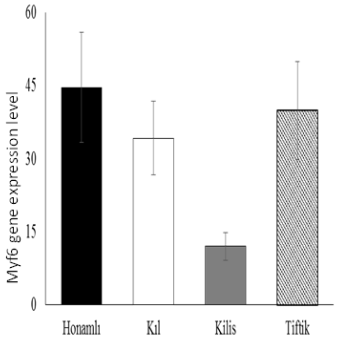

Figure 3. Expression levels of myogenic factor 6 (Myf 6) gene in Semitendinosus skeletal muscle of Honamlı, Hair, Kilis and Angora kids. The bars above the bars represent the standard error of the means and the difference between bars with different letters is significant ($P < 0.05$).

Pierzchała et al. reported in 2011 that the expression of the MYF6 gene in the skeletal muscle of PIE was higher than in the growth of other breeds and that their findings in the skeletal muscles of pigs were consistent with those indicated in his 2010 study, as they found that there was no significant relationship between age and the level of expression of the MYF5 and MYF6 genes. Pierzchała et al. Results of their study in 2011 also suggested that, as in the PIE breed, higher MYF6 gene expression may be associated with higher muscularity of carcasses.

This study The significantly different expression was presented of MYF5 and MYF6 in goat skeletal muscles. To our knowledge, this study is one of the first analyses of age- and breed-dependent relations of goat MYF5 and MYF6 expressed in postnatal period of skeletal muscle growth.

Significant differences between MYF5 and MYF6 expressions allowed us to select both candidate genes for further trait-associated studies. The further identification of causal polymorphism and determination of functional role are even more challenging, since there are many different molecular mechanisms through which expression activity of specific genes in myogenic cells can be regulated.

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DETECTION OF *SUKKULA* RETROTRANSPOSON IN DOMESTIC GOOSE BY IRAP-PCR METHOD

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Abstract

The remains of the domestication of the geese can be traced back to 7,000 years ago. Geese are birds that can gain significant weight on a limited diet. Domestic geese are primarily descended from greylag geese (Anser anser) and swan geese (Anser cygnoides). The aim of this study was to detect the mobility of barley-specific Sukkula retrotransposon in domestic goose genotypes. For this purpose, the present and the movements of this retrotransposon in 3 different populations of domestic geese (Chinese x Embden cross, Turkish White, and Turkish Multicolor) were determined by the IRAP-PCR technique. Sukkula polymorphism rates were between 0 and 73% in all samples. Intrapopulation genetic polymorphism rates were also 0-27% in Chinese x Embden crossbred and 0-50% in Turkish Multicolor. Moreover, only monomorphic bands were observed in Turkish White. Retrotransposon polymorphism and genomic instability are considered significant rearranging mechanisms under environmental stress. Retrotransposon markers have been demonstrated to be more informative and polymorphic compared with other marker systems. This study is the first report to determine and also analyse the movements of barley-specific Sukkula retrotransposon in the genome of the domestic goose (Anser anser domesticus). Thus, this study could be one of the initial steps for further research in evolutionary relations and horizontal transfer of mobile genetic elements.

Key words: *Mobile genetic elements, Sukkula, horizontal transposon transfer, polymorphism, population*

YESTERDAY, TODAY AND FUTURE OF EMBRYO PRODUCTION AND TRANSFER IN THE WORLD AND TÜRKİYE

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Abstract

Nowadays, embryo transfer application takes attention as the most powerful biotechnological technology used to improve especially in cattle breeds and to some extend in small ruminants in the world. The bovine embryo transfer industry arose in North America in the early 1970's. Using together with genomic evaluation developed in the late 2000's, embryo transfer application increases the benefits getting from embryo transfer technology. Today, embryo transfer is commonly utilized in countries which want to improve livestock farming. In the world, a total of 1.172.851 cattle embryos were transferred in 2020. In Türkiye, as a result of the continuation of an import-based livestock mentality, very strict and unnecessary official regulations related to embryo transfer, incomplete understanding of genetic value, our shortcomings in proper data logging , our inability to effectively fight important diseases in animal husbandry such as brucella and tuberculosis, the acceptance of standard prices in breeding sales rather than pedigree in the livestock market, and our inability to create a quality bull production model at an international level, embryo transfer technology is quite behind in terms of the use under the field conditions. However, as a result of the modern farm established recently and the correct farm management practices achieved in these enterprises, demands for embryo transfer technology have begun to emerge from livestock businesses to seek better genetics. One of these demands is to use the embryo transfer technology to improve the herd genetic capasity. With the embryo transfer technology to be applied by experts, our dependence on foreign sources in the need for high quality breeding heifers and semen in our country will decrease and export will be in question. Consequently, embryo transfer technology is the most advanced application and especially by using with genomic evaluation together will help to improve the genetic capacity of livestock.

Key words: *Embryo production, embryo transfer, Türkiye, world*

TOOLS, APPLICATIONS, AND LIMITATIONS OF RECOMBINANT DNA TECHNOLOGY IN LIVESTOCK PRODUCTION

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Abstract

The advent of recombinant DNA (rDNA) technology revolutionized the development of biology and led to a series of dramatic changes. The use of recombinant technologies has been proposed as an alternative to improve livestock production systems for more than 25 years; thus, understanding the use of recombinant technology could help to improve public acceptance. Recombinant DNA technology is a series of procedures that are used to join together (recombine) DNA segments. A recombinant DNA molecule is constructed from segments of two or more different DNA molecules. Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, but differs in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called chimeric DNA. rDNA technology uses palindromic sequences and leads to the production of sticky and blunt ends. Proteins that can result from the expression of recombinant DNA within living cells are termed recombinant proteins.

Key words: *recombinant DNA technology, rDNA, restriction enzymes, vectors, plasmids*

INTRODUCTION

Recombinant DNA technology is a technique which changes the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, the process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. This recombinant DNA then has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences. Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

A rDNA molecule is constructed from segments of two or more different DNA molecules. rDNA molecules are formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

Under specific conditions, a recombinant DNA molecule can enter a (host) cell and replicate

there – either on its own or after it has been integrated into a chromosome. Recombinant DNA was first achieved in 1973 Herbert Boyer, of the University of California at San Francisco, and Stanley Cohen, at Stanford University, who used E. coli restriction enzymes to insert foreign DNA into plasmids.

TOOLS OF RECOMBINANT DNA TECHNOLOGY

After the successful isolation, purification, electrophoresis of DNA, specific tools are needed for the successful creation of recombinant DNA molecules. These tools are:

- Restriction Enzymes
- Vectors or Cloning Vehicles
- Viable or Competent Host
- DNA Ligase
- Alkaline Phosphatase (AP)

Restriction Enzymes

The restriction enzymes employed in recombinant DNA technology play a vital role in determining the location for the insertion of the desired gene into the vector genome (Shinde et al. 2018). Restriction enzymes fall under a larger class of enzymes called Nucleases. There are two types of restriction enzymes used in recombinant DNA technology, namely Endonucleases and Exonucleases.

In 1963, Steward Linn and Werner Arber isolated two enzymes which restricted the growth of bacteriophage in E. coli (Modrich, 1979). One of these enzymes added methyl groups to DNA, and the second one cut DNA; the second enzymes was called "restriction endonuclease".

Isolation of restriction endonuclease whose functionality depended on a particular nucleotide sequence was done by Hamilton Smith, Tom Kelly and K. Wilcox in 1968 (Kelly and Smith 1970; Smith and Wilcox 1970). This enzyme was isolated from bacteria Heamophilus influenzae and termed Hind II. It was observed that Hind II always ligate DNA molecules at specific place by recognizing a particular nucleotide sequence of six base pairs (bp).

Types of Restriction Enzymes

Exonucleases: These enzymes remove nucleotides from the ends of DNA.

Endonucleases: These enzymes make cuts at specific positions on the DNA.

A restriction endonuclease identifies a specific base pair sequence in DNA called a restriction site (see Figure 1) and slices the DNA – through the hydrolysis (see Figure 2) of the phosphodiester backbones – within the DNA sequence. Additionally, Magnesium ions (Mg 2+) are crucial for cleavage. Restriction enzymes are found in prokaryotes and provide protection to host cells by destroying foreign DNA that enter into it. In this instance, restriction enzymes act as a part of the defense mechanism known as the Restriction Modification System (RMS).

Restriction endonucleases serve as the tools for cutting DNA molecules at specific sites, and this process is requisite for gene cloning or recombinant DNA technology. The recognition site or sequences varies from different restriction enzymes (Goodsell, 2002).

The restriction modification system has two components; the first component is a restriction enzyme that selectively recognizes a particular DNA sequence and destroys any DNA bearing the sequence.

The second component is a modification enzyme. This enzyme, unlike the restriction enzyme, adds a methyl group to one or two bases within the sequence identified by restriction enzyme. Consequently, once a base in the DNA is modified by the inclusion of a methyl group, the restriction enzymes cannot identify can cleave that DNA. Using this method, bacteria can protect their chromosomal DNA from cleavage by restriction enzymes. Therefore, bacteria possess sets of restriction endonucleases and corresponding methylases.

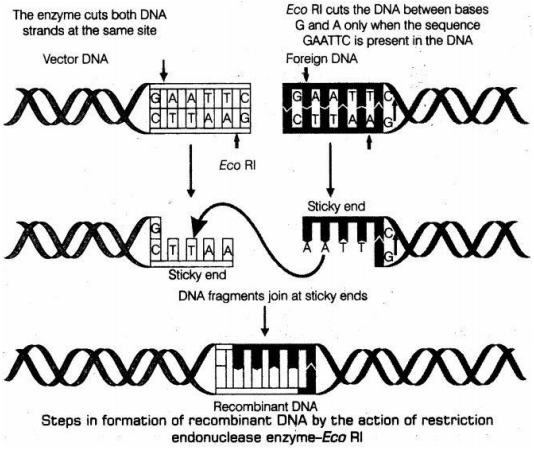

Figure 1. Action of restriction endonuclease, Eco RI, cuts the DNA bases between G and A only when the sequence "GAATTC" is present in the DNA. Source: Biology Discussion

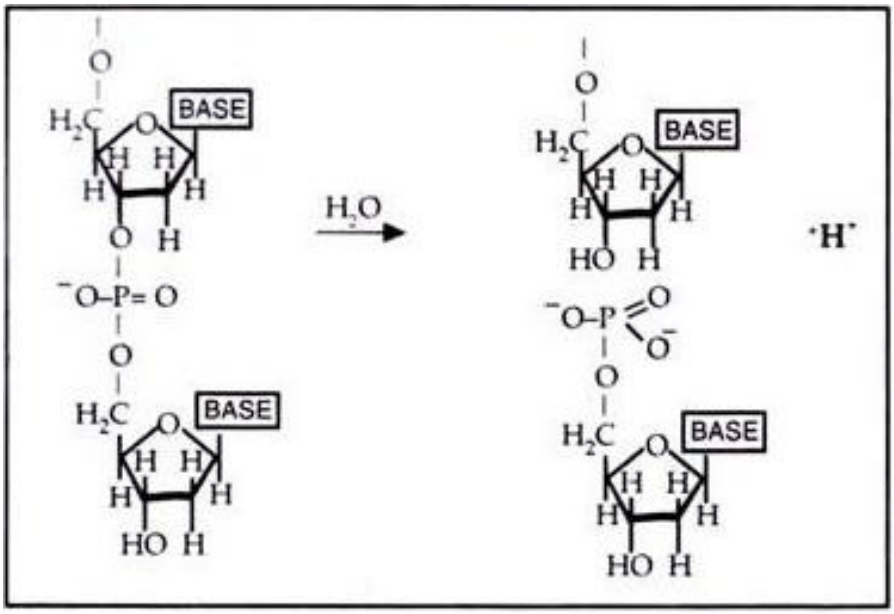

Figure 2. Hydrolysis of 3′-O-P bond in a nucleotide by a restriction endonuclease.
Source: Biology Discussion.

Types of Restriction Endonucleases

Three main types of restriction endonucleases are known – namely, Type I, Type II and Type II – with slightly different modes of action. Type II restriction enzymes are used in recombinant DNA technology because of their in vitro application; they can be used to recognize and cleave specific DNA sequences, usually having 4 to 8 nucleotides (see Figure 3). More than 350 different Type II endonucleases with 100 different recognition sequences or sites are known. The first Type II enzyme isolated was Hind II in 1970 (Leonen, 2019).

Microorganisms	*Restriction enzymes*	*Cleavage sites*	*Cleavage products*
Bacillus amy-loliquefaciens H	*Bam* HI	5-GGATCC-3 3-CCTAGG-5	5-G GATCC-3 3-CCTAG G-5
B. globigii	*Bgl* II	5-AGATCT-3 3-TCTAGA-5	5-A GATCT-3 3-TCTAG A-5
Escherchia coli RY13	*Eco* RI	5-GAATTC-3 3-CTTAAG-5	5-G AATTC-3 3-CTTAA G-5
Haemophilus influenzae Rd	*Hin* dIII	5-AAGCTT-3 3-TTCGAA-5	5-A AGCTT-3 3 -TTCGA A-5
H. parainfluenzae	*Hpa* I	5-GTTAAC-3 3-CAATTG-5	5-GTT AAC-3 3-CAA TTG-5
Klebsiella pneumoniae OK 8	*Kpn* I	5-GGTACC-5 3-CCATGG-3	5-GGTAC C-3 3-C CATGG-5
Streptomyces albus G	*Sal* I	5-GTCGAC-3 3-CAGCTG-5	5-G TCGAC-3 3-CAGCT G-5
Staphylococcus aureus 3AI	*Sau* 3AI	5-GATC-3 3-CTAG-5	5- GATC-3 3-CTAG 5

Figure 3. Some examples of Type II restriction enzymes alongside their sources and recognition sequences.

The recognition nucleotide sequences for Type II restriction enzymes form palindromes with rotational symmetry (see Figure 4). In molecular genetics, a palindrome occurs when the base sequence(s) of one DNA strand represents the mirror image of the base sequence(s) of the opposite DNA strand. Note here that the base pairs read the same on both DNA strands when the orientation of reading is kept the same.

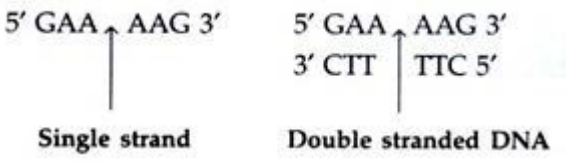

Figure 4. Sequence Palindrome with no rotational symmetry, with a mirror-like

palindrome on the left, and a double-stranded palindrome on the right.

However, in palindromes with rotational symmetry, the second half of the complementary DNA double helix is the mirror image of base sequence in the first half of another strand (see Figure 5). That is, the nucleic acid sequences in both strands of the DNA helix are the same when read from either 5′ or 3′ end of both strands in DNA duplex.

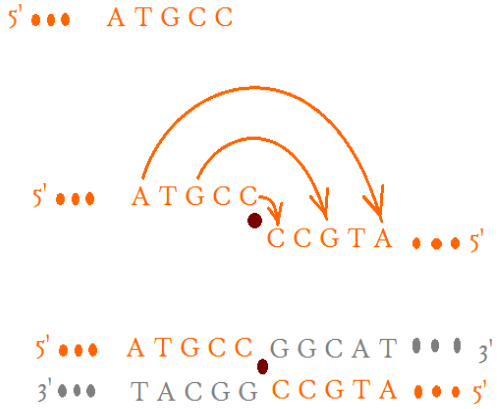

Figure 5. Palindrome with twofold rotational symmetry: A, nucleotide sequence with complementary symmetry; and B, an inverted repeat palindrome.

Many restriction enzymes identify specific palindromic sequences and cleave. For example, Eco R1 recognizes the palindromic sequence (see below):
5′ – GAATTC – 3′
3′ – CTTAAG – 5′
The top strand reads 5′ – GAATTCC – 3′, while the bottom strand reads 3′ – CTTAAG – 5′. If the DNA strand is turned upside down, the sequences remain the same for the 5′-3′ direction and 3′-5′ direction: 5′ – GAATTC – 3′ and 3′ – CTTAAG – 5′. Inverted repeat palindromes are of greater biological significance and more common than mirror-like palindrome
Eco R1 cleaves DNA molecules of two plasmids because of theirs DNA's similar recognition sites. Therefore, the circular form of DNA (of plasmids) become linear in both cases (that is, in both strands). Such linear DNAs can stick together to form a single recombinant DNA molecule. This singular process is the backbone of recombinant DNA technology. Eco R1 cleavage produces "sticky" ends, while Sma l restriction enzymes produce "blunt" ends.

Types of Cleavage Produced by Restriction Enzymes

Blunt-End Producing Restriction Enzymes: Restriction enzymes such as Sma I, isolated from Serratia marcescens, cleave both DNA strands at exactly at the same nucleotide position almost at the centre of the recognition site, resulting in blunt or flush ends.
Sticky-End Producing Restriction Enzymes: Some other restriction enzymes cut the recognition site asymmetrically, producing short, single-stranded hanging structures – otherwise known as stick or cohesive ends. The sticky ends make it possible for the DNA molecules to join together due to base pairing. Eco R1 cleaves recognition sequence at various points.

VECTORS OR CLONING VEHICLES

Vectors are DNA molecules that can replicate in a host cell and into which the DNA fragment to be cloned – known as DNA insert – is integrated for cloning. Vectors assist in carrying and integrating the desired gene. Vectors are a very important part of the tools of recombinant DNA technology (Shinde et al., 2018). Plasmids and bacteriophages are commonly used in recombinant DNA technology because they possess a very high copy number.
Cloning of a foreign fragment of DNA in bacteria is possible because of the ability of cloning vectors or carriers to continue "living" after additional sequences of DNA have been inserted into their genome. The insertion of new DNA sequences produces a hybrid, chimeric or recombinant vector, which contains, in part, the additional or inserted foreign fragment of DNA. When chimeric vectors are cloned in bacteria, they replicated exactly the same way as the original vectors. Thus, a large amount of DNA molecules is produced. The inserted DNA simultaneously replicates with the multiplies with the remaining portions of the chimeric vectors and copies of the original foreign DNA can be retrieved from the filial generation(s). Different vectors has different insert sizes (see Table1).

Characteristics of DNA Molecules that Can Act as Vectors

In recombinant DNA technology, vectors consist of an origin of replication: this is a sequence of nucleic acids from where replication commences; a selectable marker: it constitutes genes which show resistance to certain antimicrobials (e.g., ampicillin); and, cloning site: the sites identified by restriction enzymes and where the desired DNA molecules and, consequently, genes are inserted.

Table 1. Some of vector types with their insert size

	Vectors	Insert Size (kb)
1.	Plasmid	0.5 – 8
2.	Bacteriophage Lambda	9 – 23
3.	Cosmid	30 – 40
4.	BAC	50 – 300
5.	YAC	2500 – 10000

Origin or Replication (Ori): It represents the sequence from where replication started. Additionally, this sequence controls the copy number of linked DNA. To produce several copies of target DNA, cloning should be done in a vector where origin facilitates high copy number; it should bear an origin or replication due to which it is able to replicate within the host cell. In other words, the vector should be able to multiply autonomously. Owing to this, any foreign DNA introduced into the vector will also replicate during this process.

Selectable Marker: To act as a vector, a DNA molecule should be able to incorporate a selectable marker gene. This selectable marker gene enables the identification of host cells that bear the vector from those that do not. A selectable marker helps in removing non-transformants and selectively allowing the proliferation of the transformants. Examples of selectable markers are: genes that code for antibiotic resistance (for examples, ampicillin, chloramphenicol, tetracycline or terramycin); genes that code for enzymes such as β-galactosidase (product of lac Z gene), which can be identified by colour reaction. Many alternative selectable markers have been developed, which can differentiate recombinants from non-recombinants by their ability to produce colour. This process involves inserting recombinant DNA into the coding sequences of enzyme β-galactosidase. As a result, the enzyme become inactivated. This step is known as "insertional inactivation." chromogenic substrate affects blue-coloured colonies in the absence of inserted DNA in plasmids. If the inserted DNA is present, it leads to insertional inactivation of enzymes β-galactosidase. Therefore, colonies fail to produce any colour and such colonies represent the recombinant colonies.

Cloning Site: For restriction endonuclease, a vector should bear mono- or oligo- recognition sequences. Gene cloning becomes difficult and more complicated when there are many recognition sites. So, in recombination DNA technology, less (recognition sites) is more.

Additionally, a vector should be easy to isolate and purify. Cloning vectors should be relatively smaller in size, as large molecules breakdown during purification, making it difficult to manipulate. Vectors should bear at least one restriction enzymes recognition site. The recognition site makes the insertion of foreign DNA into the vector during the generation of recombinant DNA molecule possible.

Plasmids and (bacterio)phages, as mentioned above, are the commonly used vectors for cloning purposes in prokaryotes, particularly bacteria.

Plasmids as Vectors

Plasmids are the most widely used cloning vector for gene manipulation in bacteria (see Figure 6). Plasmids are circular, double-stranded extrachromosomal DNA molecules that are self-replicating. Some plasmids may have one or two copies per cell. However, plasmids may be present in greater numbers, usually about 15 to 100 per cell. Plasmids possess a replication control system that maintains them in the bacterium at a characteristic level.

Generally, there are two types of plasmids: single copy and multicopy plasmids. Single copy plasmids are kept at one plasmid per host genome, while multicopy plasmids exist under a "relaxed" replication control system – meaning, they replicate in very large amounts, about 1000, per cell when the bacteria stop growing. This type of plasmids is often used as cloning vectors.

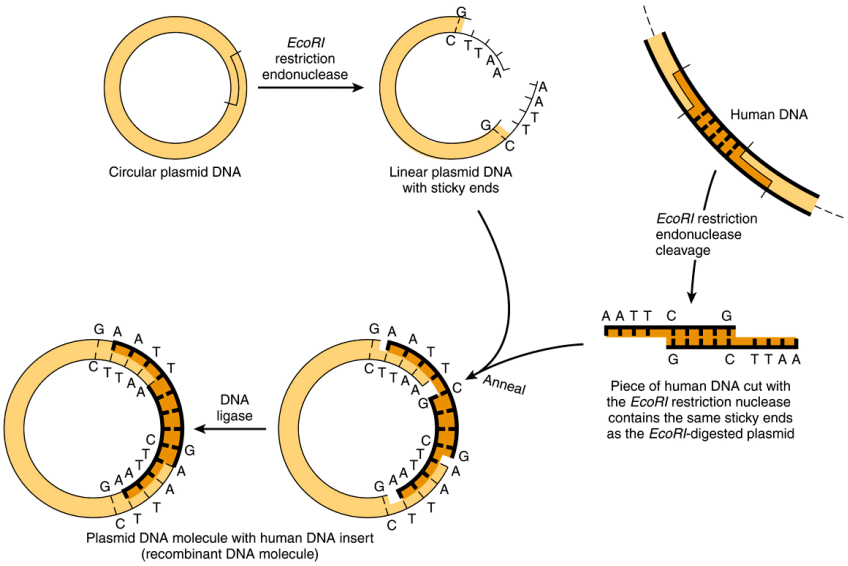

Figure 6. Foreign DNA sequences can be inserted into plasmids vectors by cleaving the circular plasmid with restriction endonuclease.

Three Widely Studied Bacterial Plasmids are:

1. F-Plasmids: They are responsible for conjugation.
2. R-Plasmids: They carry genes for antibiotic resistance.
3. Col Plasmids: Such plasmids encode bacteriocins, proteins that can kill other bacteria (e.g., E. coli).

After the cleavage transforms the circular plasmids into a linear molecule, then both ends of the linear plasmids are joined to the ends of the foreign DNA with the help of DNA ligase. The result is chimeric plasmid. The chimeric plasmid is transferred into a bacterium where it multiplies and perpetuates indefinitely.

An ideal cloning plasmid vector has three properties:

1. Low molecular weight.
2. Ability to confer readily with selectable phenotypic traits on host cells.
3. Several recognition sites for large number of restriction endonuclease.

Phages as Vectors

Bacteriophages or phages are vectors that infect bacterial cells by infusing their DNA into these (bacterial) cells. Two phages that have been extensively genetically modified for development of cloning vectors are M13 and Lambda (λ).

DNA of wild-type phage contains several target recognitions sited for most of the commonly used restriction enzymes. Therefore, it is unsuitable as cloning vector. Derivatives of the wild-type phage have, thus, been produced which either have a single target site in their DNA at which foreign DNA can be inserted, resulting in a chimeric DNA – these are known as "Insertional" phage vectors. If the phage has a pair of recognition sites that can be removed and replaced by foreign DNA – these are called "Replacement" phage vectors.

What enables one to create "replacement" phage vectors is the fact that the phage 'head' can accommodate only about 5% more than its normal complement of DNA and so prevents too long foreign DNA from being packaged into it. To overcome this problem, a fragment of phage DNA that does not carry essential phage genes is removed to increase the space within the phage DNA and is replaced by foreign DNA.

However, the chimeric or recombinant-DNA is packaged into phage head coat in vitro. The principle of packaging in vitro is to supply the ligated recombinant-DNA with high concentrations of phage head precursor, packaging-proteins and phage tail. The packaging allows the recombinant-DNA to be introduced into the host bacterium by the normal processes of phage infection (transduction), i.e., phage absorption followed by DNA injection.

Phage M13 Vectors

This kind of vector is used for producing single stranded copies of cloned DNA, which are especially suited for DNA sequencing (see figure 7). M13 vectors do not kill the cells but forms turbid plaques due to growth retardation of infected cells. M13 is a filamentous phage that infects E. coli having F-pili. Its genome is single stranded circular DNA of 6407 base pairs.

Foreign DNA can be introduced into the genome of M13 without causing any disruption of the essential genes. When M13 phage DNA enters into E. coli host replicative form (RF), a double-stranded form is created. It multiplies until 100 copies are formed. Now the DNA replication is symmetrical, and it begins producing single

stranded copies of the genome and comes out the cell as M13 particles.

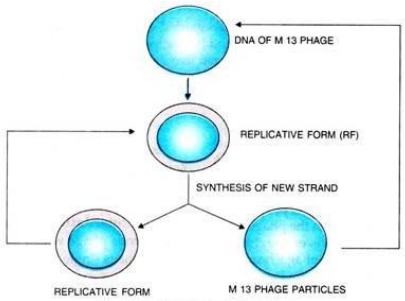

Figure 7. Synthesis of M13 Vectors; source: Biology Discussion.

Cosmids

Cosmids are created by combing certain features of plasmid and the "cos" sites of lambda phage. They were constructed to add some of the advantages of phage vectors to the plasmid vectors; the cos sites introduce in vitro packaging system to the plasmid vector. The cosmid vectors provide an efficient means of cloning large fragments of foreign DNA much more than a Lambda phage can contain. When injected into a bacterium, the recombinant DNA of a cosmid circularizes like phage DNA, but multiplies as a normal plasmid without the expression of any phage functions. Cosmid vectors are useful for creating libraries of DNA fragments of eukaryotes due to their ability to contain large fragments of DNA.

Phasmids

Phasmids also contain phage DNA, including its at its recognition site. Like cosmids, they have been constructed to exploit the benefits of both Lambda and plasmid vectors. The phasmid may be inserted into a phage DNA in the same way by which phage DNA inserts into the bacterial chromosome during the lysogenic phase of its life cycle.

YAC Vectors

Yeast Artificial Chromosomes (YAC) are being used as cloning vectors to clone DNA fragments of more than 2500 Mb in size (see Figure 8). They were highly used in mapping larger genomes for the Human Genome Project.

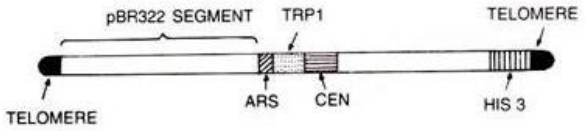

Figure 8. A typical YAC; it is linear, has telomeric sequence at its two ends, and, in addition, has ARS and a CEN sequences. Source: Biology Discussion.

BAC Vectors

Bacterial Artificial Chromosomes (see Figure 9) or BAC are used as vectors that are based on natural extrachromosomal plasmid of E. coli. The F-plasmid vector bears genes for replication and maintenance of F-factor – a selectable marker and cloning sites.

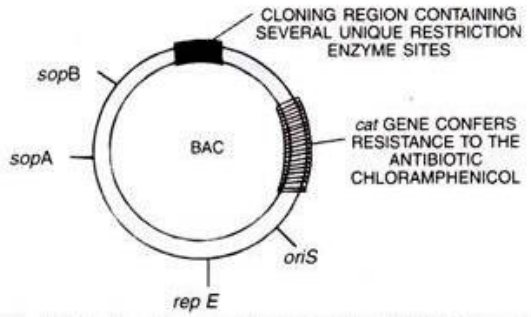

Figure 9. Genetic map of BAC vector. The genes oriS, repE, sopA and sopB are involved in plasmid replication. Source: Biology Discussion.

Shuttle Vectors

Shuttle vectors are plasmids capable of moving genes between two organisms (see Figure 10). One of the organisms is a prokaryote such as E. coli, and the other is a eukaryote like yeast. Such vectors bear unique origins of replication for every cell type; however, they should have separate markers for transformed host cells harbouring the vector.

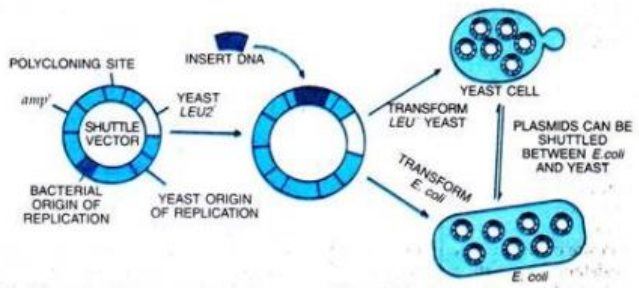

Figure 10. A shuttle vector. The vector contains both bacterial and yeast origin or replications, amp' (ampicillin resistance gene for selection in E. coli) and LEU 2 – a gene in the yeast pathway for leucine biosynthesis. Source: Biology Discussion.

Competent or Viable Host

For propagation of DNA molecules, host cells are important. Host cells such as E. coli, yeast, plant and animal cells are being used.

The most popular and widely used bacterium is E. coli. And it is due to the following reasons:

- E. coli is a gram-negative bacterium; hence, it is easy to handle and grow in the lab.
- It can accept an array of vectors.

Under optimal conditions, bacteria double their number every 20 minutes. When bacteria proliferate, the recombinant DNA also reproduces. Eukaryotic cells are also being used as host cells for expression of eukaryoticin; this leads to proper folding of polypeptide chain into an exact 3-D form.

As the cell membrane does not allow DNA pass through, due to its hydrophilic nature. Bacterial host cells are manipulated to take in plasmid. To achieve this, bacteria are treated with divalent cations like Calcium (Ca2+), enhancing the efficiency of entry of DNA into the bacterium through the cell wall's pores. Alternatively, recombinant DNA is forced into such cells by incubating the cells with recombinant DNA ice, then giving the combination (of cells and recombinant DNA) a heat shock (42oC) and putting them back on ice again.

DNA Ligase

DNA ligase forms diester bonds between adjacent nucleotides. This enzyme links two fragments of DNA by covalent bonds. The enzyme used in cloning experiment is T4 DNA ligase which is coded by phage T4.

Alkaline Phosphatase (AP)

This enzyme is used to check undesired self-ligation of vector DNA molecule. During the cloning process when vector DNA molecule is digested by restriction enzyme, cohesive or sticky ends of vector may not join with foreign DNA and may lead to recircularization of plasmid. When cleaved DNA fragments are given the treatments of AP, terminal 5′ phosphate group is removed. This 5′ phosphate group is essential at the site for ligation. Self-ligation fails to occur because of the absence of 5′ phosphate group. For ligation to happen, the presence of 5′ phosphate group is required at the DNA site. AP is used to remove the phosphate group from 5′ end of DNA, leaving a free 5′ hydroxyl group.

APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY TO LIVESTOCK PRODUCTION

The demand for animal-source protein is poised to reach its apogee in 2050; consequently, animal products (such as milk, meat and egg) must double – if not triple – to meet the increasing needs of the world population (Balehgn et al., 2020). Genetic engineering, in the form of recombinant DNA (rDNA) technology, and synthetic biology have been suggested as potential tools for improving living organisms and biological system (Sandler, 2020), including livestock animals (Pech-Cervantes et al., 2020). Recombinant DNA technologies have ushered improvements in the study, characterization, and commercialization of recombinant proteins to improve agro-industrial processes (Rosano and Ceccarelli, 2014; Gerber et al., 2015). DNA recombinant technologies has been integrated into the food industry as a means of improving the organoleptic properties of animal-source food, reduce food wastage and mitigate the environmental impact of livestock production (Pech-Cervantes et al., 2020).

Broadly, the rDNA technology has several advantages, owing to the promising results of various research. For example, it can alter a single gene locus possibly without disrupting the remaining genome. This singular quality makes rDNA of great value to agriculture, specifically animal and plant breeding and genetics. rDNA alongside gene editing is the foundation for producing transgenic animals. Additionally, scientists can employ rDNA technology in the production of recombinant proteins (including feed enzymes and hormones), peptides, vaccines, amino acids, fatty acids and vitamins by bacteria like E. coli (see Table 2).

The costs of producing recombinant proteins are low and the benefits are vast. rDNA technologies can modify bacterial genome to: produce (feed) enzymes for feed fermentation (Demirci et al., 2014); eliminate antimicrobial resistance by generating enzymes to remove the mediating molecules in bacteria (da Costa et al., 2018); and develop vaccines by separating protein antigens using specific monoclonal antibodies, synthesis of protein antigens by cloned genes, and synthesis of peptides to be used as vaccines (Nagaich, 2015).

rDNA technologies could help reduce production cost in pig farming by increasing the availability of feed-grade amino acids, which can substantially reduce the content of protein in diet; thereby, decreasing the excretion of nitrogen into the environment. A reduction in dietary protein content by a 1% unit (e.g., from 16% to 15% crude protein) can decrease the excretion of total nitrogen (in urine plus feces) from growing pigs by 8.5% (Lenis, 1999).

Recombinant hormones are one of the most widely studies recombinant proteins for improving performance and reproduction in meat and dairy ruminants (Pech-Cervantes et al., 2020). Recombinant bovine somatotrophin (rBST)

is considered one of the first biotechnology products applied in the livestock industry (Bauman, 1992). rBST improves the immunological response of ruminants by increasing the concentrations of IGF-1 in the serum, as well as gluconeogenesis in the liver (Bauman, 1992; Silva et al., 2015). A meta-analysis of effects rBST on milk production, reproductive performance, and the health status of dairy cow showed that the inclusion of rBST increased milk production by more than 10% in primiparous cows and 15.6% in multiparous cows (Dohoo et al., 2003, a, b). The use of recombinant gonadotrophin and follicle-stimulating hormone improves assisted reproduction in livestock (Adams and Boime, 2008; Hesser et al., 2011) and enhances superovulation in ruminants, respectively (Fidler et al., 1998; Sanderson and Martinez, 2020).

Recent research reports indicate that transgenic cows secreting lysostaphin in milk prevented the onset of mastitis by Staphylococcus aureus, unlike non-transgenic cows (Wall et al., 2005). These results are of economic importance because reducing clinical and sub-clinical mastitis could potentially reduce the production costs of antibiotic treatments in dairy cows. The use of recombinant tumor necrosis factor (rbTNF) has been proposed to improve immune response and performance of beef and dairy cattle after periparturient period (Kushibiki et al., 2003; Bradford et al., 2009). These studies – in conjunction with plethora of other studies – support the idea that recombinant immune cells could help improve the health status and performance of ruminants.

Table 2. The Use of Recombinant DNA Technology in Livestock Production

Product	Function	Author
Porcine Growth Hormone (Somatotrophin)	Enhances lean tissue growth	Chung *et al.* 1985
Vaccines	Regulates metabolism; treats diseases	CAST, 2008
Antibodies	Controls viruses (e.g., African Swine Fever)	CAST, 2008
Phytases	Hydrolyses phytate in plants; increases the Digestion of minerals and proteins in diets	Pandey *et al.*, 2001
Enzymes for feed fermentation	Digests complex carbohydrates and proteins	Opazo *et al.*, 2012 Demirci *et al.*, 2014
Enzymes for degrading AMR mediators	Enhances animal growth and feed efficiency	de Costa *et al.*, 2018

Limitations of Adopting Recombinant DNA Technology to Livestock Production

1. Recombinant organisms are population of clones; that is, they are vulnerable in the exact same way. In other words, a single disease or pest can exterminate the entire population quickly.
2. Hypothetically speaking, recombinant DNA technology can lead to the creation of a superbug.
3. The end-consumers, humans, worry about the safety of (genetically) modifying food and medicines using recombinant DNA technology.
4. Genetically modified organisms destroy or upset the native species when they are introduced into the population.
5. Resilient animals can theoretically give rise to resilient pathogens, which can be difficult to control.
6. Public and ethical concerns impose high barriers for marketing and acceptance, causing the passing of legislations in certain to stop the sales and circulation of rDNA technology products. For example, in the European Union since 1999, recombinant hormones have been forbidden (Dohoo et al., 2003; Lamas et al., 2019).

CONCLUSION

Although, the prospects of recombination DNA technology are advantageous, many of these technologies are highly experimental. Therefore, the feasibility of these technologies should be properly examined before a wide scale implementation is adopted.

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SOME GENES AND CHEMICAL COMPOUNDS EFFECTIVE IN HOST PREFERENCE OF THE HONEYBEE ECTOPARASITE VARROA DESTRUCTOR

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Abstract

Ectoparasitic mite Varroa destructor, which causes high colony loss, is the biggest threat to the honeybee. Chemical drugs, which are used in large quantities against Varroa cannot provide a solution to the problem. At the same time, the gaining resistance of Varroa to chemical drugs has caused new problems. For these reasons, alternative and definitive solutions are sought in the struggle against Varroa.

In recent years, scientists and beekeepers have focused on the identification and breeding of resistant honeybee genotypes. Inhibition of reproduction of Varroa is a common strategy in varroa-resistant genotype breeding programs. Reproduction of Varroa is only possible through synchronization in the best way with the development of the host honeybee. Some hormones and proteins produced by the honey bee are thought to play an important role in the initiation of the Varroa reproductive cycle.

It is important to reveal host preference mechanisms of Varroa in order to interfere with the reproductive behavior. Showing high host specificity Varroa can distinguish drone larvae from worker larvae, and nurse bees from forager bees. Uncovering the molecular mechanism underlying this selectivity will form the basis for breeding of bees resistant to Varroa. Recent studies have shown that some genes, pheromones and other low-density volatile compounds may play a role in host preference. From this point of view, some genes and chemical compounds that are effective in the host preference of Varroa are addressed in this study.

Key words: *honey bee*, *Varroa destructor*, *host-parasite interaction*, *genes*, *hormones*

INTRODUCTION

Honey bees which are of great importance for the healthy and balanced functioning of the ecosystem; In addition to the production of valuable bee products such as honey, beeswax, pollen, royal jelly and propolis, they play a greater role in the pollination of both cultivated and wild plants than other insect species (Free, 1970; McGregor, 1976; Hung et al., 2017). It is reported that 70% of the plants in the world are pollinated by bees and more than 80% of this is done by honey bees (Özbilgin, 1999). Recently, many factors threaten honey bee colonies, including poor nutrition, pressure from ecto- and endoparasites, increased bacterial and viral diseases, and synergistic pesticide interactions.

The ectoparasite mite *Varroa destructor*, which feeds on the fat body and hemolymph of bees, is the most important pest of *Apis mellifera* and plays a central role in honey bee losses (Ramsey et al., 2019). Varroa mites infest the honey bee brood cells before they are sealed and reproduce there. As the adult bee emerges from the cells, the female mites that cling to it also come out together and can be transferred to another bee or another bee larvae (Rosenkranz vd. 2010; Shimanuki vd. 1994). However, Varroa poses a great threat to the beekeeping industry not only because of its direct harmful effects, but also because it is a vector of important bee viruses and many pathogens.

Beekeepers commonly use chemicals such as coumaphos, Tau-fluvalinate, Formamidine amitraz to control Varroa mite. However, the desired results cannot be achieved due to the fact that beekeepers do not use drugs at the right time and synchronized and Varroa develops resistance to chemicals (Pettis, 2004; Maggi et al., 2010). On the other hand, breeding of honey bee colonies resistant to Varroa has been the main goal of many researchers due to the residue problem and other negativities caused by chemical control.

Varroa destructor is an ectoparasite with high host specificity (Xie et al., 2016). In addition to being able to distinguish between honey bee races, Varroa can also distinguish drone larvae from worker larvae and nurse bees from

forager bees (Guzmán-Novoa et al., 1999; Aumeier et al., 2002). Varroa mites clearly prefer nurse bee when given the choice between forager bee and nurse bee (Kraus, 1993; Kuenen and Calderone, 1997; Piccolo et al., 2010). Varroas is affected also by age in the selection of host larvae. For example, it prefers older larvae to younger ones (Aumeier et al., 2002). In the strugle against Varroa, as a different perspective, elucidation and manipulation of this host preference mechanism of Varroa can be evaluated as a potential for the development of desired effective methods. Although there are only very few studies available on this subject, chemical cues are known to play an important role. Host preference is based on both low volatility compounds such as cuticular hydrocarbons and pheromones which are volatile compounds emitted by honey bees (Eliash et al., 2014). These social insects, which provide most of their communication with pheromones, also determine the hierarchical relations in the hive with these chemicals (Bortolotti and Costa, 2014).

Some Genes and Chemical Compounds Effective in Host Preference

Host preference is an important step for Varroa reproduction and it meticulously realizes host preference. Juvenile hormone (JH) is thought to be a chemical compound that plays an important role in initiating the reproductive cycle in Varroa. The main products of the JH pathway are JH and MF (methyl farnesoate), two key hormones synthesized by JH acid O-methyltransferase (JHAMT) (Li et al., 2013). For the first time reported by Aurori et al. (2021) that juvenile hormone (JH) acid O-methyltransferase (JHAMT) may be effective in the host preference of Varroa. it has been reported that higher expression of the JHAMT gene, which is involved in the production of volatile compound MF, which can act as a pheromone in honey bees, and which is in the final stage of the juvenile hormone pathway, attracts Varroa to drone brood cells.

Because of Varroa mites live in the dark, chemical signals rather than visual signals are thought to play a role (Li et al., 2022). Le Conte et al. (1989) in their study in which they determined the effects of methyl and ethyl esters of straight-chain fatty acids on the behavior of Varroa, extracted from the cuticle of both drone and worker larvae of A. mellifera; showed that methyl linolenate, methyl palmitate and ethyl palmitate attracted Varroa in vitro conditions. They reported that Varroa was particularly attracted to drone larvae by methyl palmitate between these esters. In yet another study, palmitic acid identified on newly hatched worker larvae was reported to attract Varroa mites (Rickli et al., 1994).

Kraus (1990), using Y-tube wind channel and wax tube choice tests, reported that most of the odor produced by honeybee sting glands and alarm pheromone components have a repellent effect against mites. 1-octanol has also been reported as an important chemical trigger for the leave of Varroa from the bee.

Pernal et al. (2005) reported that the honey bee Nasonov pheromone compound geraniol and nerolic acid have a repellent effect in the host selection process and that even small differences in chemicals cause significant differences in mite activity. In another study, Piccolo et al. (2010) reported that (Z)-8-heptacene for female varroa, which is found in higher amounts in the cuticles of foragers compared to nurses, it has a Varroa deterrent effect.

Varroa prefer drone cells more than worker cells, rarely queen cells. There are both physical and chemical reasons for this situation. Investigating the chemical basis of low mite density in queen bee cells, Drijfhout et al. (2005) reported that crude extracts and fractions of royal jelly deter Varroa mites. Octanoic acid found in royal jelly has been reported to play an effective role in repelling varroa from queen cells (Nazzi et al., 2009).

CONCLUSION

Bee diseases and pests continue their devastating effects all over the world. The European Union has established project funds with broad participation and high budgets in order to protect bee losses and health (COLOSS: Prevention of Honey Bee Colony Losses and FAO803). As a result of the studies, it has been seen as a sustainable, healthy and economical solution to develop resistant lines against honey bee diseases and parasites especially Varroa.

The genes and chemical compounds that are effective in the host-parasite interaction that we have summarized have the potential to develop new and sustainable control strategies by manipulating their pathways. Unfortunately, molecular studies of Varroa have been limited to identifying genes responsible for behavioral resistance to Varro, measuring immune responses and gene expressions of Varroa-infected individuals, and phylogenetic studies. Molecular studies on the determination of the dynamics affecting the host selection of Varroa mites are almost lacking. Molecular methods are of great importance in understanding host-

parasite preference interactions, parasite control and prevention of parasitism. With the inclusion of molecular methods in breeding programs, it will be possible to develop genotypes that mites do not prefer, and the harms of chemical drugs especially to bees, human health and the environment will be prevented.

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MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE DEVELOPMENT IN CHICKENS

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Abstract

Poultry, which accounts for approximately 30% of global meat consumption, is an important dietary source of animal protein. Because its low-price, low-fat content and no religious restrictions, chicken has become an increasingly consumed meat all over the world. Therefore, increasing the growth rate of broilers has always been one of the goals that breeders pursued, and the growth rate has increased significantly over the last few decades. Increasing intensive chicken production and developing modernization in line with consumer demands have led researchers to investigate molecular mechanisms affecting growth in chickens. Understanding the genetic basis of growth traits of chickens is certainly important, as growth traits will be a good indicator of predicting amount of meat and timing of sexual maturity. On the other hand, determining the molecular factors affecting growth has become an important goal in developing countries in order to contribute to the economically sustainable breeding of local chicken breeds with unique meat quality and taste. Though growth characteristics are considered to be important economic traits in broiler production, the underlying genetic mechanisms of chicken growth traits are still unclear. Growth in chickens, a polygenic quantitative trait, is regulated by some major genes such as MSTN (Myostatin), GH (Growth Hormone), IGFI (Insulin Like Growth Factor-1). Therefore, it is quietly important to enlighten the genetic mechanisms of skeletal muscle development in chickens. Many studies have been conducted on skeletal muscle development in chickens using qRT-PCR, transcriptome, and RNA sequencing technology. In recent years, especially RNA sequencing technology has been widely used to explore the genetic mechanisms and biological pathways affecting skeletal muscle development in chicken. In this review, some molecular studies on genes that play role in skeletal muscle development in chicken are summarized.

Key words: *genetic basis of chicken growth, skeletal muscle development, RNA-sequencing, Next Generation Sequencing*

INTRODUCTION

In the last few decades, poultry meat has been almost 30% of global meat consumption (Cao et al., 2020), and it is predicted that it will reach 41% by 2030 (OECD-FAO, 2021). Of course, there are several reasons why chicken meat is so preferred; its low price, low cholesterol and fat levels, delicious taste and no religious restrictions (Cao et al., 2020). Therefore, producers aim to increase their production with a lower cost in order to meet the increasing demand for chicken meat with the human population from past to present.

With the increasing human population until 2001, the demand for poultry meat also increased and breeders tend to intensive poultry production systems (especially for broiler) to meet this demand (Fraser, 2008). In the intensive poultry production system, animals were kept in special closed environments and automation was used for many routine tasks (Fraser et al. 2001). In this way, not only the number of birds per unit area has been increased, but also fast- growing broilers were produced in a shorter time by using genetic selection, heterosis and some food additives. This is such a rapid growth that the 1957-strain broilers had been reached a body weight of 1815 grams in (at best) 101 days, while the 2001-strain broilers had been reached the same weight in just 32 days (Havenstein et al., 2003; Bagés et al., 2015). Zuidhof et al. (2014) studied growth and productivity parameters by comparing two control unselected strains representative of 1957 or 1978 broilers with a commercial broiler. Between 1957 and 2005, they reported that broiler growth increased by over 400% (Figure 1). All these findings demonstrate the affect and power of genetic selection in poultry.

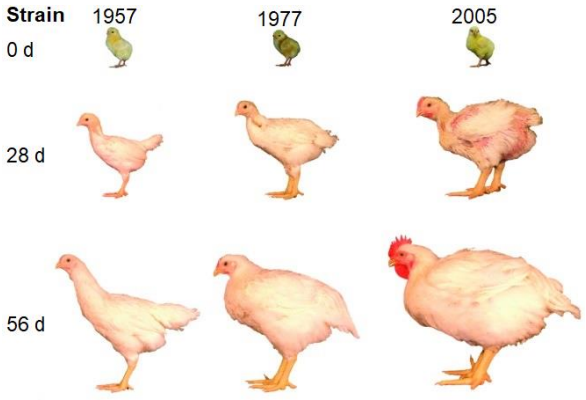

Figure 1. Age-related changes in size of strains unselected since 1957 and 1978, and Ross 308 broilers (2005) (Zuidhof et al., 2014).

This rapid growth of chickens and keeping them in intensive breeding systems has created an increasing response from consumers. In the last few decades, increasing consumer's awareness and concerns about animal welfare have directed consumers towards poultry products obtained from free-raising systems (Joubert, 2013). While this situation has led producers to improve meat quality (more delicious, juicy and tenderness), to develop new chicken meat products (Zhang et al., 2017; San et al., 2021), and to rear local chicken breeds, it has led researchers to investigate the molecular mechanisms that regulate growth of fast and slow growing chickens (Chen et al., 2015; Zhang et al., 2017; Chen et al., 2019; He et al., 2019; Zhang et al., 2019).

Understanding the genetic basis of chickens' growth traits is essential, since growth traits can be a very powerful predictor of meat quantity and timing of sexual maturity (Goto et al., 2019). On the other hand, identification of molecular factors affecting growth has become an important goal to contribute to the commercial breeding of local chicken breeds with unique meat quality and flavor in developing countries (Zhang et al., 2017; Liu et al., 2020). Furthermore, many studies have been conducted on the genes involved in growth, the biological pathways in which genes take part; the gene-gene network, and the detection of genes with major effect (Chen et al., 2015; Zhang et al., 2017; Kong et al., 2017; Chen et al., 2019; Liu et al., 2019; He et al., 2019; Zhang et al., 2019; Wu et al., 2020; San et al., 2021). Especially with developments in the fields of genomics and next generation sequencing (NGS), these studies have accelerated. In this context, many studies have been conducted on chicken skeletal muscle development by using qRT-PCR (quantitative real-time PCR), transcriptome, and RNA sequencing technology. In this review, some molecular studies on genes that play a role in skeletal muscle development in chickens are summarized.

SOME MOLECULAR STUDIES ON CHICKEN SKELETAL MUSCLE GROWTH

Skeletal muscle formation is based on a multi-step process known as myogenesis and occurs in chickens in two stages, embryonic and postnatal (Li et al., 2018; Shi et al., 2022). Muscle precursor cells originate from the somite during the embryonic stage and undergo differentiation and proliferation to form myoblasts. Myoblasts are induced by specific myogenic transcription factors to form multinucleated myotubes after proliferation, migration, and fusion (Shi et al., 2022). Finally, the myotubes turn functionally mature into fast-twitch and slow-twitch fibers (Braun et al., 2011; Buckingham et al., 2017). In this process, it is very crucial for poultry muscle development as the future number and structure of muscle fibers are determined (Ran et al., 2021). Muscle fibers undergo hypertrophy at the postnatal stage. In the hypertrophy process, muscle fibers convert to protein and activate the muscle satellite cells. In addition to all these developmental processes, skeletal muscle development is also associated with the regulation of multiple myogenic genes (Relaix et al., 2012; Scaal et al., 2018; Liu et al., 2021).

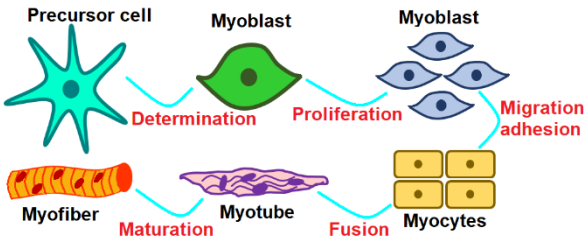

Figure 2. Myofiber formation during skeletal muscle development in chicken (Figure was modified from Nawaz et al. (2021)).

The best models that can be used to investigate the mechanisms underlying the regulation of myogenesis rate and muscle growth are chickens reared for meat (broiler) and egg production (egg) (Zhang et al., 2019). The researchers revealed that the slow growth rate of layers is related to the higher expression of slow-type muscle-related genes (*MB, MYH7B, TNNI1, MYL3,* and *MYL2B*) (Zheng et al., 2009). Differently expressed genes obtained from microarray hybridization analysis were revealed to be associated with satellite cell proliferation and differentiation and muscle hypertrophy (*MUSTN1, FHL2, FGFR2, HS6ST2,* and *CSRP3*) (Zheng et al., 2009). It was determined that the expression level of *MUSTN1* increased in

hypertrophic muscle after exercise and was more expressed in broilers than in layers (Kostek et al., 2007). Thus, this gene is thought to be a key regulator of skeletal muscle hypertrophy (Zheng et al., 2009). On the other hand, *FBXO22, FBXO30, UCHL1, RNF12, HERC4, RLD5* and *HERC5* genes were less expressed in broilers compared to laying hens. Given that these genes are associated with protein degradation, it is assumed that they are responsible for muscle mass in broilers compared to laying hens (Zheng et al., 2009; Mohammadabadi et al., 2021). Thirteen genes (*CDKN2B, ACTC1, MYH15, TNNI1, TNNI2, TNNT2, CCK, CXCL14, MDK, PENK, CSRP2, MFAP5*, and *UCHL1*) were identified as candidate genes related to myoblast proliferation and differentiation in chickens (Nihashi et al., 2019). Wu et al. (2020) revealed that *ANXA1, COL1A1, MYH15, TGFB3*, and *ACTC1* genes were related to chicken skeletal growth. Three commonly differentially expressed genes (*ADAMTS20, ARHGAP19*, and Novel00254) were detected in fast-growing and slow-growing groups of Jinghai yellow chickens (4 weeks of age). In another study on slow and fast-growing chicken strains, KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) analysis revealed that many genes such as *SNCG, MCL1, ARNTL, PLPPR4, VAMP1* were associated with cell proliferation and differentiation, muscle growth, and cell division (Chen et al., 2019).

Many studies have been conducted to investigate the relationship between the growth characteristics of chickens and the genetic characterization of genes affect growth. Polymorphism studies have shown that *PIT1, PRLR, IGF1, INS* and *MSTN* genes are associated with growth and development in chickens (Zhou et al., 2005; Lei et al., 2007; Nei et al., 2008; Bhattacharya et al., 2012; Dushyanth et al., 2016; Liang et al., 2019).

In recent years, RNA sequencing and qRT-PCR have been the most widely used tool to identify genes that affect the growth and development of hybrid and domestic chicken breeds. Chen et al. (2015) reported that *FOXO3* was predominantly expressed in breast and leg muscle of native (slow growing) and commercial (fast growing) chicken breeds but, its mRNA level in slow-growing chickens was significantly higher than in fast-growing chickens. It was suggested that *FOXO3* in slow-growing chickens contributed to their lower growth performance. Furthermore, KEGG and GO analyses showed that CEBPB, FBXO32, *FOXO3* and *MYOD1* played key roles in chicken growth.

Myostatin protein (MSTN), also called growth differentiation factor-8, is predominantly expressed in skeletal muscle cells and functions as a negative regulator of skeletal muscle development (muscle fiber number and diameter) in poultry. Bhattacharya et al. (2015) determined that the expression of *MSTN* at 2 weeks of age was quite low in the layer breed compared to the broiler breeds, while it was expressed quite high at the 4th and 7th weeks. In the obtained findings, they emphasized that the structural differences between the breeds and the negative regulatory effect of Myostatin protein on growth with different expression levels may be the only main factor playing a role in muscle development.

Calpains are cytoplasmic cysteine proteases that need Ca^{2+} to be active. They are involved in various calcium-regulated cellular processes such as signal transduction, cell proliferation and differentiation, membrane fusion, and apoptosis (Huang and Wang, 2001; Sorimachi and Suzuki, 2001). Calpain 3 (CAPN3) is a muscle-specific intracellular protease involved in the calcium-dependent proteolytic system. Zhang et al. (2012) compared the *CAPN3* mRNA level in different tissues (heart, liver, breast muscle, leg muscle, and brain) of a broiler chicken breed and a Sichuan Mountains black bone chicken breed. Findings revealed that the *CAPN3* mRNA level was mostly detected in the breast and leg muscle tissues of all studied individuals, and that the *CAPN3* gene played an important role in the regulation of muscle development in chickens.

Transcriptomics is an analysis that reveals the complete expression profile of all RNAs (mRNA, rRNA, tRNA, miRNA, siRNA, circRNA, lncRNA etc.) present in a given cell population and at a given time (Munshi and Sharma, 2018; He et al., 2019). While miRNAs are highly conserved non-coding small RNAs, mRNA are coding small RNAs involved in protein synthesis. But, both RNAs play critical role in the growth of chicken skeletal muscle. Whereas mRNA directly takes part in protein production, miRNA regulates gene expression as directly or indirectly (Liu et al., 2019). MicroRNAs (miRNAs) are important regulators involved in muscle growth and development. MicroRNA mainly regulates the proliferation and differentiation of myoblasts and thus also regulates the skeletal muscle phenotype in animals (Chen et al., 2020). Chen et al. (2020) revealed that ggmiRNA-454 was a time-dependent (early and late embryonic stage) and tissue-differential expression miRNA. Furthermore, it was stated that gga-miRNA-454 targeting the myotube-associated protein *SBF2* inhibited myoblast differentiation. The expression level of miR-203 in chicken embryonic skeletal muscle increased between 10-16 embryonic days, while its expression sharply decreased after embryonic day 16 and stopped.

The weights of embryo skeletal muscle and histological profiles revealed that miR-203 expression correlated with muscle embryonic development (Luo et al., 2014).

In an mRNA-miRNA interaction study performed in the chest muscles of Chinese Qingyuan partridge chickens, glycolysis/gluconeogenesis process was only enriched (Liu et al., 2019). Several candidate miRNA-gene pairs (gga-miR-499-5p/SOX6 and gga-miR-196 5p/CALM1 etc.) were identified that may affect muscle fiber performance. *SOX6* and *CALM1* genes were target of gga-miR-499-5p and gga-miR-196-5p, respectively. Gga-miR-193-3p also inhibited *PPARGC1A* in chicken muscles. Consequently, these miRNAs could regulate the chicken muscle fiber phenotype by targeting sequences in these genes.

MyomiRs are mainly composed of miR-1 and miR-133 families (include miR-1/miR-1-2/miR-206 and miR133a /miR-133b) and they are a muscle-enriched group of miRNAs (Horak et al., 2016; Mok et al., 2017; Luo et al., 2021). The expression of MRFs in the developing chicken neural tube induced the expression of miR-1 and miR-206, while the lack of *Myf-5* causes a decrease in myomiR expression in developing somites (Sweetman et al., 2008).

Circular RNAs (circRNAs) are non-coding RNA, and their form is a closed loop. CircRNAs can be produced from anywhere in the genome (exons, introns, untranslated regions, non-coding RNA loci also intergenic and antisense transcripts) (Chen et al., 2020; Shi et al., 2022). Some exonic circRNAs (eciRNA) can up-regulate the expression of miRNAs target gene by competing with mRNAs for miRNA binding like miRNA sponges (Ouyang et al., 2018a). Ouyang et al. (2018a) stated that chicken skeletal muscle at embryonic day 11, 16, and post-hatching day 1, circSVIL was differentially expressed. By acting as miR-203 sponges, circSVIL elevates *c-JUN*, and *MEF2C* expression levels, thereby promoting proliferation and differentiation of myoblasts (Ouyang et al., 2018b). In the other study demonstrated that circFGFR2, which is a product of *FGFR2* gene, was differentially expressed during chicken embryo skeletal muscle development (Chen et al., 2018). One of the circRNAs that promotes the proliferation and differentiation of chicken myoblast cells is CircHIPK3 that sponges miR-30a-3p and prevents it from binding to *MEF2C* (Chen et al., 2019).

LncRNAs are length of <200 nucleotides and are not generally translated into protein (Derrien et al., 2012). LncRNAs (Long non-coding RNAs) play a crucial role, as and circRNAs, in muscle development and disease (Chen et al., 2020). The lncRNA Six1, which plays a role in the cis-acting regulation of the *Six1* gene, promotes cell proliferation and muscle growth in chicken (Cai et al., 2017). Lnc-IRS1 (Long non-coding RNA synthezed by insulin receptor substrate 1 gene) regulates myoblast proliferation and differentiation in vitro, while also controlling muscle mass and muscle fiber counts in vivo. In addition, its expression increases with myogenic differentiation (Shi et al., 2022). On the other hand, Lnc-IRS1 functions just like ceRNA (competing for endogenous RNAs), competing with miR-15b-5p and MiR-15c-5p and regulating the expression of the *IGF1* downstream receptor *IRS1*. Thus, its expression increases in hypertrophic broilers and activates the IGF1-PI3K/AKT pathway to prevent muscle atrophy. In this way, it supports myoblast proliferation and differentiation (Li et al., 2019).

DISCUSSION

Chicken is widely preferred by consumers in many countries due to its delicious taste, nutritiousness and delicate meat quality, as well as ease of cooking. The increasing demand for poultry meat has encouraged the production of chickens with higher economic value. In order to meet the increasing demand, both selection and crossbreeding were applied, and intensive production systems were used in order to increase the growth rate of chickens and thus increase muscle mass. However, in order to increase economic efficiency, it has been focused on the research of molecular mechanisms that affect the growth characteristics of chickens. On the other hand, consumers who are more aware of the welfare of animals raised in intensive systems have started to search for delicious and different chicken meat products. Therefore, researchers have carried out many studies in order to gain economic value for domestic chicken breeds, which have a unique taste and meat quality. The molecular mechanisms underlying skeletal muscle development in chickens as well as in other organisms are quite complex and have not yet been fully elucidated. Growth, like other traits of economic importance, is regulated by many genes. However, studies have identified some major-acting genes involved in these processes and many RNAs that regulate these genes. Mechanisms underlying skeletal muscle development in chickens should be investigated more in order to both include local chicken breeds with unique flavors in the trade and to create a market for changing consumer profile.

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SPIRULINA (Arthrospira platensis) EXTRACT PROMOTES MOTILITY, MICROSCOPIC, AND ANTIOXIDATIVE PARAMETERS OF RAM SEMEN DURING REFRIGERATED STORAGE

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Abstract

This study investigated the effect of spirulina ethanolic extract (SEE) on the quality of ram semen during low-temperature liquid storage and the relationship between sperm features. Ejaculates were collected from five Djallonké rams, pooled, extended with Tris-egg yolk (TEY) enriched with 0 (control), 20 (SEE20), 40 (SEE40), and 80 µg/mL (SEE80) of SEE to reach the concentration of 200 × 10^6 spz/mL, and stored at 4°C for 72 h. Extended semen samples were assessed for total motility, progressive motility, sperm motion characteristics, viability, membrane integrity, and morphology at 6, 24, 48, and 72 h of storage. Moreover, malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase, (SOD) and catalase (CAT) levels were measured at 72 h of storage. The enrichment of TEY with SEE at 40 and 80 µg/mL, improved sperm total motility at 48 and 72 h of storage (P<0.05). Also, all SEE-treated samples evidenced higher progressive motility in comparison to the control at 48 and 72 h (P<0.05). SEE80 group showed the highest percentages of viability (76.26 ± 0.90%) and membrane integrity (58.19 ± 1.50%); whereas, SEE40 demonstrated the lowest percentage of morphological abnormality (18.14 ± 1.01%) at 72 h of storage. SEE did not influence NO levels; however, at 40 µg/mL, it reduced MDA concentration and improved SOD and CAT activities (P<0.05). Total motility was positively correlated to progressive motility (r = 0.69, P<0.01), viability (r = 0.91, P<0.01), and membrane integrity (r = 0.49, P<0.05); while, morphological abnormality was negatively correlated to the other sperm parameters. Furthermore, MDA was negatively correlated to total motility (r = - 0.91, P<0.01), progressive motility (r = -0.70, P<0.01), and viability (r = -0.91, P<0.01), and positively correlated to morphological abnormality (r = 0.70, P<0.01). An entirely opposite figure was recorded for SOD. Overall, the results indicated that SEE, especially at 40 µg/mL, can protect ram semen against liquid storage-associated damages. Furthermore, positive and negative correlations exist between semen parameters.

Key words: *Liquid storage, Ram semen, Spirulina, Semen quality*

INTRODUCTION

Liquid semen storage represents a practical and affordable alternative to freeze-thawing especially in developing countries where farmers are smallholders, mainly relying on extensive practices, and more prone to loss of production as a consequence of global warming. Liquid semen storage consists of the reduction of the metabolism of sperm cells thereby extending their shelf life. Coupled with artificial insemination (AI), this assisted reproductive tool allows the acceleration of lambing program and the dissemination of genetics of high merit, prevents disease transmission associated to natural mating, and can considerably improve food security thereby assisting with poverty alleviation and household income security.

However, like freeze-thaw process, liquid semen storage is associated to the decrease of sperm quality which arises as a consequence of oxidative stress (Gundogan et al., 2011). Indeed following semen collection, sperm cells are exposed to *in vitro* conditions and inherent sources of stress (light, temperature, dilution, and pressure to name a few), leading to the overproduction of reactive oxygen species (ROS). Furthermore, the dilution process reduces semen antioxidant capacity (Bilodeau et al., 2000) in which superoxide dismutase (SOD) and catalase (CAT) play essential roles. Besides, ram spermatozoa are devoid of synthesis abilities as

they loss their antioxidant-rich cytoplasm during the differentiation step of spermatogenesis, hence they cannot compensate the deficient antioxidant capacity (Bucak et al., 2007; Eslami et al., 2017). When ROS surpass the detoxifying capacity of semen, oxidative stress occurs. In excess, ROS attack membrane phospholipids, proteins, carbohydrates, DNA, and respiration process, leading to the loss of membrane functionality, DNA integrity, mitochondrial activity, motility, antioxidant activities, and increase of lipid peroxidation (LPO) (Peris-Frau et al., 2020; Kameni et al., 2021), hence the deleterious effects on sperm quality. Moreover, the abundance of polyunsaturated fatty acids (PUFAs) in cellular membrane makes ram spermatozoa particularly vulnerable to LPO, thus the accumulation of toxic products such as malondialdehyde (MDA) during semen storage (Eslami et al., 2017; Zarei et al., 2021). Therefore liquid semen storage suffers from insufficient antioxidant capacity to prevent the generation and/or scavenge excess ROS, maintain homeostasis, and ultimately prevent the loss of sperm quality.

To face this limitation, enrichment of semen extenders with plant extracts rich in antioxidant molecules has shown to be effective in reducing storage-associated damages of semen and consequent gain in fertility after AI (Allai et al., 2016; Abadjieva et al., 2020).

Research directed towards the filamentous cyanobacterium blue-green alga *Arthrospira platensis* commonly known as spirulina, which is an abundant source of bioactive molecules, has provided evidence of its robust antioxidant capacity (Kannan et al., 2014) and its beneficial effect against oxidative stress (Karadeniz et al., 2008; Sorelle et al., 2020). Spirulina belongs to the substances that are listed by the US Food and Drug Administration under the category Generally Recognized as Safe and its typical composition as percentage dry weight can be summarized as: 50% -70% protein, 15 - 25% carbohydrates, 6 - 13% lipids, 4.2 - 6% nucleic acids, and 2.2 - 4.8% minerals (Belay, 2002). Additionally, spirulina possesses PUFAs and other potent antioxidants such as carotenoids, vitamins (B and E), spirulans, C-phycocyanin which is particularly accountable for the antioxidant activity, and allophycocyanin (Estrada et al., 2001). These chemicals makes this alga a rich source of biomolecules with strong antioxidant potential.

With this background, the objectives of the present study were to assess the effect of the supplementation of Tris-egg yolk (TEY) extender with different concentrations of SEE on ram sperm motility, motion characteristics, viability, membrane integrity, and morphology of the Djallonké breed during liquid storage at 4°C for different time intervals (6, 24, 48, and 72 h). Moreover, levels of MDA, an indicator of LPO, nitric oxide (NO), SOD, and CAT were evaluated at 72 h of storage. The correlations between sperm quality parameters, LPO, NO, SOD, and CAT were also investigated.

MATERIALS AND METHODS

Spirulina ethanolic extract (SEE) was prepared by soaking 400 g of spirulina powder in 2 L of ethanol 96% at 4°C in the dark. The soaked material was stirred every 12 h. After 72 h, the mixture was filtered using qualitative filter paper (Whatman 113V, England). The filtrate was evaporated to total dryness by vacuum distillation on a rotary evaporator at 45°C and the resulting extract stored in the dark at 4°C.

Ejaculates from 5 healthy Djallonké rams (2.5 - 3 years, 38 ± 2 kg) were collected via electro-ejaculation. Ejaculates with colour score ≥ 3, volume ≥ 0.75 mL, concentration ≥ 2.5 × 10^9 spz/mL, and mass motility score ≥ 3 were pooled and diluted at 37°C with TEY (2.666 g Tris, 0.44 g glucose, 1.398 g citric acid in 100 mL distilled water, and egg yolk 12% (v/v). penicillin and streptomycin (0.05 mg/mL)) supplemented with 0 (control), 20 (SEE20), 40 (SEE40), and 80 µg/mL (SEE80) of SEE to reach the concentration of 200 × 10^6 spz/mL. Extended samples were stored at 4°C for 72 h and assessed for sperm quality after 6, 24, 48 and 72 h of storage. Oxidative stress indicators (LPO, NO, SOD, and CAT) and total protein content were evaluated at 72 h.

Sperm motility and motion characteristics were assessed by computer-assisted sperm analyser (CASA) with a warmed stage at 37°C (Sperm Analyze Vista, version V1.12 Maya, Guangzhou, China). Extended samples were further diluted using Tris-based extender without egg yolk to 25 × 10^6 spz/mL at 37°C. An aliquot (10 µL) was placed on a warmed slide and covered with a cover slip. For each sample, 4 – 5 fields per drop were analysed at 200 × and a minimum of 200 spermatozoa were evaluated as described by Eslami et al. (2017). The semen variables included in the analysis were total motility (TM, %), progressive motility (PM, %), curvilinear velocity (VCL, µm/s), straight line velocity (VSL, µm/s), average path velocity (VAP, µm/s), linearity (LIN, %), and straightness (STR, %).

Sperm viability was evaluated with eosin-nigrosin staining (Evans and Maxwell, 1987). Thin smears, made in duplicate, were prepared by mixing 10 µl of semen (diluted at 25 × 10^6 spz/mL with Tris-based without egg yolk) with 20 µL of eosin-nigrosin (eosin-Y 1.67 g, nigrosin 10 g, sodium citrate 2.9 g, dissolved in 100 mL distilled water)

on a warm slide (37°C) and immediately spread with another slide. After air drying, viability percent was estimated by counting a minimum of 200 cells from 3 - 4 different fields with bright-field microscopy (400 ×). Spermatozoa showing partial or complete purple colour were considered non-viable and only spermatozoa showing white colour, indicative of strict exclusion of the stain were considered to be alive.

The same slides were used to determine sperm morphology; with similar microscopic settings. A minimum of 200 sperm cells per slide were examined and morphological abnormalities included head, midpiece, and tail defects (Zarei et al., 2018).

Sperm functional membrane integrity was assessed following the principle described by Revell and Mrode (1994). Extended semen sample (20 µL) was mixed with 200 µL of pre-warmed (37°C) 100 mOsm hypoosmotic solution (9 g fructose, 4.9 g trisodium citrate per litre of distilled water) which was prepared daily and kept at 4°C. The mixture was incubated at 37°C for 60 min. After incubation, the sample was gently mixed. Smears were realized in duplicate. A drop (15 µL) of the treated mixture was smeared on a pre-warmed slide and air-dried. A minimum of 200 spermatozoa were counted in 4 - 5 different microscopic fields at 400 × magnification. Spermatozoa with swollen or coiled tails were considered to have functional membranes; whereas, sperm cells showing no swollen or coiled tails were considered to have defective plasma membranes.

At 72 h of storage, spermatozoa were separated from the dilution medium by successive centrifugations (550 g for 10 min, 550 g for 10 min, and 3000 g for 30 min). The resulting supernatant was used to quantify total protein content using Chronolab kit, MDA, NO, SOD, and CAT levels as per Kodjio et al. (2016), Griess (1879), Misra and Fridovich (1972), and Sinha (1972), respectively.

The experiment was conducted in 6 replicates and statistical analyses of the data were performed using R statistical package, version 4.2.0. One-way analysis of variance (ANOVA) was used to determine the difference among means at each time point. Changes in different variables over time were evaluated using repeated measure ANOVA. In case of significant difference, the Tukey post hoc test was used to separate means. The results were reported as mean ± standard error of the mean and values of $P<0.05$ were considered statistically significant. Pearson correlations between parameters were evaluated by combining data for all treatments.

RESULTS AND DISCUSSION

This study demonstrated that enrichment of TEY with SEE can enhance the preservation of sperm TM, PM, and sperm motion characteristics VSL and VAP during refrigerated storage (Tables 1 and 2). The results reported herein are consistent with previous reports regarding the supplementation of extenders with plant extracts during storage at low temperature (Allai et al., 2016; Wen et al., 2019) and cryopreservation (Merati and Farshad, 2020), but contrary to other (Taşdemir et al., 2020). The comparison of the results of sperm motility and motion characteristics obtained from different experiments is challenging considering the variety of sperm concentrations in the samples and diluents used (Câmara et al., 2011).

Table 1. Percentages of total and progressive motility of spermatozoa in ram semen stored at 4°C for 72 h in Tris-egg yolk extender supplemented with spirulina ethanolic extract

Parameters	Treatments	Storage duration (h)			
		6	24	48	72
TM	Control	80.49 ± 1.88^{Aa}	76.49 ± 1.11^{Ba}	69.86 ± 1.37^{Bb}	66.53 ± 0.93^{Bb}
	SEE20	82.54 ± 1.67^{Aa}	79.73 ± 0.94^{ABab}	77.24 ± 1.19^{Ab}	70.96 ± 1.55^{ABc}
	SEE40	84.83 ± 1.16^{Aa}	78.97 ± 1.57^{ABb}	79.40 ± 1.43^{Ab}	71.79 ± 1.25^{Ac}
	SEE80	84.94 ± 1.06^{Aa}	82.83 ± 0.90^{Aa}	77.42 ± 1.14^{Ab}	74.62 ± 0.90^{Ab}
PM	Control	62.49 ± 1.26^{Ba}	60.55 ± 1.18^{Ba}	51.39 ± 0.92^{Bb}	43.94 ± 0.90^{Cc}
	SEE20	66.78 ± 1.18^{ABa}	63.15 ± 0.84^{ABab}	61.36 ± 1.24^{Ab}	53.72 ± 0.71^{Ac}
	SEE40	66.33 ± 1.15^{ABa}	63.32 ± 2.63^{ABa}	60.64 ± 1.68^{Aa}	48.50 ± 1.28^{Bb}
	SEE80	69.38 ± 1.37^{Aa}	68.75 ± 1.29^{Aa}	60.82 ± 1.38^{Ab}	53.96 ± 0.96^{Ac}

TM: Total motility; PM: Progressive motility; SEE20, SEE40, and SEE80: 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender.

A, B, C Values with different superscripts indicate significant differences ($P<0.05$) within groups at each time point.

a, b, c Values with different superscripts indicate significant differences ($P<0.05$) within groups over storage time.

Motility has been documented as one of the essential sperm parameters for fertility (Kasimanickam et al., 2011), especially in AI procedures that require sperm cells to move within the reproductive tract of the females to reach the ovum. Effective semen storage relies on reversible decrease in motility and metabolic activity of sperm cells following cooling at lower temperatures; however, exposure of sperm cells to artificial conditions amplifies the generation of ROS which normally arises as a consequence of aerobic conditions where live sperm cells are involved (Agarwal et al., 2005). As the ROS accumulate and reach a critical concentration, oxidative stress occurs and provokes an irreversible loss of motility, inhibition of fructolysis and respiration in sperm cells

(Salamon and Maxwell, 2000), hence the decrease over time in sperm motility and motion characteristics as observed in the present study. Additionally, motility, which is an energy-dependent function, is particularly associated to mitochondrial activity and therefore may also decrease as a consequence of insufficient supply of energy from mitochondria which impairment drives to adenosine triphosphate (ATP) depletion. In fact, sperm mitochondrion is particularly sensitive to cooling process and this sensitivity results in disturbance in ATP transport with consequent reduction in motility (Zarei et al., 2021).

Interestingly, all SEE treated samples showed greater PM in comparison to the control sample from 48 h onwards (Table 1) Progressive motile sperm cells represent the spermatozoa fraction that can effectively move within the female reproductive tract once insemination is performed. Therefore, by reducing the loss of PM, SEE may improve the fertilization rate of chilled semen. Enrichment of extenders with SEE at 80 µg/mL beneficially affected VSL and VAP respectively at 72 h and 24 h (Table 2). The preservation of these sperm attributes can be ascribed to the capacity of the bioactive components present in SEE to inhibit the generation and/or scavenge ROS in excess. Particularly SEE bioactive components may inhibit the mitochondrial outer membrane enzyme monoamine oxidase that catalyses the oxidative deamination of biogenic amines, producing a large amount of H_2O_2 that contributes to an increase in the steady state concentrations of reactive species within both the mitochondrial, matrix and cytosol (Cadenas and Davies, 2000). In this way, SEE may restore the balance between the amounts of ROS produced and scavenged, and consequently preserve the metabolic activity of sperm cells. It is well known that spirulina is a rich source of bioactive ingredients among which vitamin E which is considered as an essential component of the sperm antioxidant defence system, hence one of the major protector against oxidative stress and LPO (Yousef et al., 2003). SEE, thanks to the presence of vitamin E which is liposoluble, may have inhibited the peroxidation of PUFAs abundant in ram sperm membrane.

Viability, as assessed by dye exclusion, allows to discriminate the necrozoospermia from the total lack of motility associated to structural deficiencies in the tail zone (Chemes and Rawe, 2003). The results of this study evidenced the beneficial influence of SEE especially at 40 and 80 µg/mL on sperm viability during storage (Table 3). Natural herbs cladodes (Opuntia ficus indica) and green tea (Camellia sinensis) used as additives to semen extenders improved viability (Allai et al., 2016; Mehdipour et al., 2016). During semen storage, the accumulation of ROS above the detoxifying capacity of spermatozoa leads to peroxidative damage of membrane proteins, phospholipids, and PUFAs (Peris-Frau et al., 2020; Kameni et al., 2021), hence the loss of membrane integrity and subsequent cell death. This phenomenon is particularly prominent in ram because of the abundance of PUFAs in sperm plasma membrane (Bucak et al., 2007).

Table 2. Kinematic parameters of spermatozoa in ram semen stored at 4°C for 72 h in Tris-egg yolk extender supplemented with spirulina ethanolic extract

		Storage duration (h)			
Parameters	**Treatments**	6	24	48	72
VCL	Control	109.08 ± 2.88^{Aa}	97.70 ± 5.78^{Aa}	71.40 ± 3.79^{Ab}	62.46 ± 2.83^{Ab}
	SEE20	112.18 ± 3.45^{Aa}	100.88 ± 3.27^{Aa}	83.46 ± 3.76^{Ab}	72.73 ± 2.95^{Ab}
	SEE40	109.85 ± 3.27^{Aa}	97.55 ± 3.16^{Aa}	75.03 ± 3.27^{Ab}	68.23 ± 3.11^{Ab}
	SEE80	111.18 ± 4.55^{Aa}	99.89 ± 4.71^{Aa}	83.60 ± 2.89^{Ab}	74.46 ± 3.89^{Ab}
VSL	Control	25.20 ± 1.19^{Aa}	19.09 ± 1.28^{Ab}	17.41 ± 1.63^{Ab}	14.95 ± 1.31^{Bb}
	SEE20	23.65 ± 1.01^{Aa}	22.12 ± 1.01^{Aa}	20.10 ± 1.05^{Aa}	20.29 ± 1.01^{Aa}
	SEE40	23.05 ± 1.58^{Aa}	21.74 ± 1.63^{Aa}	20.98 ± 1.92^{Aa}	18.32 ± 1.38^{ABa}
	SEE80	24.52 ± 1.82^{Aa}	23.98 ± 1.42^{Aa}	22.24 ± 1.53^{Aa}	21.11 ± 1.21^{Aa}
VAP	Control	36.86 ± 1.03^{Aa}	28.26 ± 1.36^{Bb}	24.39 ± 0.98^{Ab}	19.90 ± 0.96^{Ac}
	SEE20	34.58 ± 1.34^{Aa}	30.36 ± 1.23^{Aba}	24.26 ± 1.46^{Ab}	22.18 ± 1.02^{Ab}
	SEE40	33.64 ± 1.65^{Aa}	30.32 ± 1.61^{ABab}	26.59 ± 1.87^{Abc}	22.45 ± 1.41^{Ac}
	SEE80	35.02 ± 1.53^{Aa}	33.84 ± 1.46^{Aa}	27.75 ± 1.51^{Ab}	24.11 ± 1.22^{Ab}
LIN	Control	26.34 ± 3.22^{Aa}	26.81 ± 2.78^{Aa}	22.62 ± 1.95^{Aa}	23.32 ± 1.48^{Aa}
	SEE20	27.45 ± 1.89^{Aa}	23.11 ± 1.62^{Aab}	21.26 ± 1.28^{Ab}	21.26 ± 1.16^{Ab}
	SEE40	26.99 ± 3.20^{Aa}	26.66 ± 2.84^{Aa}	22.01 ± 2.06^{Aa}	21.24 ± 1.82^{Aa}
	SEE80	27.96 ± 2.38^{Aa}	26.18 ± 2.12^{Aa}	25.09 ± 1.79^{Aa}	22.16 ± 1.26^{Aa}
STR	Control	67.85 ± 1.83^{Aa}	69.54 ± 1.41^{Aa}	65.23 ± 1.18^{Ba}	70.91 ± 1.67^{Aa}
	SEE20	68.85 ± 2.03^{Aa}	68.31 ± 2.43^{Aa}	69.98 ± 1.51^{ABa}	71.61 ± 1.58^{Aa}
	SEE40	68.65 ± 1.96^{Aa}	70.72 ± 1.86^{Aa}	71.50 ± 1.27^{Aa}	69.78 ± 1.37^{Aa}
	SEE80	69.61 ± 1.55^{Aa}	71.64 ± 0.92^{Aa}	69.63 ± 1.48^{ABa}	68.30 ± 1.26^{Aa}

VCL: Curvilinear velocity (µm/s); VSL: Straight line velocity (µm/s), VAP: Average path velocity (µm/s), LIN: Linearity (%); STR: Straightness (%); SEE20, SEE40, and SEE80: 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender.

$^{A, B}$ Values with different superscripts indicate significant differences ($P<0.05$) within groups at each time point.

$^{a, b, c}$ Values with different superscripts indicate significant differences ($P<0.05$) within groups over storage time.

The improvement of sperm viability in the present study may be essentially linked to the bioactivity of phycocyanin which has been documented as the compound mainly responsible for the antioxidant activity of spirulina thanks to its strong radical scavenging properties (Estrada et al., 2001). Phycocyanin and other chemicals present in spirulina may have scavenged the ROS generated in excess, hence reducing ROS detrimental action on sperm

membrane constituents, inhibiting LPO, and ultimately preserving sperm viability as noticed in this investigation. Furthermore, SEE may have inhibited the release of cytochrome C from the mitochondria to the cytosol, release which is the initiation point of the apoptosis cascade (Silva, 2006). Besides improving the antioxidant defence of spermatozoa, SEE may have strengthen the levels of phosphoinositide-3 kinases which have been documented as potent stimulators of several anti-apoptotic effectors, hence preventing cell death (Oudit et al., 2004).

The results of the current work indicated that, SEE at 80 µg/mL improved sperm functional membrane integrity at 6, 24, and 72 h (Table 3). This observation may be associated to the capacity of SEE to inhibit the generation of free radicals and their negative action on lipid bilayer interactions and proteins' anchorage to the bilayer, ultimately preventing the loss of physiological function.

For satisfactory results following AI in small ruminants, the threshold of 15% has been suggested as the maximum critical percentage of sperm morphological defects (Rehman et al., 2013). In this study, as the storage duration was extended, the percentages of sperm morphological abnormalities increased. This result is in accordance with previous reports (Gundogan et al., 2011; Gheller et al., 2018). However, enrichment of extenders with SEE at the intermediate concentration (40 µg/mL) showed to be effective at reducing sperm morphological defects (Table 3). While many studies have reported no effect following extender supplementation with antioxidant compounds on sperm morphology (Amini et al., 2019; Zarei et al., 2021), arguing that morphology is mainly related to spermatogenesis, others have highlighted positive effects (Allai et al., 2016; Rateb, 2018). It seems as the dynamics of sperm morphology expands beyond the scope of spermatogenesis, and morphology alteration may also be prevented thanks to the antioxidative potential of SEE. Moreover, the nature, chemical composition and incorporation level of the antioxidant compound coupled with the variety of methodologies used to assess sperm morphology may account for the discrepancy observed.

Under low LPO rates (sub-toxic conditions), cells initiate their maintenance and survival through intrinsic antioxidant defence systems or signalling pathways activation that up-regulate protein antioxidants resulting in an adaptive stress response. On the other hand, under medium or high LPO rates (toxic conditions), the magnitude of oxidative stress exceeds repair capacity, and the cells induce apoptosis or necrosis (Ayala et al., 2014). Therefore, by moderating the rate of LPO as observed in the present work, SEE may have created sub-toxic conditions that favour the increase of enzymatic antioxidant activities and the preservation of sperm quality.

Table 3. Percentages of sperm viability, membrane functional integrity, and abnormal morphology in ram semen stored at 4°C for 72 h in Tris-egg yolk extender supplemented with different concentrations of spirulina ethanolic extract

Parameters	Treatments	Storage duration (h)			
		6	24	48	72
Viability	Control	81.25 ± 1.11^{Ba}	78.62 ± 1.23^{Aab}	75.25 ± 0.94^{Bb}	70.22 ± 0.55^{Bc}
	SEE20	84.00 ± 1.07^{ABa}	82.37 ± 0.86^{Aab}	79.37 ± 1.16^{ABb}	73.78 ± 0.64^{Ac}
	SEE40	86.12 ± 1.38^{Aa}	82.12 ± 2.59^{Aa}	80.94 ± 1.93^{Aab}	74.37 ± 1.25^{Ab}
	SEE80	88.37 ± 1.25^{Aa}	84.51 ± 0.81^{Aab}	81.25 ± 1.11^{Ab}	76.26 ± 0.90^{Ac}
Membrane functional integrity	Control	63.99 ± 0.43^{Ba}	$60.59 \pm .080^{Bb}$	56.89 ± 0.85^{Ac}	51.91 ± 1.15^{Bd}
	SEE20	65.43 ± 1.41^{Ba}	62.23 ± 1.73^{Bab}	59.82 ± 1.11^{Abc}	56.72 ± 1.06^{ABc}
	SEE40	62.89 ± 1.08^{Ba}	59.47 ± 0.92^{Ba}	57.61 ± 1.38^{Aab}	52.94 ± 2.36^{ABb}
	SEE80	73.21 ± 1.02^{Aa}	67.53 ± 1.58^{Ab}	60.86 ± 1.21^{Ac}	58.19 ± 1.50^{Ac}
Abnormal morphology	Control	9.69 ± 0.85^{Aa}	14.00 ± 0.79^{Ab}	18.19 ± 0.68^{ABc}	23.64 ± 0.58^{ABd}
	SEE20	10.94 ± 0.92^{Aa}	12.58 ± 0.62^{Aab}	15.69 ± 0.45^{Cb}	20.22 ± 1.15^{BCc}
	SEE40	11.98 ± 0.87^{Aa}	13.31 ± 0.94^{Aab}	16.02 ± 0.70^{BCbc}	18.14 ± 1.01^{Cc}
	SEE80	12.61 ± 0.96^{Aa}	14.56 ± 0.86^{Aa}	20.15 ± 0.69^{Ab}	27.02 ± 0.97^{Ac}

SEE20, SEE40, and SEE80: 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender.

$^{A, B, C}$ Values with different superscripts indicate significant differences ($P<0.05$) within groups at each time point.

$^{a, b, c, d}$ Values with different superscripts indicate significant differences ($P<0.05$) within groups over storage time.

Recent investigations have evidenced the increase of the antioxidant capacity of semen and the inhibition of LPO following addition of plant extracts to extenders during semen storage (Wen et al., 2019, Taşdemir et al., 2020). Likewise, the enrichment of TEY extender with SEE increased SOD and CAT levels and inhibited MDA production during cooling storage at 4°C (Figure 1).

The correlation analysis revealed that TM was positively related to PM, VCL, VSL, viability and sperm membrane functionality (Table 4). Similar relations have been established in earlier reports (Câmara et al., 2011; Singh et al., 2014). The positive correlations among sperm quality parameters reported herein may be linked to the involvement of sperm membrane in the maintenance of these parameters. On the other hand and as reported by Gupta and Singh (2018), morphological abnormality was negatively correlated to other sperm quality parameters.

Impaired morphology, especially at the level of the tail which is the spermatozoon structure in charge of locomotion, may lead to decrease motility. In addition, morphology impairment may alter normal metabolism with reduction of energy production and accumulation of toxic products, and subsequent loss of sperm motility, viability, and membrane integrity.

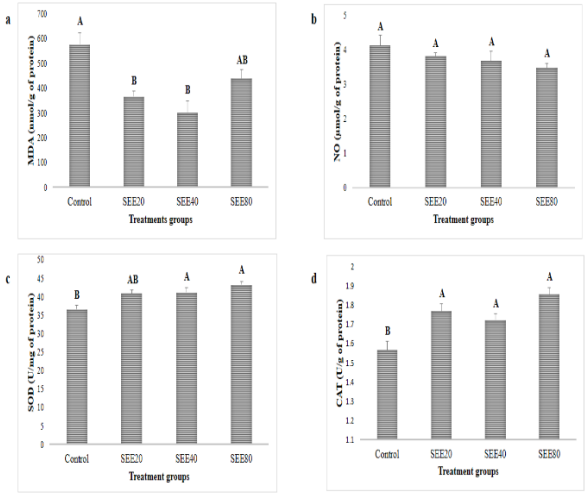

Figure 1. MDA (nmol/g of protein) (a), NO (µmol/g of protein), SOD (U/mg of protein), and CAT (U/g of protein) values after 72 h of liquid storage at 4°C of ram semen extended in Tris-egg yolk supplemented with different concentrations of spirulina ethanolic extract.
SEE20, SEE40, and SEE80: 20, 40, and 80 µg of spirulina ethanolic extract per mL of extender.
A, B Values with different superscripts indicate significant differences (P<0.05) among groups.

Table 4. Correlations (r) among ram sperm parameters after 72 h of liquid storage at 4°C of ram semen in Tris-egg yolk

	PM	VCL	VSL	VAP	LIN	STR	Viab	SFM	Abn
TM	0.69[a]	0.62[a]	0.49[b]	0.40	-0.24	-0.312	0.91[a]	0.49[b]	-0.65[a]
PM		0.50[b]	0.48[b]	0.24	-0.15	0.17	0.81[a]	0.44	-0.61[a]
VCL			0.31	0.58[a]	-0.32	-0.55[b]	0.64[a]	0.54[b]	-0.99[a]
VSL				0.42	0.48[b]	0.06	0.53[b]	0.32	-0.35
VAP					-0.004	-0.11	0.39	0.30	-0.57[a]
LIN						0.48[b]	-0.14	-0.18	0.33
STR							-0.37	-0.19	0.51[b]
Viab								0.48[b]	-0.68[a]
SFM									-0.56[a]

TM: Total motility (%); PM: Progressive motility (%); VCL: Curvilinear velocity (µm/s); VSL: Straight line velocity (µm/s); VAP: Average path velocity (µm/s), LIN: Linearity (%); STR: Straightness (%); Viab: Viability (%); SFM: Spermatozoa with functional membrane (%); Abn: Morphological abnormality (%).
[a] Correlation is significant at the 0.01 level (P<0.01).
[b] Correlation is significant at the 0.05 level (P<0.05).

MDA was negatively correlated with sperm TM, PM, viability and positively correlated to morphological abnormality; whereas, the totally inverse figure was observed for the SOD (Table 5). These results are consistent with previous findings (Kadirve et al., 2014). Alvarez and Storey (1992) reported that semen samples with highest viability after freeze-thawing were characterized by high SOD activity and a strong correlation between loss of SOD activity and loss of motility and membrane integrity, and concluded that the loss of sperm quality might be at least partly mediated by SOD.

Table 5. Correlations (r) of ram sperm parameters with oxidative stress indicators after 72 h of liquid storage at 4°C of ram semen in Tris-egg yolk

Oxidative stress parameters	Sperm parameters									
	TM	PM	VCL	VSL	VAP	LIN	STR	Viab	SFM	Abn
MDA	-0.91[a]	-0.70[a]	-0.49[b]	-0.67[a]	-0.35	0.26	0.45[b]	-0.91[a]	-0.43	0.70[a]
NO	-0.21	-0.35	-0.19	-0.41	-0.35	0.13	0.27	-0.28	-0.12	0.42
SOD	0.66[a]	0.52[b]	0.36	0.70[a]	0.51[b]	-0.41	-0.37	0.55[b]	0.52[b]	-0.74[a]
CAT	0.38	0.14	0.04	0.26	0.30	-0.29	-0.18	0.33	0.59[a]	-0.25

TM: Total motility (%); PM: Progressive motility (%); VCL: Curvilinear velocity (µm/s); VSL: Straight line velocity (µm/s); VAP: Average path velocity (µm/s), LIN: Linearity (%); STR: Straightness (%); Viab: Viability(%); SFM: Spermatozoa with functional membrane (%); Abn: Morphological abnormality (%); MDA: Malondialdehyde (nmol/g of protein); NO: Nitric oxide (µmol/g of protein); SOD: Superoxide dismutase (U/mg of protein); CAT: Catalase (U/g of protein)
[a] Correlation is significant at the 0.01 level (P<0.01).
[b] Correlation is significant at the 0.05 level (P<0.05).

CONCLUSION

In conclusion, SEE at 40 µg/mL can improve ram semen storage by reducing the loss of sperm motility, viability, morphology, and membrane integrity during chilled storage at 4°C for up to 72 h. Moreover SEE can inhibit LPO and stimulate enzymatic antioxidant SOD and CAT at 72 h of storage. Significant positive and negative correlations exist between sperm and oxidative stress parameters. It will be worth to assess the underlying mechanism(s) of the SEE protection of semen during storage and conduct AI trial with SEE treated semen.

ACKNOWLEDGEMENTS

This work was supported by the International Foundation for Science, grant N° I-3-B-6120-1.

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COMPARING THE EXPRESSION STABILITY OF CRISPR/CAS9 MEDIATED KNOCKED IN GENES IN TWO SAFE HARBOR LOCI IN THE GENOME OF CHICKEN SOMATIC CELLS

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Abstract

Little attention has been paid to find the precise location of transgene integration in the chicken (Gallus gallus domesticus) genome in order to achieve a reliable and persistent expression over time. Identification and characterization of "Genomic Safe Harbor" (GSH) loci could be a major area of interest within the field of transgenic technology. A GSH locus that allows the persistent and reliable expression of a knocked-in gene without epigenetic silencing and disruption of the functions of internal genes would be very important and central to the development of bioreactor transgenic livestock. In this study, using bioinformatics, gene expression atlas, and Hi-C data, GSH regions including cROSA-like and cHIPP-like were predicted in the chicken genome. Then, we used CRISPR/Cas9 technology to integrate the EGFP transgene under the control of CMV or delta CMV promoter in the predicted GSH regions as well as OVA as a non-GSH locus of the chicken somatic cells. In contrast to our expectation, the transgene expression under the control of a CMV promoter in a non-GSH locus outperformed the one integrated into the GSH locus. This result suggested that when the expression of a transgene is controlled by a CMV promoter may act independently from its chromosomal position effects. Replacing the CMV promoter with delta CMV altered the expression level in favor of GSH loci. To decipher the behavior of GSH loci, isogenous clones harboring delta CMV promoter-driven EGFP were isolated. The results revealed that bi-allelic expression levels of EGFP controlled by delta CMV promoter were significantly higher in GSH loci than those of the non-GSH locus. Also, we found that terminally-differentiated somatic cells that have integrated the transgene cassette in the GSH region can express EGFP for more than 6 months. In conclusion, we found that the cROSA-like and cHIPP-like loci as GSH regions could be reliably exploited for consistent production of recombinant proteins in the future.

Key words: *CRISPR/Cas9, Transgenesis, Genomic safe harbor locus, Chicken cells*

CRISPR/Cas9- AND dCas9-MEDIATED MANIPULATION OF NATIVE PROMOTERS IN CHICKEN CELLS

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Abstract

Avian transgenesis has served as a suitable approach to generate bioreactors for the manufacturing of recombinant proteins. Production in chicken cells comes with significant advantages over other systems including providing the human-like glycosylation on target proteins. In this regard, the oviduct-specific ovalbumin promoter has been one of the ideal candidates to drive the expression of transgenes. Previous plasmid-based studies on the regulatory sequences of the ovalbumin promoter have led researchers to exploit ovalbumin regulatory elements out of their native genomic context (ex situ) to direct transgene expression in the transgenic chicken bioreactors. In this study, we used CRISPR/Cas9 technologies to engineer the genome and epigenome of the ovalbumin promoter and coding sequence in a non-oviduct chicken cell. We show that CRISPR-mediated deletion of some distal ovalbumin promoter sequences (SDRE and NRE elements) can lead to the significant expression of the ovalbumin gene (more than 104-fold compared to that in the wild-type DF1 cell), and also a knocked-in reporter, can function in an estrogen-independent manner. Also, this study is the first record of the CRISPR/Cas9 guided epigenome engineering in chicken cells. We showed that epigenome modulation using CRISPR-mediated transcriptional activation (CRISPRa) can activate the ovalbumin gene in DF-1 cells (non-oviduct cells). In conclusion, through this research, we were able to use the predominant ability of the ovalbumin promoter to produce exogenous proteins via CRISPR-based genome and epigenome engineering in chicken cells.

Key words: *Chicken fibroblast, Ovalbumin promoter, CRISPR/Cas, Regulatory sequences, Gene editing*

INSERTION AND ACTIVATION OF A REPORTER INTO THE 3'-UTR REGION OF THE OVALBUMIN GENE IN CHICKEN FIBROBLASTS USING CRISPR/DCAS9-MEDIATED TRANSPOSITION

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Abstract

CRISPR technology is the most robust genome manipulation tool that allows researchers to generate animals with new features. Using CRISPR technology various animals with an improved immune system, disease resistance, and the ability in producing drugs have been generated. In transgenic chicken, under the control of the ovalbumin gene promoter, biopharmaceutical proteins are produced in the oviduct and secreted into the egg white. In this study to exploit the promoter of the ovalbumin gene, we inserted a promoterless reporter gene in the 3'-UTR region of this gene in chicken DF1 cells using CRISPR-HDR. Then, we applied our recently developed CRISPR/dCas9-mediated transposition technology to incorporate a ubiquitous promoter (CMV), upstream of ovalbumin exon 1 and to activate this promoter in DF1 cells. Our results showed that the 3'UTR region of the ovalbumin gene is a suitable candidate to support the expression of the transgene in chicken bioreactors. Also, our introduced dCas9-piggyBac transposition system provided a useful tool for studying transgene expression in non-oviduct cells. These methods provide useful tools for the generation of transgenic chicken bioreactors.

Key words: *CRISPR/Cas9 system, Biopharmaceutical proteins, CRISPR/dCas9-mediated transposition, Oviduct-specific expression*

INTESTINAL FATTY ACID BINDING PROTEIN (I-FABP) GENE VARIATION IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*)

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Abstract

Fatty acid binding proteins (FABPs), which belong to the multigene family, play an important role in homeostasis, lipid uptake and transport in tissues. Intestinal fatty acid binding protein (I-FABP) is a small cytosolic protein and is highly active in intracellular fatty acid metabolism in fish gut. The European Sea bass (Dicentrarchus labrax) is an important commercial marine fish species in the Mediterranean region. In the present study, the partial I-FABP gene region of European sea bass was sequenced for detecting single nucleotide polymorphism (SNP) using DNA sequencing. We identified one SNP (g.2450T>C) in the noncoding region of the I-FABP gene in European sea bass. After approval with further studies, the *g.2450T>C locus in I-FABP which could affect growth and muscle fat content, can be used for marker-assisted selection (MAS) studies in European sea bass.*

Key words: *SNP, FABP gene, teleost, variation*

INTRODUCTION

FAPBs, which are long-chain fatty acids, consists of 126 to 137 amino acids and their molecular weight is around 150 kDa (Chen and Shi, 2009; Venold et al., 2019). Growth and development in animals are affected by the intracellular transport of long-chain fatty acids (Besnard et al., 2002; Zhang et al., 2019). The intestinal fatty acid binding protein (I-FABP) gene is involved in the synthesis, uptake and intracellular transport of triglyceride-rich lipoproteins in the intestine (Chen and Shi, 2009; Levy et al., 2001). The fatty acids and dietary lipids supply most of the energy needed for vital activities such as the growth, development, swimming and reproduction of fish (Andrea et al. 2000).

The I-FABP has been shown as an important candidate gene for the meat quality of livestock (Dawood et al., 2021). Besides, it has been reported that there are significant associations between the single nucleotide polymorphisms (SNPs) of the I-FABP gene and growth characteristics of fish species such as growth and abdominal adiposity (Xia et al., 2013; Zhou et al., 2019).

European sea bass, which belongs to the Moronidae family, lives from the north-eastern Atlantic Ocean to the Mediterranean and the Black Sea (Vandeputte et al., 2019). Turkey is the first producer of European sea bass in the world, it was 149,000 tons in 2021 (TUİK, 2021). Increasing the growth rate and muscle fat content of *D. labrax* stocks is important for sustainable aquaculture. Molecular markers are very effective tools in breeding programs of aquaculture species.

The aim of this study is to reveal the SNPs in the European sea bass I-FABP gene region by DNA sequencing.

MATERIALS AND METHODS

A total of 80 European sea bass samples were randomly taken from a processing factory in Urla- İzmir. These fish samples were reared in the same cage environment from Çeşme-İzmir.

Muscle tissue samples were collected from each fish and preserved at -20°C until DNA isolation. Genomic DNA was extracted by using the High Pure PCR Template Kit (Roche, Germany) following recommended protocols in the Ege University, Faculty of Fisheries, Laboratory of the Molecular Genetics and Fish Breeding. The concentration and purity of the genomic DNA samples were measured by spectrophotometer.

The primer sequences of partial region of I-FABP gene were designed for European sea bass based on whole genome shotgun sequence (Accession number CBXY010015347) using Primer-BLAST algorithm (https:// www.ncbi.nlm.nih.gov/tools/primer90blast/) from GenBank using the Primer3 program (http://bioinfo.ut.ee) (NCBI, 2022). Primer sequences of I-FABP gene are F: 5'-TCCAGGGTGCGGAATTTACT -3' and R: 5'-

CCTTCAACGGCAACTGGAAA -3'. PCR was performed in a 50-µL volume containing 50 ng genomic DNA, 0.5 µM of each primer, 2× MyTaq Mix (Meridian Bioscience, USA), 0,5 U Taq Hot Start DNA (Bioline) polymerase and distilled water. The thermal profile consisted of initial denaturation at 95°C for 4 min; 37 cycles of amplification, including 95°C for 45 s, 56°C for 45 s and 72°C for the 90 s and final extension at 72°C for 10 min. The PCR products were checked on 2% agarose gel using horizontal electrophoresis and the gels were stained using RedSafe (iNtRON) (Figure 1).

The genotyping of the SNPs in the partial region of the I-FABP gene was performed by 3500XL Genetic Analyzer System (Applied Biosystems, USA). The sequence results were aligned and controlled by ChromasPro Version 2.1.10 (Technelysium Pty. Ltd. Australia). Differences of gene sequences between individuals were detected using BioEdit (Hall, 1999). The genotype distributions of nucleotide polymorphisms of I-FABP sequences were tested with the "HardyWeinberg" package in R software (R version R-3.4.3).

The sequence data obtained for I-FABP gene region and the reference sequences taken from GenBank were used in the reconstruction of the phylogenetic tree based on Maximum Likelihood (ML) method applying HKY nucleotide substitution model. Phylogenetic evolutionary analyses of I-FABP gene region of European sea bass were conducted using MEGA version 11 (Tamura K, Stecher G, and Kumar S 2021).

RESULTS AND DISCUSSION

The genetic variation at 754 bp of the partial I-FABP gene was amplified by PCR and it was shown in Fig. 1. The amplified gene region is located between 1845 and 2598 bp in the reference sequence (CBXY010015347) (NCBI GenBank).

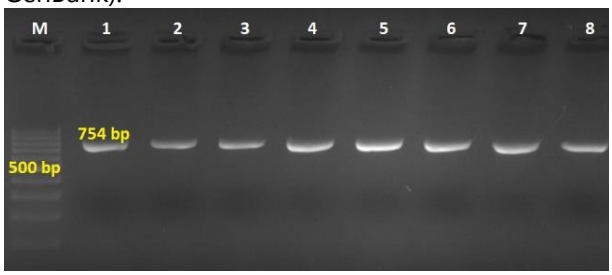

Figure 1. Electrophoresis image of the PCR amplicons of I-FABP gene for the 8 European sea bass samples. M: Marker

European sea bass I-FABP gene contains four exons and three introns that encode 132 amino acids (KJ130030) (NCBI, 2022). In this study. PCR products of the I-FABP gene region were investigated with DNA sequencing method and a g.2450T>C change was detected in the noncoding region (Figure 2). More polymorphisms are found in intronic regions, because they are under less selection pressure than exonic regions of genes (Özcan Gökçek and Işık, 2020; Özcan Gökçek et al., 2020). Moreover, SNPs in non-coding regions can affect transcription and translation of mRNA splicing and regulate the gene expression (Pagani and Baralle, 2004). For this reason, it is recommended to investigate the relationship between detected SNP (g.2450T>C) in the I-FABP gene and the yield characteristics of European sea bass. Xia et al. (2013) reported that a SNP (SNP1245) detected in the exon 3 of the IFABP-a gene of Asian sea bass (*Lates calcarifer*) has a significant relationship with the growth characteristics. Zhou et al. (2019) found that I-FABP on chromosome 24 affects the body thickness (BT) of *L. crocea* by influencing the fatty acid metabolism in the intestine and abdominal fat accumulation.

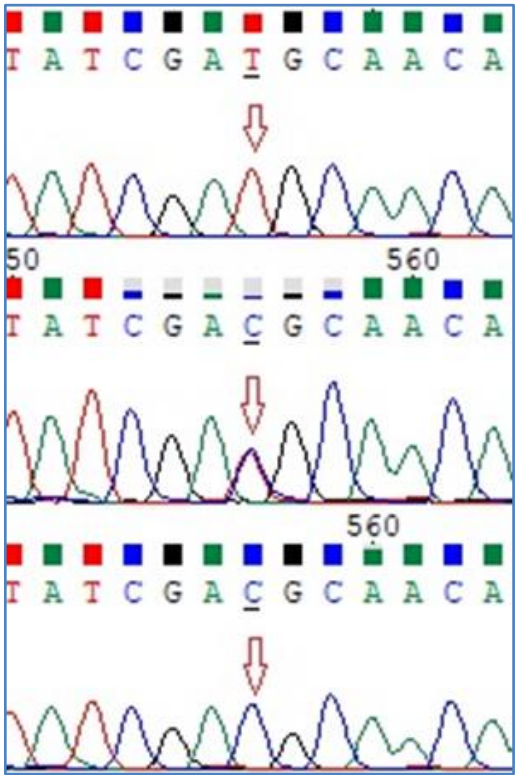

Figure 2. Partial sequencing results for SNPs g.2450T>C in noncoding region of I-FABP gene

In this study, the g.2450T > C locus of I-FABP gene was in Hardy–Weinberg equilibrium. The genotype and allele frequencies of the European sea bass I-FABP gene were shown in Table 1.

Table 1. Allele and genotype frequencies of I-FABP gene region in European sea bass

Loci	I-FABP Genotypes				Allele Frequency		χ^2*
		TT	TC	CC	T	G	
g.2450T>C	O	50	25	5	0.78	0.22	0.58
	E	48.83	27.34	3.83			

Note: $\chi 2$ (0.05; 1) $p < 0.05$

According to ML analysis of the evolutionary relationship of the I-FABP sequences obtained from the present study with the other fish species retrieved from GenBank are shown in Figure 3. The ML tree based on HKY nucleotide substitution model revealed D. labrax, Epinephelus fuscoguttatus, Larimichthys crocea and partial region (1845-2598) of D. labrax whole genome shotgun sequence in the same clade.

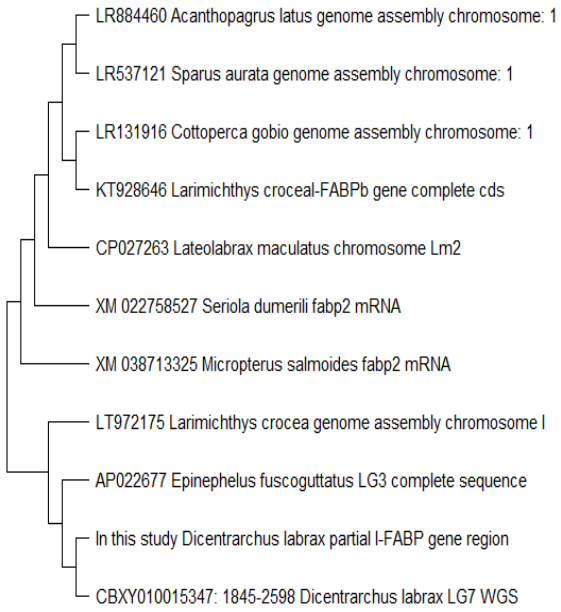

Figure 3. The phylogenic tree of the sequences of I-FABP (*fabp2*) gene in different species retrieved from GenBank database.

CONCLUSION

The SNP (g.2450T>C) detected in the current study and potential SNPs that can be found in other regions of the I-FABP gene of European sea bass and their relationships with harvest traits such as growth, muscle fat content and body thickness should be investigated.

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EMBRYO BIOTECHNOLOGIES IN SHEEP: ACHIEVEMENTS AND NEW IMPROVEMENTS

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Abstract

To date, large-scale use of multiple ovulation and embryo transfer (MOET) programmes in ovine species, even significant progress have been made, are still showed several limitation due to high costs of hormonal stimulation and treatment which determine in many cases unpredictable results among the breeds. For these main reasons, the full application in large-scale systems, for the development of breeding program, is still far away. However, the intense basic and applied research on sheep breeding has provided over the last 50 years the foundation for the development of assisted reproductive technologies (ARTs), which have significantly increased the efficiency of reproduction in flock breeding. The new prospects offered by in vitro embryo production (IVEP) through collection of oocytes post-mortem or by repeated ovum pick-up from live females suggested an alternative to MOET programmes and may be more extensively used, moving from the exclusive research in the laboratory to field application. Several advances have been obtained in these years, exploring, and improving the methods for oocytes collection in vivo and post-mortem from adult and juvenile lambs; developing efficient system for in vitro maturation, fertilization and embryo culture. More specifically it has been evaluated the possibility to obtain embryos used after transfer, to potentially reduce the generation interval, speeding the rate of genetic improvement. Recently, significant progress on embryo freezing have been proposed and they might allow wider propagation of valuable genes in small ruminants populations and may be used for constitution of flocks without risks of disease. Moreover seminal experiments on embryo manipulation, cloning by nuclear transplantation, and transgenic animal production in sheep have largely contributed to establish the methods currently applied in many animal models, showing their limits and future perspective. Finally, the new era of gene editing might offer innovative perspectives in sheep breeding, but the efficient application is nowadays limited by the need of specialized operators and to the high costs for embryo manipulation and molecular biology analysis.

Key words: *Embryo, biotechnology,fertilization,in vitro*

WHOLE GENOME SEQUENCING OF A CALF SHOWING PHENOTYPICAL FINDINGS OF COMPLEX VERTEBRAL MALFORMATION (CVM)

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Abstract

Complex Vertebral Malformation (CVM) has been reported as a disease of Holstein-Friesian cattle breeds with an autosomal recessive pattern of inheritance. The disease has been associated with a mutation on the SLC35A3 gene, which is localized in the range of 43,294,662-43,248,939 on bovine chromosome 3 (BTA3). The point mutation c.559G>T is a missense mutation causing V180F amino acid change. Another mutation (g.43268369G>T) in the Montbeliarde cattle breed is also associated with CVM. About 80% of homozygous mutant fetuses are aborted before the 260th day of gestation. Growth retardation, vertebral malformation, bilateral symmetrical arthrogryposis of carpal and metacarpophalangeal joints are among the most important clinical findings of the disease. In Holstein cattle breeds 17 haplotypes, which resulting in abortion and stillbirth, have been identified with homozygous recessive inheritance pattern. Among in the 17 haplotypes HH5 and HH6 have been reported as phenocopies of CVM. Beside that 2% of the genotyped bulls in the French cattle population were found heterozygous for c.559G>T CVM mutation, moreover 86% of this bull population were heterozygous for HH5. A stillborn calf with CVM phenotype in a farm in Kastamonu province is subjected to the study. The blood of the calf´s mother, stillborn calf were sent in for genetic analysis. The necropsy findings were compatible with the CVM and anatomical presentation of the vertebra showed malformation characteristics. However, the c.559G>T missense mutation was detected neither in dam nor in the calf by direct mutation analysis. Therefore, to have a better view of the genome whole-genome sequencing (WGS) analysis was used. Bioinformatic analysis of the WGS revealed silent mutations in the SLC35A3 gene. In this study, preliminary findings of the stillborn calf with CVM phenotype from Turkey will be presented for the first time. The study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Directorate of Science Fellowships and Grant Programmes (BİDEB) project number 1919B012102959.

Key words: *GWAS, Haplotypes, HH5, Stillbirth, V180F*

INVESTIGATION OF THE EFFECT OF ALLELES OF BMP15 AND GDF9 GENES ON THE LITTER SIZE IN KANGAL AKKARAMAN SHEEP BY PCR-RFLP METHOD**

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Abstract

BMP15 and GDF9, two members of the transforming growth factor-β (TGF-β) superfamily, are key genes involved in increasing the ovulation rate. These genes are produced by the ovary. Furthermore, these two oocyte-specific factors induce mitosis along with differentiation in follicular somatic cells during follicular development via a paracrine signaling pathway. It has been reported that heterozygous sheep mutants for both GDF9 and BMP15 genes are fertile and that the effects of these mutations on laying rate are additive. Homozygous mutant sheep have been reported to be sterile due to inappropriate sexual differentiation. BMP15, also known as the GDF9B (FecX) gene, is on the X chromosome and encodes bone morphogenetic protein 15, which plays an important role in follicular development in sheep. The GDF9 gene, on the other hand, consists of 1 intron and 2 exons on the 5th chromosome in sheep. It has been reported that the twinning rate of Kangal Akkaraman sheep is around 22%. The sample of this planned study consisted of blood to be collected in K3EDTA tubes of Kangal Akkaraman sheep who gave birth to at least two twins (n:96) and at least two singletons (n:96). DNA isolation was carried out by the standard phenol-chloroform method. For the PCR-RFLP reactions, for the FecXB, FecXG, FecXI, FecXH alleles of the BMP15 gene, respectively: BstDEI, HinfI, XbaI, Ah1I; For the FecG allele of the GDF9 gene, HhaI restriction enzymes will be used. In this study, it was aimed to genotype the alleles in the BMP15 and GDF9 genes by PCR-RFLP method and then to determine the effect on the number of lambs per birth by logistic regression analysis.
***The genetic material of this project was obtained from the herds of the "TAGEM/58KAK2012-08" subproject of the Domestic Animal Breeding in the Hands of Public Breeding of Kangal Akkaraman Sheep in Sivas Province. We thank the Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies. This study was supported by Erciyes University, Scientific Research Projects Coordination Unit (Project No: TSA-2022-11745).*

Key words: *BMP15, GDF9, Kangal Akkaraman, litter size, PCR-RFLP*

THE REVIEW OF THE GENETIC DIVERSITY IN AWASSI SHEEP BREEDS IN FERTILE CRESCENT BASED ON MICROSATELLITES

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Abstract

Many domestic breeds belonging to different animal species, such as sheep, are in danger of extinction, as farmers prefer high-yielding breeds as well as intense selection pressure. This fact leads to the conduct of appropriate breeding and conservation programs all over the world. Revealing genetic diversity is the first step in implementing breeding and conservation programmes. This is the first comprehensive study aimed at reviewing the current genetic diversity and evolutionary relationships in the indigenous Awassi sheep of the Fertile Crescent based on previous studies of microsatellite markers. The original Awassi sheep has tremendous genetic diversity compared to other domestic sheep breeds reared in different regions of the world. On the other hand, the population size of the original Awassi sheep has decreased, and the local breed is crossed with exotic breeds. Until now, many microsatellite marker studies have been carried out for Awassi sheep breeds in different countries such as Turkey, Jordan, Iraq, Saudi Arabia, Israel and Egypt. According to these studies based on microsatellite markers, it is believed that crossbreeding practices lead to genetic erosion of the original Awassi sheep, while a decrease in population size will negatively affect genetic diversity in the future. Small farmers can raise the local Awassi sheep separately from the exotic flocks. This kind of application might stop genetic erosion and make it possible for local herds to be raised in places where they are naturally suited.

Key words: *genetic polymorphism, microsatellite markers, Awassi, Fertile Crescent*

INTRODUCTION

The wide variety of sheep breeds present in the Fertile Crescent is a reflection of its ecological richness (The Arab Center for the Studies of Arid Zones Dry lands, 1997). Domesticated farm animals are essential for food production and agriculture, contributing 30–40% of the worldwide agricultural economy. Farm animals are rapidly losing their genetic resources, and the world loses two breeds of its priceless domestic variety each week despite their enormous economic contribution (FAO, 2000). The variety of livestock genetic resources should thus be maintained and documented urgently, and suitable conservation and sustainable use plans should be developed, especially in developing nations (Hannotte and Jialin, 2005). Around the world, several regional varieties of farm animals are in danger of becoming extinct. Native breeds of farm animals, despite their unique characteristics and adaptability to diverse and often adverse environmental conditions are at risk due to their lower profitability and corresponding declining use (Polak et al., 2021). According to FAO data, there are currently around 8800 livestock breeds in the world (http://www.fao.org/dad-is/en; 09.11.2019), of which 17% have been assessed to be endangered. The high level of genetic variability needed to address unanticipated future issues of livestock production systems is nonetheless facilitated by these breeds (Olschewsky and Hinrichs, 2021). One of the major objectives of breeders nowadays is to find and preserve the genetic diversity of local livestock breeds for a variety of reasons. For instance, genetic variety is required to provide milk and meat from various livestock species to fulfill present and future demand (Toro and Caballero, 2005; Verrier et al., 2005). In this regard, Awassi sheep breeds are critical because they meet a large portion of the demand for both meat and milk in the countries of Fertile Crescent (Galal et al., 2008).

Sheep farming plays a significant role in the Middle East, as it does around the globe, since it feeds the local populations with milk and meat (Galal et al., 2008). It is known that a balanced and healthy diet depends not only on the consumption of crop products but also on livestock products obtained from different species, such as sheep. In the Fertile

Crescent, sheep breeding is mainly focused on the Awassi breed and other breeds that have been improved, such as the improved Awassi, in order to obtain a high milk yield. Since the milk and meat production of the Baladi Awassi breed is low, farmers prefer exotic breeds (Abdullah et al. 2002), which has led to a significant decrease in the population size of the Baladi breed (Al-Atiyat et al., 2012). Today, it is thought that there are between 46 and 49 native breeds of sheep in Arab countries (FAO, 1995; The Arab Center for the Studies of Arid Zones Dry lands, 1997). The most common are the fat-tailed and fine-tailed woolly sheep, as well as the hairy, fat-tailed sheep. Also, the native Awassi breed has added to the genetic resources of animals and has been an important part of the culture of the Fertile Crescent region.

Widespread acceptance exists for microsatellites as effective tools for assessing genetic diversity and divergence within and across populations (FAO, 2011). Analyzing genetic diversity at the DNA level in different animal breeds has become easier because to the use of molecular DNA technologies (Al-Atiyat, 2009). Microsatellite DNA markers fall under this category since they are polymorphic and randomly dispersed across the genomes of livestock species (Karaca et al., 1999; Ahmadi et al., 2007; Sulaiman et al., 2011; Ismoyowati and Purwantini, 2011). These markers have also been effectively used to research the genetic links between and within populations of sheep, as well as biodiversity (Romanov and Weigend, 2001; Rosenberg et al., 2001). Gives accurate details on allele frequencies and profiles for a single DNA sample that may be taken from blood or tissue. The majority of sheep genetic diversity studies utilizing microsatellite markers, however, may not be as helpful as anticipated in supplying the necessary knowledge for creating suitable and sustainable sheep breeding programs and conservation plans.

By evaluating the previous studies, this review aimed to reveal current genetic diversity and phylogenetic relationships among Awassi sheep breeds reared in Fertile Crescent. In addition, current genetic diversity of Awassi sheep breeds was compared to other sheep breeds raised in different countries of the world and some solid solutions for future challenges were proposed.

MOLECULAR TECHNIQUES TO DETECT GENETIC DIVERSITY

Molecular markers, particularly DNA-based markers, may be used to estimate genetic diversity accurately. A molecular marker is a DNA sequence that can be tracked and has a known position on the chromosome. Other genetic diversity research involves generating molecular markers, and the information collected may be used to quantify links between species and other genetic diversity research (Hoshino et al., 2012).

Microsatellites have been used to detect genetic diversity in Awassi sheep breeds raised in various countries, including Turkey (Yilmaz et al. 2014), Israel (Lawson Handley et al. 2007), Jordan (Al-Atiyat, 2012), Egypt (Elbeltagy, 2015), Syria (Elbeltagy, 2015), Iraq (Raheem Alnajm, 2021), Saudi Arabia (Mahmoud, 2020).

GENETIC DIVERSITY AMONG AWASSI SHEEP

Genetic diversity and population structure aid in guiding breed development programs to meet current production needs in various environments, allowing for long-term genetic improvement, and facilitating adaptation to changing breeding objectives, and devising measures of livestock breed utilization and conservation (Notter, 1999; Dalvit et al., 2008). The fundamental goal of conservation initiatives, in general, is to maintain as much genetic variation as possible (Hoban et al., 2018). In conservation projects, priority must be given to breeds with fewer individuals and those in danger of extinction. Unfortunately, the Awassi has taken its place among the genotypes of indigenous genetic resources requiring a protection project due to their declining numbers. In this respect, it is essential to identify the genetic makeup of the Awassi breed, which is valuable to the region, and to determine the position of this breed among genetic resources, especially its genetic distance from domestic genotypes and its position on the phylogenetic tree (Al-Atiyat et al., 2014; Al-Atiyat and Aljumaah, 2018).

So many researches have been carried out to detect the genetic variation among Awassi sheep breeds in Fertile Crescent as a result of both declining population levels and heightened awareness of the significance of genetic conservation of local breeds (Table 1).

The most comprehensive microsatellite study (29 microsatellite loci) of native Turkish Sheep breed was carried out by Ozmen et al. (2020). The test for Hardy-Weinberg equilibrium (HWE) showed a significant deficit of heterozygosity in six investigated sheep populations when considering all loci. In comparison to prior research, the study undertaken by Ozmen et al. (2020) is of considerable value since it contains a larger number of microsatellite markers, a larger number of people, and a superior sampling approach. It is clear that it shows the genetic diversity and

population structure of native Turkish sheep breeds, which have been shown to have a lot of genetic diversity and not much inbreeding. Yilmaz et al. (2014) used a total of 18 different microsatellite markers to detect genetic diversity in nine native Turkish sheep breeds (Awassi, Cine Caparı, Karakas, Karya, Karayaka, Morkaraman, Norduz, Sakız and Tuj). The authors point out that that they are adequately polymorphic and suitable for studying diversity in Turkish sheep breeds. The Turkish sheep breeds exhibit higher within-breed variance than between-breed variation. In another study (Soysal 2005) in Türkiye, a total of 45 alleles were observed based on three microsatellites in five breeds. The authors concluded that the Awassi sheep from Israel and Turkey had a larger average number of alleles per microsatellite than the Icelandic, French, German, and Hungarian sheep breeds studied. They also concluded that the Turkish Awassi is more diverse than others.

In Jordan, the first attempt to detect genetic diversity via microsatellite markers was carried out by Al-Atiyat (2012). Despite the three flocks being small in size, they had effective selective mating and gene flow, and they matched with the Hardy-Weinberg Equilibrium, strongly proving the usefulness of loci as a genetic tool in population genetics analyses of sheep. From a genetic conservation point of view, it is recommended to maintain the genetic diversity of small flocks when genetic migration of good genetic resources is absent. Al-Atiyat (2014) used the microsatellite markers to detect the genetic diversity and differentiation of sheep populations in Jordan. The results obtained when studying the breed of Awassi sheep in Jordan from ten different geographic regions revealed that these sheep have shown genetic diversity in relation to the microsatellite used, and they have been divided into four major genetic groups instead of ten different geographic areas due to genetic convergence between them. Mahmoud (2015) used microsatellite markers to detect genetic differentiation between Awassi sheep of Jordan and Merino sheep from Australia. The author highlighted that the most genetically variable flock was found to be the Awassi middle region flock, which had more alleles per locus and higher He. This might be as a result of gene flow into this flock. Overall, the data shows that Awassi sheep differ from Merino sheep in terms of evolution. Jawasreh et al. (2018) investigated the genetic and population structure of native (Awassi) and foreign (Romanov, Charollais, Assaf, and Suffolk) sheep breeds by using microsatellite markers. According to the author, the findings showed that the tested breeds have significant genetic variability.

Table 1. Genetic diversity parameters in Awassi Sheep breed

Country	Ho	He	Na	PIC	F_{IS}	NoM	References
Türkiye	0.23	0.66	8.172	0.80	0.660	29	Ozmen, et al. (2020).
	0.72	0.82	11.39	0.87	0.144	18	Yilmaz et al. (2014)
	0.73	0.74	8.67	-	0.0145	3	Soysal et al.(2005)
	0.67	0.75	6.69	-	0.103	13	Elbeltagy et al. (2015)
Jordan	0.67	0.70	6.17	0.65	-	6	Al-Atiyat (2012)
	0.67	0.73	12.67	-	0.078	6	Al-Atiyat (2014)
	0.67	0.76	-	0.65	0.13	6	Mahmoud et al. (2015)
	0.90	0.70	3.5	0.60	-	9	Jawasreh et al.(2018)
	-	0.80	5.4	-	0.057	19	Al-Atiyat et al. (2018)
Saudi Arabia	-	0.74	8.8	-	0.074	19	Al-Atiyatet al. (2018)
Egypt	0.72	0.79	10.92	-	0.09	13	Elbeltagy et al. (2015)
Syria	0.70	0.74	7.08	-	0.047	13	Elbeltagy et al. (2015)
Israel	0.64	0.75	19.9	-	0.134	23	Lawson Handley et al. (2007)

Ho: observed heterozygosity; He: expected heterozygosity; Na: number of alleles; PIC: Polymorphic information content; F_{IS}: Inbreeding coefficient; NoM: number of loci studied.

Al-Atiyat et al. (2018) with the microsatellite markers, the genetic structure and diversity of five Saudi Arabian sheep (Harri, Najdi, Naemi, Arb, and Rufidi) and a group of Awassi sheep from Jordan were looked at. The Arb sheep was the most different from the other breeds, while the Jordan Awassi sheep was the most similar to the Naemi sheep, which shows that they come from the same place. Elbeltagy et al. (2015) investigated levels of within-and between-population genetic diversity in indigenous sheep populations in Egypt, Turkey, and Syria using microsatellite markers. The authors emphasized that this genetic structure was the result of genetic stock exchange along the Nile River valley and the Mediterranean Sea coast, but that there was little gene flow between flocks found in Northern, Central, and Southern Egypt across the Western desert. Lawson Handley et al.

(2007) were used the microsatellite markers to explore the genetic structure of sheep breeds in Israel. The authors said that heterozygosity was just a little bit higher in southern breeds than in northern breeds. This shows that variety decreases as you move away from the center of domestication in the Near East.

PHYLOGENETIC RELATIONSHIPS AMONG AWASSI SHEEP

Microsatellite markers are effective for detecting genetic variation as well as revealing phylogenetic links between various livestock breeds. They've been used to look at phylogenetic links between various breeds of sheep (Raheem Alnajm et al., 2021), chicken (Karsli et al., 2019), goat (Ceccobelli et al., 2020), and cattle (Demir et al., 2019) using genetic distance, factorial correspondence analysis (FCA), and structural analysis. In this section, we talked about how Awassi sheep breeds evolved based on research that used microsatellite markers. One of the most significant topics in molecular evolution right now is reconstructing the evolutionary history of populations. Previous investigations using microsatellite markers were used to evaluate phylogenetic linkages among sheep breeds.

In various research, phylogenetic trees based on genetic distance values were generated for Awassi sheep breeds, in which Al-Atiyat et al. (2018) and Raheem Alnajm et al. (2021) showed that Awassi and Naemi were closer, while Jawasreh (2011) reported that Blackface Awassi and Black Najdi were closer compared to other sheep breeds. And also, Harri individuals showed genetic closeness to Awassi Adam et al. 2015. Recent research (Yilmaz et al., 2014) showed that the Awassi and Morkaraman breeds are part of the same group in the phylogenetic tree. It is not surprising since both the Awassi and Morkaraman breeds are raised in Eastern Anatolia, Turkey. On the other hand, Awassi sheep breeds were clearly separated from exotic breeds such as Merino flocks and from all other Turkish sheep in the phylogenetic tree, which is in accordance with the breed origins (Al-Atiyat, 2015; Ozmen et al., 2020). These results agree with the known history of the populations regarding their faraway geographical location and the long evolutionary time of their common ancestors. A previous study about the genetic distance between Awassi and Merino sheep reported a significant differentiation between Awassi sheep and the Merino Spanish breed (Arranz et al., 2001). Recently, phylogenetic tree diagrams showed the Awassi fat-tailed sheep were separated from the other species, including Merinos. (Ozdemir et al., 2011; Musthafa et al., 2012).

CONCLUSION AND FUTURE GOALS

It's critical to preserve both within- and between-breed variety in order to be prepared for agricultural difficulties in the future. Choices about breed priority, conservation, and management are all based on the traits of genetic diversity, which can be better understood by making and using genotyping tools. Since 2005, diversification studies have included a substantial variety of various (mainly cattle) breeds from all over the globe. For this, several diversity parameters were calculated using various genotyping approaches. Microsatellite methods predominated with a use of 48%, but SNP-array and WGS usage rose between 2016 and 2020. In this study, the genetic diversity and evolutionary relationships of the Awassi sheep breed on the basis of microsatellite markers were reviewed by verifying previous studies. It has been observed that the Awassi breed has great genetic diversity compared to other breeds of sheep bred in different regions of the world due to the geographical location in which the breed is spread (Fertile Crescent). The Awassi sheep breed is a valuable genetic resource that is important to the sheep industry in more than 30 countries.

There are still some challenges to the genetic diversity of the Awassi breed. Today, Awassi sheep breeds are replaced with high-yielding exotic breeds by crossing Awassi with other native and exotic breeds in their countries of origin. So, the Awassi has taken its place among the genotypes of indigenous genetic resources requiring a protection project due to their declining numbers. These facts lead to genetic erosion in local breeds, which possess unique characteristics needed for the sustainable use of local breeds in the future.

To prevent the genetic erosion of Awassi sheep, further work is needed to reveal more information on the sheep population structures and to help start sustainable breeding programs and policies involving the decision on pure or crossbreeding. In this respect, it is essential to identify the genetic diversity of the Awassi breed, which is valuable to the region, and to identify its position among genetic resources, especially its genetic distance from domestic genotypes and its position on the phylogenetic tree. Moreover, not only microsatellite markers but also new molecular techniques based on next-generation sequencing (NGS) should be adopted by researchers to reveal and detect genetic diversity corresponding to a more significant part of the genome.

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IN OVO STIMULATION WITH BIOACTIVE SUBSTANCES CAUSES CHANGES IN GENE EXPRESSION AND METHYLATION OF CAECAL TONSILS IN CHICKENS

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Abstract

The caecal tonsils are a cluster of lymphoid tissue near the bird's cecum. They are the organs that generate the organism´s immune response. The caecal tonsils are functionally and anatomically essential organs of the gut-associated lymphoid tissues (GALT). They are adjacent to the intestinal microbiota, exposing them to microbe-associated molecular patterns. The GALT has developed mechanisms to control intestinal pathogens´ host response to colonization. Commensal bacteria are recognized, and then GALT activates a transient and non-inflammatory immune response. Bioactive substances have begun to be used to control pathogens and enhance the immune response. These include probiotics, prebiotics, and synbiotics. It has been proven that stimulation of bioactive substances in ovo on day 12 of egg incubation provides early contact between GALT and beneficial gut bacteria of chicken. This study aimed to determine the effect of in ovo stimulation with bioactive substances on immune-related gene expression and DNA methylation in caecal tonsils of two different chicken genotypes. On day 12 of incubation, a probiotic - Lactococcus lactis, a prebiotic - galactooligosaccharide, and a synbiotic - a combination of both - were injected into the eggs of Ross 308 broiler chickens and native polish breed chickens. Caecal tonsils were collected post mortem on day 42 of breeding. Isolated RNA was used for gene expression analysis (RT-qPCR), while DNA was used for global methylation and gene-specific methylation (qMSP). The results showed changes in methylation of 6 genes: SYK, ANGPTL4, TNFRSF14, IKZF1, CYR61, SERPING. The expression these genes were down-regulated, especially after prebiotic and synbiotic in ovo injection. Global methylation analysis showed differences between two different genotypes of analyzed chickens. The results indicate substance-dependent and genotype-dependent stimulation of chickens and epigenetic silencing of gene expression in caecal tonsils.

Key words: *Probiotic, Prebiotic, Epigenetics, Transcriptomics, in ovo stimulation*

COMPARISON OF MS2 RNA CARRIER INFLUENCE FOR MILK microRNA ISOLATION

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Abstract

MicroRNAs are short non-coding RNAs that regulate gene expression by post-transcriptional regulation. Considering the theoretical and clinical importance of microRNAs, it will be very beneficial to eliminate the question marks and optimize the extraction of milk microRNAs. They can be observed in many biofluids such as urine, saliva, serum, plasma, and milk. Our study is about milk microRNAs, which are of great importance for the health of offspring as well as the contribution they can provide to the industry. The first step in obtaining microRNA from milk is usually to obtain total RNA of good concentration and purity. We used two different amounts of starting material (200 µl and 400 µl) and MS2 RNA addition to investigate the impact of the input material size and carrier RNA effect on total RNA extraction efficiency.
The highest total RNA concentration was obtained using MS2 with an input volume of 200 µl. There is a statistically significant difference between the groups in the presence and absence of MS2 (P<0,001). The result suggests that the use of MS2 RNA can help optimize extraction in biological fluids where the RNA level is relatively low, such as skim milk.

Key words: *microRNA, skim milk, MS2, RNA isolation*

INTRODUCTION

MicroRNAs are short (18-25 nt) non-coding RNAs that regulate gene expression by post-transcriptional regulation. MicroRNAs have been shown to be essential for numerous biological processes such as cellular proliferation, migration, invasion, apoptosis, differentiation, and tumorigenesis. MicroRNAs can be encountered not only in the cell but also in the extracellular space. They can be observed in various biological fluids such as urine, saliva, serum, plasma, and milk (Santulli, 2015; Anfossi et al., 2013). Milk microRNAs are active in 2 ways as in other biological fluids; 1) encapsulated in exosomes, milk fat globules, and milk cells 2) found free in skim milk associated with Ago-2 protein (Anfossi et al., 2013; Melnik et al.)

Milk is vital for the offspring in the first months of life. Although there are differences in components between species, milk basically consists of 4 main components: lipid, water, lactose, and protein. These components also vary individually depending on genetic and environmental factors such as nutrition, stress, illness, and lactation period. In addition to providing the energy needed by the offspring of all species, milk also supports the immune system, facilitates digestion, and supports growth (Fox, 2011; Knight, 2018).

MicroRNAs can be synthesized from mammary epithelial cells in skim milk, lipid and cell fractions of the milk. Environmental conditions can affect all mentioned biological processes by altering the expression of these microRNAs in milk.

The efficient and accurate acquisition of milk microRNAs depends on many factors, from the collection and storage of milk samples to the input volume used in the extraction.

On the other hand, microRNA extraction is closely related to the yield and concentration of total RNA to be obtained. Therefore, there is a need for reproducible optimization that incorporates all these factors, especially in RNA extraction from biofluids. The carrier used in the study is RNA from MS2 bacteriophage MS2 improves binding to the column membrane and increases the yield of extracted RNA from samples with low RNA concentration such as the skim milk fraction of the milk used in this paper. This study aims to examine the effect of starting material amount and MS2 RNA usage on total RNA concentration and purity.

MATERIALS AND METHODS

Milk Collection and Sample Handling

Milk samples were collected from 35 healthy heifers (Holstein) and drawn into 50 mL RNase-

free tubes. The samples were then aliquoted in 1.5 mL tubes and stored at -80°C until analysis. Skim milk was obtained by centrifugation of 1.5 mL milk samples at 12000 x g for 5 minutes at 4°C then immediately processed for RNA isolation with different input material size 200 µl (n = 17); 200µl + MS2 (n = 45); 400µl (n = 13) and 400µl + MS2 (n = 15).

RNA isolation

The miRNeasy Serum/Plasma Advanced Kit (Qiagen #217204) was used for RNA isolation, according to the manufacturer's protocol, with the following modifications: For each 200 µL of starting material, 1.5 µL of MS2 RNA (Roche #10165948001) was mixed with 60 µL of Buffer RPL; Columns were air-dried for 10 minutes to avoid ethanol carry-over before the elution step. Isolated RNA was stored at -80°C. RNA purity was estimated by measuring the absorbance at 260 nm and 280 nm (A260/A280) using Nanodrop ND-2000.

RESULTS AND DISCUSSION

Tables 1 and 2 represent the interpretation of the RNA concentration and A260/280 value of all groups. Our results demonstrated that the presence of MS2 and different input volumes among MS2-present samples increased the total RNA concentration in skim milk. (Table-1) (P<0,001). For A260/280 values, no statistically significant difference was found between 400 input volumes and any group. On the other hand, 200 input volumes differed significantly with all groups with MS2. (Table-2). A260/280 absorbance means of all groups were outside the acceptable limit (1.8-2.2) (Imbeaud et al., 2005). However, when the percentage of values above 1.8 were taken, the 200 µl + MS2 group was quite prominent with 51.1% (Table-3).

In terms of nucleic acid concentration, no statistically significant difference was found between the input volume of 200µl and 400µl. Based on these data, it is possible to say that using MS2 RNA during the isolation of total RNA from milk positively affects RNA concentration. The most successful result of the RNA purity value was achieved with 200µl + MS2 input volume. According to the data obtained, since 400µl input volume does not show a significant difference in RNA purity with the use of MS2 RNA, 200µl input volume could be the appropriate method for RNA extraction.

Table 1. The relationship between Nucleic Acid Concentration and MS2 RNA Carrier for skim milk

Nucleic Acid Concentration (ng/µL)			
Method	**N**	**Mean ± SEM**	**Statistical significance (ANOVA)**
200µl	**17**	**6,40 ± 0,90^{a}**	**P<0,001**
400µl	**13**	**8,34 ± 0,73^{a}**	
200µl+MS2	**45**	**21,68 ± 1,69^{b}**	
400µl+MS2	**15**	**36,08 ± 3,31^{c}**	

Note: 200µl and 400µl: *volume of milk used for extraction;* ***200µl+MS2 and 400µl+MS2:*** *milk samples with MS2 RNA;* ***N:*** *Sample Size;* ***SEM:*** *Standard Error of the Mean* ***a, b, c:*** *the values with different superscript letters in a column are significantly different (p<0.05)*

Table 2. The relationship between A260/280 absorbance and MS2 RNA Carrier for skim milk

A260/280 absorbance			
	N	**Mean ± SEM**	**Statistical significance (ANOVA)**
200µl	**17**	**1,44 ± 0,06^{a}**	**P<0,01**
400µl	**13**	**1,57 ± 0,09^{ab}**	
200µl+MS2	**45**	**1,71 ± 0,03^{b}**	
400µl+MS2	**15**	**1,69 ± 0,03^{b}**	

Note: 200µl and 400µl: *volume of milk used for extraction;* ***200µl+MS2 and 400µl+MS2:*** *milk samples with MS2 RNA;* ***N:*** *Sample Size;* ***SEM:*** *Standard Error of the Mean* ***a, b:*** *the values with different superscript letters in a column are significantly different (p<0.05)*

Table 3. Percentage of A260/280 values over 1,8

A260/280 absorbance Percentage		
	N	**Percentage of values over 1.8**
200µl	**17**	**11,8%**
400µl	**13**	**23,1%**
200µl+MS2	**45**	**51,1%**
400µl+MS2	**15**	**26,7%**

Note: 200µl and 400µl: *volume of milk used for extraction;* ***200µl+MS2 and 400µl+MS2:*** *milk samples with MS2 RNA;* ***N:*** *Sample Size*

In the kit protocol, the recommended input volume is 200 µl, and the maximum volume is 600 µl. As stated in the kit, increasing the initial volume may increase the RNA concentration but may decrease the RNA purity. Alsaweed et al. (2015) concluded that skim milk contains lower RNA concentrations than other milk fractions

(lipid and cell). There was no significant difference between 200 and 400 µl in skim milk samples may be caused by the material used is the region of the milk with the lowest concentration of free RNAs. Therefore, using MS2 RNA may assist in optimizing such biological fluids. Ramón-Núñez et al. (2017) isolated microRNA from plasma using yeast and MS2 RNAs as carriers with two different kits, including the Qiagen miRNeasy kit. The Qiagen kit with the MS2 RNA gave the highest concentration of RNA. The low RNA purity in our samples may be due to low RNA concentration and contamination of the guanidinium salt included in the kit protocol (Ahlberg, 2021).

CONCLUSIONS

This study showed that using MS2 carrier in RNA isolation from skim milk increases RNA concentration. In all, the MS2 RNA method is promising to gain eligible RNA in skim milk to support further molecular experiments.

ACKNOWLEDGEMENTS

We would like to extend our sincere thanks to Aytaç AKÇAY for his statistical support.

One of the authors, Kardelen Karaman, would like to thank the Council of Higher Education of Turkey for 100/2000 CoHE Doctoral Scholarship.

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A2 MILK, THE HUMAN ASPECT OF GENETIC VARIATION IN CATTLE

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Abstract

Recently, a new type of cow's milk has been commercialized in the markets, called A2 milk. this call comes from the allelic composition at chromosome 6. The only difference between A1 and A2 milk results from the polymorphysme at the 67 amino acid chain. In this position, A2 milk has a proline amino acid, while A1 milk has a histidine amino acid. The variants A1 and A2 are generally the most well-known variants while there are eleven other genetic variants of β-casein which are A3, A4, B, C, D, E, F, H1, H2, I and G. Proteins are one of the most important components of milk, especially casein have received the greatest attention as they are the source of bioactive opoide peptides called beta-casomorphine 7. Peptides are released through enzymatic digestion of casein and whey proteins. More precisely, this bioactive peptide is produced by sequential gastrointestinal digestion of bovine A1 variants proteins, while this phenomenal is not present in variant A2. In milk hydrolyzed with beta casein variant A1, the percentage of BCM-7 is higher than milk type A2. Studies have shown that A1 milk can be harmful to health not only for adults but also for infants and that β-casein A2 becomes a safer choice following the relationship between disease risk and consumption of the BCM 7 peptide. Indeed, epidemiological studies suggest that the released BCM 7 peptide is a risk factor for the development of diseases in humans, including an increased risk of type 1 diabetes and cardiovascular disease, but this has not yet been established by other studies fully demonstrated by the researchers. On the other hand, A2 milk has been suggested as an appropriate substitute for A1 milk since populations consuming milk containing high levels of the A2 beta-casein variant have lower rates of these diseases.

Key words: *A2 milk, Milk allergies, Milk production*

SOME CANDIDATE GENES ASSOCIATED WITH DISEASE RESISTANCE IN POULTRY

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Abstract

In intensive poultry production systems, high expenditures are made for the sanitation and prevention of disease factors that have negative effects on the poultry immune system. Since bacteria and virus-borne disease agents cause significant economic losses for the poultry industry, studies have been the focus on to increase genetic resistance chickens. Therefore, studies have been mostly focused on some candidate genes associated with disease resistance, B and T cells, MHC (major histocompatibility complex), MHC-B locus genes and SAL1 genes, which can be used as molecular markers. In this review, some major candidate genes that are associated with the resistance to highly mortal epidemic diseases in poultry flocks will be discussed.

Key words: *Chicken Immune System, Disease Resistance, Gene, MHC, Molecular Marker*

DETERMINATION OF GROWTH PERFORMANCE OF LAMBS AND FERTILITY CHARACTERISTICS OF KARAYAKA SHEEP RAISED IN TOKAT

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Abstract

This study was carried out to determine the growth and reproductive performance of Karayaka sheep in Tokat province within the scope of National Animal Breeding Project. Data were collected from 27432 Karayaka lambs of 28885 Karayaka sheep which were born during 2017 to 2021 birth season. In this study, the live weights, survival rates of the lambs in various periods and reproductive traits of Karayaka ewes were investigated. In this study, the live weights, daily live weight gain, survival rates (90th day) and reproductive traits of Karayaka ewes were investigated. The averages of reproductive traits of Karayaka sheep such as infertility, fertility, twins, fecundity, litter size and survival rate were 12.6 %, 87.4 %, 14.8 %, 0.94, 1.09 and 93.2 % respectively. The effects of year, sex and type of birth on the birth weights and the live weights of 90 days (weaning) of Karayaka lambs were found to be significant. Daily live weight gain was affected only by year and sex.

Key words: *sheep, karayaka, lamb, weight, fertility*

INTRODUCTION

Karayaka sheep one of the native breeds of Turkey extensively raised in coastline of Black Sea Region especially in Sinop, Samsun, Ordu, Giresun, Trabzon, and in passageway of Black Sea to inland Anatolia regions such as Tokat and Amasya. Karayaka sheep is named after Karayaka town of Tokat approximately 300 years ago according to history. Karayaka sheep made up %3-4 of the sheep population of Turkey (Şen at. al., 2009). This sheep breed is well suited to the harsh climate, poor pasture and severe conditions that are the characteristics of the hills and uplands in the region. They are carpet-wool breed kept also for meat production. The body color of Karayaka sheep is white, and there might be black and brown plaque in head, ear, leg and body and occasionally black or brown animals are seen (Ulutaş et. al.,2009).

Sheep breeding is an important livestock branch with meat, milk, fleece, leather and fertilizer yields. Meat production has come to the fore in today's economic conditions. Lamb yield is one of the most important factors affecting meat production. In summary, fertility is one of the factors that determine profitability in sheep breeding (Tamer and Şirin, 2021).

In the researches, it has been determined that Karayaka sheep have a wide variation in terms of yield characteristics. Birth weight, weaning (90^{th} day) and 180^{th} day live weights were determined as 2-6 kg, 19.5 kg and 29.6 kg, respectively (Ertuğrul, 1985; Ünal et al., 2003). In another study, birth weight, 56^{th} and 140^{th} day live weight were found as 3.68 kg, 14.93 kg and 25.46 kg, respectively. Adult live weight varies between 32-65 kg in sheep and 50-85 kg in rams (Ulutaş et. al., 2011).

In this study, it was aimed to improve the fertility of Karayaka sheep and the live weight of lambs within the scope of the National Animal Breeding Project.

MATERIALS AND METHODS

This study were used Karayaka sheep within the scope of The National Animal Breeding Project as animal material. Data were collected from 27432 Karayaka lambs of 28885 Karayaka sheep which were born during 2017 to 2021 birth season. As fertility traits were calculated birth rate, infertility rate, twinning rate, fecundity, litter size and survival rates in sheep (Kaymakçı and Sönmez, 1996).

Lambing rate: (ewes lambed/ ewes mated) × 100

(2) Twins birth rate: (twin-born / ewes born) × 100

(3) Infertility rate: infertile ewes/ ewes exposed × 100

(4) Survival rate: number of lambs alive at three month/number of lambs born X 100

(5) Fecundity: lambs born/ ewes mated

(6) Litter size: lambs born/ number of lambing ewes

The birth and 90th day (weaning) weights of the lambs were determined according to year, sex and birth type. The birth weights of the lambs were determined within 24 hours at the latest after birth.

The Minitab 13.0 program was used to perform an analysis of variance on the progeny yield results. Tukey's multiple comparison test was used to compare the means.

RESULTS AND DISCUSSION

Some reproductive traits and survival rate of Karayaka sheep are given in Table 1.

Table 1. Some fertility characteristics and survival rates of Karayaka sheep.

Yaer	Birth Rate (%)	Twins Birt Rate (%)	Fecundity	Litter Size	Infertility Rate (%)	Survival Rate (90th day) (%)
2017	80	6.25	0.84	1.06	20	94
2018	80	11.82	0.92	1.15	20	92
2019	93	5.40	0.98	1.06	7	95
2020	92	11.73	1.02	1.13	8	91
2021	92	3.95	0.96	1.04	8	94

In this study, the highest birth rate was reached in 2019. The highest twinning rate was 11.83 %. The infertility rate exceeded acceptable rates in 2017 and 2018. The birth rate was determined as 86.2 % in another study conducted in Karayaka sheep (Belgüzar, 2011). These birth rates are similar to the rates obtained in this study. The infertility rate (13.3%) obtained in a study (Olfaz and Saylam, 1996) is similar to the rate obtained in this study. The survival rate was determined as 80 % in the study carried out in Karayaka sheep (Akçapınat et. al., 2002). Survival rates obtained in our study was higher than obtained in Akçapınar et al. (2002).

Birth and 90th day weight according to year, sex and birth type of Karayaka lambs are given in Table 2. Liveweights are indicative of an animal's current and changing physical state and measuring changes in liveweight is useful in assessing how an animal is responding to its current situation (Baker et al., 1947). As liveweight is affected by: growth, nutrition, health, stress, pregnancy and genetics (Brown et al., 2015), research exploring these areas in sheep can use liveweight as an important variable.

The highest birth weight was reached in 2017 and 2021 ($P<0.01$). Birth weights of males were higher than females ($P<0.01$). Single lambs have higher birth weights than twin lambs ($P<0.01$).

Table 2. Birth and 90th day weight according to year, sex and birth type of Karayaka lambs

		Birth Weight (kg)		90th Day Weight (kg)	
		n	Mean ±SH	n	Mean ±SH
Avarage		28667	3,94±0,57	26414	27,86±0,06
Year					
	2017	4779	3,97±0,06[a]	4661	23,86±0,5[a]
	2018	6200	3,89±0,07[b]	5093	29,40±0,5[b]
	2019	5922	3,95±0,06[a]	5793	27,81±0,5[c]
	2020	6269	3,90±0,06[b]	5682	27,71±0,5[c]
	2021	5497	3,98±0,06[a]	5185	29,08±0,6[b]
Birth Type		**	**		
	Single	24430	3,95±0,06[a]	22552	28,45±0,6[a]
	Twins	4237	3,83±0,06[b]	3862	27,18±0,5[b]
Sex		**	**		
	Male	15050	3,99±0,06[a]	14248	29,95±0,6[a]
	Female	13617	3,86±0,07[b]	12166	26,80±0,4[b]
		**	**		

**The differences between the means shown with different letters were found to be very significant ($P<0.01$).

Birth weight averages obtained in this study were higher than the average birth weights obtained in another study conducted in Karayaka lambs (Arıtürk ve ark., 1987). In a study, the weaning (90th day) weight of Karayaka lamb was determined as 19.5 kg (Unal et. al., 2003). The 90th weight obtained in this study is higher.

CONCLUSIONS

Birth weight and 90th day weights of Karayaka lambs within the scope of The National Animal Breeding Project have improved over the years. However, some of the fertility parameters were also improved. However, the infertility rate is above acceptable limits.

ACKNOWLEDGEMENTS

This work was supported by TAGEM (National Animal Breeding Project)

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ANIMAL NEED INDEX EVALUATION IN DAIRY CATTLE: A CASE STUDY FROM SAMSUN PROVINCE OF TURKEY

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Abstract

Animal welfare is one of the principal issues to boost productivity level of dairy herds. The objective of the present study was to evaluate the practical animal need index (ANI) levels of dairy cattle reared at Practicing Farm of University of Ondokuz Mayis, Samsun, Turkey. In total, twenty assessors visually scored the farm by ANI. Locomotion ability (LA), social interaction (SI), flooring (F), indoor conditions (IC) and stockmanship effect (SE) were formed as ANI fragments. A chart with 1 to 100 points (1-25: poor, 26-50: moderate, 51-75: suitable and ≥76: excellent) was used for recording data. While the overall ANI was calculated to be 76.36±1.04 points, the highest correlation coefficient was estimated between IC and ANI (r=0.61). It was concluded that the evaluated farm was found within the excellent class, and more attention on LA and F might be suggested to elevate total ANI to the higher value.

Key words: *animal welfare, cattle, dairy farm, management.*

ANALYSIS OF THE CULLING OF SIMMENTAL HEIFERS IN THE REPUBLIC OF KAZAKHSTAN

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Abstract

According to the data of many researchers, the number of replacement heifers in the herd directly depends on the proportion of cows culled. However, the analysis of the culling of Simmental breed heifers has not been previously carried out in Kazakhstan, that is the purpose of this study. The object of the study were 7 breeding herds of Simmental breed, data collection was conducted from 2019 to 2021, the data of 7859 heifers were involved in the research. Two groups of data of young heifers aged 15-18 months (4006 heads) and older heifers aged 18-24 months (3744 heads) were formed. Based on the research, the main indicators of the causes of culling of dairy heifers were formed, which included: conformation defects (CD), low fertility (LF), hoof disease (HD), udder disease (UD), and other causes (OC) of animal culling. Most heifers of two ages are culled from the OC, 49% for 15-18 months and 57% for 18-24 months. The second significant reason for culling for heifers aged 15-18 months was CD - 23%, and for heifers aged 18-24 months was HD. At 15-18 months of age, CD and LF were equally important causes of culling, 14% each. At older ages, culling due to LF remains at about the same level of 13%, and HD decreases to 5%. At this age, since the first pregnancy ends and udder formation is more intense, 3% of the heifers leave the herd due to UD. As a result, it was determined that the total culling rate of heifers at the age of 15-18 months was 14.6±2.2%, and for 18-24 months it was 12.6±2.2%.

Key words: *culling, replacement heifers, Simmental breed, culling causes*

INTRODUCTION

The life expectancy of dairy cows in the world is on average 3-4 years, in terms of lactation is about 2 lactations.

As De Vries A. (2020) writes in his review, the natural life expectancy of cows is about 20 years, but in the last 10 years, no matter how scientists tried to improve the technology of housing, reproduction, feeding, and genetically increase the economic use of cows, they have not advanced far enough. All this makes one think that this indicator is influenced not only by the above-mentioned factors, but by the welfare and ethical use of cows. In this regard, scientific work in this area is so highly developed in the dairy countries of Europe. At the same time, he points out that reducing the use of cows may be due to economic considerations, but it is not always economically beneficial for the farmer.

Fetrow J. (2006) and others note in their review that the dairy farming industry needs a clearer or even standardized approach to reporting cow life expectancy as well as to reporting the causes of cow culling. They propose the term "Herd Turnover Rate," believing that this indicator is much more effective than the previously proposed herd replacement rate, culling rate, or attrition rate. The herd turnover rate should be calculated as the number of cows leaving the herd in a certain period divided by the time the animals are at risk, which will allow a more accurate characterization of populations.

In this regard, it is of great importance to study the factors that affect the culling of dairy breeds heifers of reproductive age in Kazakhstan. Studies of this nature have not been conducted earlier in our country.

Thus, research aimed at studying the causes of heifer culling is relevant, and it is necessary to make decisions in the conditions of the Republic of Kazakhstan.

The purpose of the research was to study the actual number of culled heifers of dairy heifers in the farms of the Republic of Kazakhstan.

MATERIALS AND METHODS

In order to analyze the actual number and causes of culled heifers of dairy direction, studies were conducted in farms located in regions of Kazakhstan with different natural and climatic zones, including East Kazakhstan, West Kazakhstan, Akmola, Aktyubinsk, Almaty, Kyzylorda, Pavlodar and South Kazakhstan regions. The data were collected in the period

from 2019 to 2021 on a herd of 7859 heifers of Simmental breed.

Based on the research, the main indicators of the culling causes of dairy heifers were formed, which included: conformation defects (CD), low fertility (LF), hoof diseases (HD), udder diseases (UD), as well as other causes (OC) of animal culling. Information was collected according to the primary documentation of the farms.

To obtain descriptive statistics, a standard MS Excel add-in "Analysis ToolPak" - the "descriptive statistics" analysis tool was used, into which sets of primary data for the years under study were transferred https://docs.google.com/spreadsheets/d/1TK40MzT432ECuh5WZ2ilwcfyxwMRBiC9sXDexv9dJzs.

RESULTS AND DISCUSSION

Culling is the removal of animals from the main herd for various reasons, such as sale, mortality, etc. In turn, culling can be voluntary and involuntary, voluntary occurs when an animal dies for various reasons, involuntary is carried out by man. At the same time culling is a big deal, it can also be called the final stage of breeding work for breeding farms. Culling from the herd can also be due to sale to other farms.

To reduce culling rates of dairy animals from the main herd, heifers must be physiologically developed and well prepared for the reproduction process at the time of insemination. The main indicators determining the period of insemination of animals are their age, live weight indicators, fatness of animals and general development of their organism. When heifers reach 65-70% of the live weight of an adult animal of the respective breed with the completion of the development of the whole organism, at which the animal acquires exterior and constitutional features, their economic maturity is formed.

The number of culling heifers of different ages for the whole period of the study is presented in Table 1.

Table 1. Number of heifers of Simmental breed culling in 2019-2021.

Age groups of heifers, months	Total, heads	Culling, heads	% of attrition
15-18	4006	603	14,6±2.2
18-24	3744	563	12,6±2.2

In total, the research involved data collected from 7 farms from different regions of Kazakhstan. In total 4006 heifers at the age of 15-18 months were counted from these 7 farms, according to the data received for 3 years 603 heifers, or on average 28.7±6.3 heads on farms, culling of this livestock. As a percentage, this figure was at 14.6±2.2% level. In the group of 18 to 24 months old bred heifers were also considered. In all 7 farms during the study period 3,744 heifers of 18-24 months old were born, 563 of them were lost or on average 26,8±7,1 heifer per farm. The proportion of heifers in this group was 2% lower than that of younger heifers and amounted to 12.6±2.2%.

Figure 1 shows the causes of heifers' culling at different ages.

As it can be seen from the chart, heifers in both age groups left the herd most often for reasons that are not common, but the number of heifers that left for these reasons increases by almost 10% with age, that is 49% versus 57%.

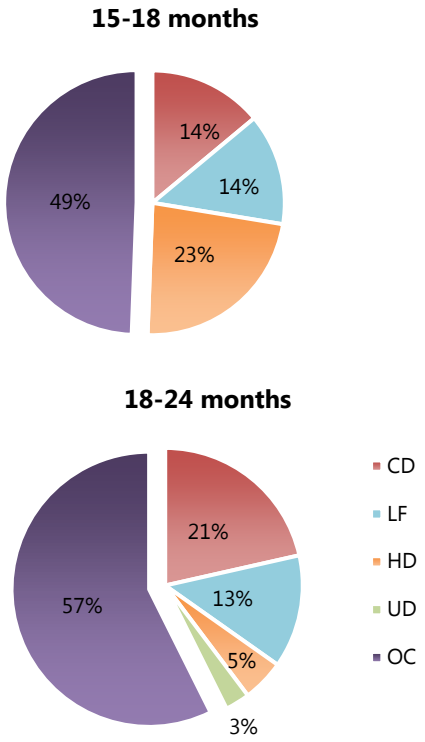

Figure 1. Causes of heifer culling at different ages

Farmers also cull more heifers with age because of exterior deficiencies, rising from 14% to 21%. Because of low reproductive performance and udder disease, heifers in both age groups drop out in equal numbers, the difference is ±1% for each reason. For example, if heifers in the 15-18 months age group are losing heifers due to low reproductive performance about 14%, then older heifers are losing 13%. For udder disease, the dropout of older heifers was 3%, whereas younger heifers are practically not dropped out for this reason, their value was less than 1%. The percentage of dropouts due to hoof disease was also ambiguous. The loss of young heifers due to hoof diseases amounted to 23% of all the heifers, while older heifers only 5%.

Our studies are similar to those conducted by different scientists in different countries and in different years.

Rilanto T. (2020), noted, that in Estonia about 26% of cows are culled from 100 cows, of which: hoof diseases 26.4%; udder diseases 22.6%, digestive diseases 18.1%; fertility problems 12.5%.

A review presented by Olechnowicz J. (2011), showed that the total culling of cows per year is 20-35% due to hoof disease, with a 40% decrease in productivity, treatment costs increase by 34%, and cow fertility decreases by 26%.

Nor N.M. (2014) in the studies carried out in the Netherlands between 2007 and 2010 on dairy herds pointed that the number of replacement heifers in a herd was directly related to the culling rate of cows in the herd. The results showed that the average culling rate was 25.4% and ranged from 23% (2007) to 28% (2010).

In an earlier study by a team of Bascom S.S. scientists (1998), it was found that low reproductive capacity was the main cause of cow culling - 35% and 11% of cows were culled due to low productivity.

Pinedo P.J. (2010) writes in his research that physiological death is the major share of culling - 20.6%, followed by low fertility - 17.7%, injuries - 14.3%, and low productivity and mastitis - 12.1%.

Thus, when looking at the overall picture, we can notice that the share of culling, as well as the reasons are different in different countries, i.e. there is no averaged data.

CONCLUSIONS

In conclusion, the results of the statistics given in the article are approximate to those given in the literature review. At 15-18 months, 14.6±2.2% have been culled, and at 18-24 months, 12.6±2.2% have been culled. The main cause of heifers' culling is various reasons, which include injuries, digestive diseases, etc., averaging 49-57%. The second most common reason for heifers at younger ages was hoof disease at 23%, and at older ages it was conformation defects at 21%. Other causes ranged from 3-14%.

ACKNOWLEDGEMENTS

The research results presented in this paper were conducted under the program funded by the Ministry of Agriculture of the Republic of Kazakhstan. Program Number: BR10764965

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MICROSCOPIC ASSESSMENT OF BULL SEMEN BY EJACULATE DENSITY AND SPERM ACTIVITY

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Abstract

Many researchers note that the reproductive ability of bulls directly correlates with the quality of sperm. At the moment, microscopic evaluation of bull semen by ejaculate density and sperm activity is relevant in Kazakhstan, which was the purpose of this study. Purebred bulls of the Kazakh white-headed breed in the amount of 127 heads of 7-10 months of age were selected for the experiment. The place of research is Akmola region, Republic of Kazakhstan. The research period is November 2021 – February 2022. In the course of the research, the results of the evaluation of the ejaculate of 127 bulls were obtained. It was found that in 20 bulls the sperm is thick (15.7%), and in 68 bulls the sperm has an average density, which is 70.6% and 94.1% more compared to thick and rare sperm, respectively. Only 4 bulls in density had rare sperm, which is 3.2% of the total number of all bulls. Allowed for use in artificial insemination, bull sperm is thick and medium, which in our study is 69.3% of the total livestock. Also, pathological forms of sperm were detected, which is 23.6% of the entire herd. Among them, 23 bulls were found to have aspermia (A), and 7 bulls had oligospermia (O). 5 bulls (3.9%) had a prepuce of the genital canal, which refers to penile abnormalities. Microscopic evaluation of sperm activity showed that the average score of sperm activity in the herd was 6.99±0.23 with a coefficient of variation of 32%, max 10 and min 2. Of the entire herd, 36 bulls have a score below 7 (28.3%), and 54 bulls are above 7 points, which is 42.5% of the total number of bulls. When the activity of bull sperm is below 7 points, sperm is not used for insemination.

Key words: *microscopic assessment, ejaculate density, sperm activity, bull.*

INTRODUCTION

Currently, all types of farm animals have violations of the reproductive function of males, which is due to both external factors (conditions of keeping, feeding, exploitation of animals, radioactive contamination of the terrain) and violations of the development of genitals in the pre- and postnatal periods of ontogenesis.

Simonik, O. (2015) point out that the fertility of bulls is often measured by the percentage of females exposed to the bull and fertilized over a period of time (usually 60-90 days).

Patel, B.R. (2013) write that sperm quality is determined by ejaculate volume, as well as sperm motility and morphology. It is important to remember that poor nutrition, extreme ambient temperatures and diseases can reduce the quality of sperm. The quality of sperm from a single bull can change over time.

Bhakat, M. (2011) write in their reviews that breeding bulls can remain fertile with high-quality sperm for more than ten years. However, breeding bulls can show infertility at any age. Low fertility can be caused by a number of factors, including damage to the testicles by frost, infection of the testicles, poor nutrition, physical injuries and genetic predisposition. Therefore, microscopic evaluation of bull semen is relevant and has great practical significance for Kazakhstan, since the fertility of bulls depends on the quality of sperm.

The aim of our research was a microscopic evaluation of bull semen by ejaculate density and sperm activity.

MATERIALS AND METHODS

Purebred bulls of the Kazakh white-headed breed in the amount of 127 heads of 7-10 months of age were selected for the experiment. By the end of the test, the bulls were about 11-14 months old. The animals were selected taking into account breed characteristics, gender, age, origin, and body weight. During the test period, the bulls were in the same feeding and keeping conditions. In our study, the ration of experimental bulls consisted of feed produced on the farm. The main food was: hay, haylage and concentrates. The feeding norms during the

period of scientific research corresponded to the breed, live weight and physiological condition of the bulls.

The place of research is Akmola region, Republic of Kazakhstan. The research period is November 2021 – February 2022.

After evaluating the newly obtained sperm by external signs, a microscopic assessment is made on the density, motility and concentration of sperm, determination of the proportion of living and dead sperm, ugly (pathological) forms.

Evaluation of sperm by density. According to the density, only freshly obtained sperm is evaluated. Distinguish between thick sperm – it is designated by the letter I, medium – C, rare – R. Density estimation is performed under a microscope by the method of a crushed drop at a magnification of 120-280 times.

Thick sperm – the entire field of view of the microscope is filled with sperm, there are barely noticeable insignificant gaps between them. The thick sperm contains over 1 B sperm in 1 ml. Average sperm – there are gaps between individual sperms, approximately equal to the length of the sperm. Such sperm contains from 400 M to 1 B sperms. Rare sperm – the gaps between sperms exceed the length of one sperm, in such sperm there are less than 400 M sperms in 1 ml. The absence of sperms-aspermia is indicated by the letter – A, an insignificant number of them is oligospermia – O.

Sperm motility is assessed according to a 10-point system. Each score is equal to 10% of the sperm with translational motion. If the sperm have a ring movement (in a circle) or only fluctuate, the quality of the sperm is evaluated by the letter K (oscillatory movement). Sperm with immobile sperms is designated by the letter H (necrospermia). Minimum indicators of sperm motility in sperm suitable for use.

Sperm collection from bulls was carried out at the age of 300 to 400 days. The tests were carried out in the morning before the feed was distributed, and at the time of the tests, the air humidity was 88%, with a pressure of 733 mmHg, with a wind speed of 3.8 m/s and a temperature of – 11ºC. To obtain descriptive statistics, the standard MS Excel add-in "Analysis Package" was used - the descriptive statistics analysis tool, to which sets of primary data for the studied years were transferred. This method is a classic one for determining the quality of the seed and assigning it a class according to Statement Standard 26030-2015.

RESULTS AND DISCUSSION

The decisive indicator of the quality of the producer bull is the results of the sperm study. When receiving sperm for the evaluation of the producer bull, they also use B. If the sperm is of poor quality, it is being evaluated again. We must not forget that after long breaks in sexual activity, the producer bull almost always releases low-quality sperm during the first coitus.

Benign sperm contains a sufficient number of living, stable in the external environment and able to participate in the fertilization of sperm; it is free from foreign impurities (blood, pus, microbes).

Insufficient development of bulls can delay puberty and potentially worsen the quality of sperm. Excessive development of bulls with a high energy diet can create problems such as excess fat in the scrotal (increased heat stress), decreased sperm production and quality, as well as the risk of digestive problems and lameness.

The results of the assessment of the density of the ejaculate are shown in Table 1.

Table 1. Assessment of the density of the ejaculate of bulls (n=127)

Ejaculate density	I	C	P	Pathological forms of sperm		
				A	O	Preputation of the genital canal
Number of heads	20	68	4	23	7	5

Table 1 shows the results of the evaluation of the ejaculate of 127 bulls. It was found that in 20 bulls the sperm is thick (15.7%), and in 68 bulls the sperm has an average density, which is 70.6% and 94.1% more compared to thick and rare sperm, respectively. Only 4 bulls in density had rare sperm, which is 3.2% of the total number of all bulls.

Allowed for use in artificial insemination, bull sperm is thick and medium, which in our study is 69.3% of the total livestock.

Also, pathological forms of sperm were detected, which is 23.6% of the entire herd. Among them, 23 bulls were found to have aspermia (A), and 7 bulls had oligospermia (O). 5 bulls (3.9%) had a prepuce of the genital canal, which refers to penile abnormalities.

The following table shows the results of bull sperm activity. According to the ejaculate assessment (Table 2), the average sperm activity score for the herd was 6.99±0.23 with a variation coefficient of 32%, max 10 and min 2. Of the entire herd, 36 bulls have a score below 7 (28.3%), and 54 bulls are above 7 points, which is 42.5% of the total number of bulls. When the activity of bull sperm is below 7 points, sperm is not used for insemination.

Table 2. Score assessment of sperm activity (n=127)

The indicator	Average	$C_{v, \%}$	Max	Min
Sperm motility, scores	6.99 ± 0.23	32	10	2

This farm was recommended to cull bulls with various pathologies, as well as bulls with an estimate of P in terms of ejaculate density and bulls with sperm activity below 7 points, since the reproductive capacity of these bulls is very low.

As noted by Ahmed H. B. et al. (2016), it should have a sperm motility of at least 30%, normal sperm morphology of 70% and a minimum scrotal circumference, depending on age. Bulls meeting the above minimum requirements are classified as satisfactory potential breeders.

Giaretta E. et al. (2017) and others write that the fertility of bulls may be the main limiting factor in the breeding program. It is estimated that in the United States and Canada, the infertility rate in bulls ranges from 15 to 25%.

Morphology of spermatozoa, as Shukla M.S. et al. say (2005), affects the frequency of pregnancy. In a recent study, bulls with less than 20% abnormal sperm had a pregnancy rate at least 4% higher than randomly selected bulls. Thus, the selection of bulls with more than 80% of normal sperm can increase the overall frequency of pregnancy in the herd.

Thus, a microscopic evaluation of the sperm of bulls will make it possible to analyze fertility and choose the best breeding bulls.

CONCLUSIONS

In conclusion, the results of our data show that the assessment of ejaculate density and semen activity are among the main indicators characterizing the suitability of bulls for insemination.

ACKNOWLEDGEMENTS

The research results presented in this paper were conducted under the program funded by the Ministry of Agriculture of the Republic of Kazakhstan. Project number: BR10865103

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CALF SHELTER MODELS AND THEIR RELATIONSHIP WITH WELFARE

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Abstract

The aim of this review article was to clarify some debatable aspects of calf housing and related welfare and behavioral consequences. There are many options for housing calves, with advantages and disadvantages. Breeders want to choose suitable housing systems to feed their calves with high comfort. The most appropriate system may vary depending on the equipment and facilities on the farm, the feeding system, labor cost and management layout. Control of disease transmission, easy sanitation, better growth, appearance of natural calf behavior and reasonable welfare are the main goals of a prosperous calf rearing system. Each of these factors can be affected by housing type and breeders can take these considerations into account when planning to build a shelter for their calves.

Key words: *calf, welfare, calf shelter*

INTRODUCTION

The placenta In recent years, studies on the effect of barns on calf welfare have increased in the world and initiatives to increase the living standard of calves have started to gain importance for producers. (Çam ve İnal, 2021). In order to reduce stress and increase the welfare level, existing shelter systems are examined and negative factors are eliminated and rearranged. Raising calves in commercial conditions makes an alteration in their behavior, which may be reflected in altered calf welfare (Nikkhah and Alimirzaei, 2022). After parturition, calves are separated from their dams and transported into individual or group stations and are kept therein until weaning. Calf hutches and other forms of individual calf housing have been widely used for the past 50 years or so to reduce transmission of disease among dairy calves and the occurrence of cross-suckling (suckling of other calves, which can spread disease and cause skin damage in severe cases) (Krawczel, 2016).

According to European regulations on the protection of calves (Directive 2008/119/CE), individual housing is only allowed for up to 8 weeks, specifying that animals must have physical and visual contact with other calves (Mainau and Blanco-Penedo, 2017). Therefore, individual homes with permanent walls or no direct physical contact between them will not meet minimum animal welfare standards. From a welfare point of view, the environment in which animals live should be adapted to their needs and allow them to express their typical behavior (Dereti et al., 2016). Dairy cattle need to learn how to interact with their environment in order to respond adequately to the environmental and management changes they will encounter throughout their lives (Mainau and Blanco-Penedo, 2017).

Numerous calf housing options are available, each with their advantages and disadvantages, but all calves should be raised in an environment that is (Anonymous, 2022): clean, dry, well drained, provided with sufficient bedding and enrichment, draught free and well ventilated, free of projections that may cause injury.

When selecting a calf housing system, you will need to consider your climate, budget and labour constraints and individual preferences. Calf housing does not have to be elaborate to be effective. The focus should be on providing calves with shelter from the weather and plenty of clean bedding. Remember, even the very best facilities will not succeed without proper management.

Within a farm, combinations of housing systems may be used for calves of different ages. For example a common hybrid system is to keep calves in smaller groups for the first 2-3 weeks followed by a larger group. Whatever system you use, calves housed in pens must have at least 1.5-2.0 m^2 of space for newborns, increasing to 2.5 m^2 or greater as calves grow (Anonymous, 2022).

In this review, it is aimed to explain some of the controversial aspects of keeping calves in different calf pens in relation to their growth and well-being from birth to weaning.

1. Individual Versus Group Housing

Group housing: Group housing is preffered as it gives calves opportunities to play, bond and learn from their peers. Group management is simpler than individual management but equally, it is harder to respond to individual needs. Calves should be grouped by size and age to reduce competition and facilitate observation and management. Small group sizes of 5-10 calves combine the advantages of group management with ease of record keeping and monitoring. Fewer pen divisions are needed, and access for cleaning is usually easier in larger pens.

Figure 1. Group housing

Table 1. A comparison of individual and group housing *(Anonymous, 2022)*

	Group housing		Individual housing	
Disease control	X	More disease risk due to increased contact between calves	√	Least risk of diarrhoea and respiratory disease
	X	Harder to monitor individuals	√	Close monitoring of each calf
			√	Easier record keeping
Cleaning and hygiene	√	Easier access for mechanized cleaning	√	Reduced exposure to faecal material
	X	Good hygiene needed to control disease	X	More labour intensive
Labour	√	Less labour intensive, easier management, suited to group feeding systems	X	Labour intensive feeding
Behaviour and growth	√	Better for social development, learning, play and exercise	√	Less cross sucking
	X	Competition for milk access between calves	X	Little opportunity for contact between calves
	√	Improved solid feed intake and weight gains before and after weaning	X	More fearful at 3 months, deficient social skills, poorer learning abilities and difficulties in coping with new situations
	X	Good ventilation is essential	X	Growth check at weaning

Individual housing: Individual housing may achieve the best disease control and allows the rearer to closely monitor each calf. However, social isolation can have detrimental effects on calves. As pens form a microclimate inside the larger housing system, it is important to consider ventilation and

Figure 2. Individual housing

draughts at the calf level within the pen. A floor area of at least 2.0 m2 should be provided for each calf in individual pens to permit self-grooming and prevent overcrowding. Calves housed in single pens should be able to see neighbouring calves, and kept in the company of other calves from three weeks of age.

Individual housing is a preferable system in many dairy farms worldwide (60% in Europe and 75% in the U.S.) because disease transmission risk is relatively lower in these systems, when compared to group housing (Ede et al., 2022). Nonetheless, natural behavior of calves may be restricted in individual housing systems (Ede et al., 2022).

Housing calves in-group pens, on the other hand, has attracted much attention in recent years. It has been reported that calves housed in-group pens are able to display their natural behaviors such as competition with other calves, playing, jumping, and running; indicating improved calf welfare (Chua et al., 2022). In addition, greater feed intake and weight gain have been observed in-group vs. individually housed calves (Ede et al., 2022).

Also, lower mortality rate was observed for individually housed calves in another study (Losinger et al., Heinrchs, 1997). Moreover, higher respiratory diseases rates in large sized group pens (8-12 calves per pen) have been reported (Svensson and Lberg, 2006). Authors have also illustrated those respiratory diseases rates could be reduced by minimizing pen size. The incidence of diarrhea was also high in large grouped calves (Losinger and Heinrchs, 1997).

However, in a recently reviewed article (Costa et al., 2016), some evidence was provided showing no detrimental effects of group housing on calf health. It seems that the effects of housing system on calf health may be multi-factorial.

Table 2. A comparison of fully enclosed and open housing *(Anonymous, 2022)*

	Full enclosed housing		Open housing	
Disease control	X	Increased disease if ventilation & climatic conditions not managed well	√	Less disease risk
Ventilation	X	Reliance on mechanical ventilation	√	Good ventilation
Shelter	√	Warmer for calves		
Cleaning and hygiene			√	Easier access for mechanized cleaning
Labour	√	Higher level of staff training and competence to operate	X	More exposure to unpleasant weather conditions
	X	Pleasant work environment		
Costs	√	Higher stocking rates	√	Cheaper construction
	X	Greater start-up costs	√	Lower energy use
	X	Higher cost per unit area		

2. Fully enclosed versus open housing

Fully enclosed: Fully enclosed, controlled climate (heated and ventilated) calf sheds are usually not justified.

Figure 3. Fully enclosed housing

Open housing: Open or partially enclosed housing that provides passive cooling is the most cost effective option in most regions. The closed sides should protect calves from prevailing winds and rain but windows can be used to improve ventilation in good weather. Remember to check natural ventilation at calf level.

Figure 4. Open housing

Table 3. A comparison of purpose-built and retro-fitted calf housing facilities *(Anonymous, 2022)*

	Purpose-built facility		Retro-fitted facility	
Cost	X	Higher start-up costs	√	Construction materials can be relatively cheap
			X	Feeders may be expensive
			X	May be compromises in existing building design or facilities available
Ventilation	√	Planning permission needed and design must be approved by a structural engineer	X	No planning permission needed although if poorly sited or managed, EPA may enforce changes
	X	Likely to be more efficient to operate		

Figure 5. Purpose-built housing

Figure 6. Retro-fitting/temporary housing

3. Purpose-built Versus Retro-fit / Temporary Housing

Purpose-built: A purpose-built shed could include: A storage area for feed, medications and equipment, A hospital area for sick calves, An area for handling calves e.g. a draughting race with crush pens or stalls, Weighing equipment, Computer facilities, Electronic scanning equipment, A loading ramp.

Retro-fitting/temporary: Temporary pens can be constructed out of steel reinforcing mesh, weldmesh or gates or hurdles. If outdoor, shelter can be provided using tarpaulin to cover one corner from prevailing winds or large hay bales. Temporary outdoor pens can easily be moved to a clean area of the paddock. Existing buildings can be converted to calf sheds, but they may need modifications. For example hay sheds can be effective calf shelters, using stacks of fodder to block the weather. The air space of the building needs to be considered when planning stocking rates, not just floor area, otherwise respiratory disease can result. In some buildings, ceiling height can severely limit air space.

Table 4. Advantages and disadvantages of hutches for calf housing *(Anonymous, 2022)*

	Advantages		Disadvantages	
Disease control	√	Good for disease control with limited contact between calves	X	Disease control can be a problem if there is poor drainage or the area is not rotated
	√	Easy observation of all calves		
Ventilation	√	Excellent ventilation		
Shelter	√	The inside is dry and protected from the weather and outside the calf is able to get limited exercise and sunlight	X	Extra shade may need to be provided in summer
Location	√	Hutches can be oriented towards the sun, or moved to locations that are most suitable according to the season		
Cleaning and hygiene	√	Synthetic materials are easy to properly disinfect; can be moved to clean ground	X	Cleaning can be very labour intensive and some corners may be hard to reach
Costs	√	Cheaper than purpose-built sheds	X	Can be quite costly, depending on source, when set-up costs are considered
Labour	√	Better work environment, with less air pollution, in good weather	X	Feed and water may need to be carted some distance, unless automated systems can be designed
			X	Carers work outdoors in all weather
			X	Twice weekly removal and replacement of bedding material may be required

4. Hutches

Hutches are used to house calves individually. Hutches made of polyethylene or fibreglass can be purchased commercially. Homemade hutches can be constructed from material such as marine plywood. Hutches are usually 1.2-1.5 m width and 2.0-2.4 m length. To provide shelter from the wind, hutches should be twice as long as they are wide. Hutches should be placed so calves can see each other. Placing hutches at least 1m apart will prevent physical contact between calves when using to control the spread of disease. Hutches should be slightly elevated to allow drainage and prevent flooding. A 15 cm layer of sand, gravel or crushed stone, or a pallet can be used to provide a base layer under appropriate bedding. Securing the feed and water buckets outside the hutch is labour efficient and helps keep the calf's pen dry. Hutches should not be placed in excessively hot, windy and wet locations, but a sunny location in winter will allow the run and part of the bedding to dry out. Light coloured, reflective hutch materials will reflect sunlight and prevent the hutch from heating up too much. During hot summer conditions hutches should be placed in a shady area, or extra shade may need to be provided.

Figure 7. Hutches

Table 5. Advantages and disadvantages of igloos for calf housing *(Anonymous, 2022)*

	Advantages		Disadvantages	
Disease control			X	Group housing can allow spread of disease if not well managed
Ventilation	√	Good ventilation		
Shelter	√	Excellent shelter with calves able to choose their preferred environment and exercise freely		
Location	√	Can be oriented towards the sun		
Cleaning and hygiene	√	Synthetic materials are easy to properly disinfect; can be moved to clean ground		
Costs	√	Cheaper than purpose-built sheds		
Labour	√	Suited to group feeding systems, such as calfeterias, for ease of management	X	Carers work outdoors in all weather

Table 6. Advantages and disadvantages of deep litter sheds for calf housing *(Anonymous, 2022)*

	Advantages		Disadvantages	
Disease control			X	Group housing can allow spread of disease if not well managed
Ventilation	√	Excellent ventilation for calves	X	Ventilation can be poor if all openings are closed to keep in the warmth
Shelter	√	Excellent shelter for calves, warm in cold climates		
Cleaning and hygiene	√	Allows easy access for cleaning equipment		
Costs	√	Fairly inexpensive		
Labour	√	Provides protection for carers from the weather; allows flexible management		

5. Igloos

Igloos are designed for groups of calves, and allow the calf to choose between a sheltered warm environment and an outside area for exercise and play.

Figure 8. Igloos

6. Deep Litter Sheds

Deep litter sheds (also known as Greenhouse barns) are available in all sorts of designs, sizes and materials. Curtains can be incorporated so sides and ends can be used

Figure 9. Deep litter housing

for controlling temperature and ventilation. They retain heat so are warm in winter, although care needs to be taken to maintain ventilation when side curtains are rolled down.

Best Time for a Group Split?

Bundling should be done before 6 weeks to benefit feed intake and weight gain (Costa et al., 2015). When the weights of calves housed in groups immediately after birth and at 3 weeks of age were compared, no difference in solid feed intake was found (Tapki, 2007). Calves mated at 3 weeks of age exhibit more social behavior than calves housed alone, and only little difference was found between calves mated from birth compared to calves mated at 3 weeks of age (Duve and Jensen, 2012).

Considering the results of these studies, it is recommended to start social grouping in the first three weeks of life.

CONCLUSIONS

It is notable that larger size grouping is still risky for pre-weaned calves, given the higher prevalence of diarrhea or respiratory issues, suggesting that smaller groups can help to reduce disease incidence. As such, pair housing could possibly mitigate the health problems of group housing while improving social behavior and feed intake of calves (Buckova et al., 2019). As morbidity and mortality rates are important indices of success in any calf rearing system, it is recommendable to keep calves in individual pens at least for the first two weeks of age. Then, they can be transported into smaller group pens, for instance, with a maximum of 6 calves in each pen. Furthermore, routine sanitation, hygiene of feeding facilities, and proper nutrition are important factors alongside the housing system to control diseases and improve dairy calf performance (Nikkhah and Alimirzaei, 2022). It is proposed that future studies test interactions of the above-mentioned factors with housing systems to help optimize calf performance and welfare.

It is not yet clear whether group housing results in higher body weight after weaning. Some studies had good results and others did not differ, but these were likely due to differences in experimental conditions (Mainau and Blanco-Penedo, 2017). However, a recent review (Costa et al., 2016) suggests that calves housed in groups with good management guidelines show better welfare conditions and better productive performance than animals housed individually. As well as the best time to house calves in groups between birth and the third week of life, more research is needed to determine whether they differ.

There is no single best way to raise calves, and there is endless variation in every system. Any calf housing system will need to be adapted to the specific conditions of the individual farm (budget, staff, facilities, preferences and climate). Remember that the purpose of all calf housing systems is to protect the welfare of the calves by providing a clean, safe and comfortable environment.

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TRAIT PREFERENCES OF INDIGENOUS KILIS GOATS UNDER EXTENSIVE MANAGEMENT CONDITIONS OF SUBTROPICAL REGION OF TURKEY

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Abstract

The milk quality and quantity are important factors for sustainability of the dairy sector for smallholder farmers, as it play fundamental role in their daily income and self-sufficiency. In extensive production systems of rural farming milk production of indigenous goats is an integral part of the small-farm economy. This study was performed to determine the lactation performance and the milk characteristics of the local Kilis does, that of raised at extensive conditions of 2 small-scale farmers of eastern Mediterranean part of Turkey. 80 heads of the Kilis goats were determined for lactation performances and milk yields., At the end of the study lactation milk yield and length were recorded as 201.12±4.26 lt and 174.65 ± 3.45 days respectively. Besides the dry matter (%), dry matter free oil (%), oil (%), protein (%), lactose (%), casein (%), urea (%), density (g), pH, free oil acids (%), citric acid (%) and freezing point (°C) in Kilis goats were recorded as, 12.11±0.32, 6.22±0.05, 4.05±0.03, 3.52±0.06, 4.00±0.05, 3.19±0.08, 0.08±0.01, 1045.68±2.19, 6.09±0.23, 4.69±0.25, 0.09±0.01 and 0.52±0.04, respectively. At the end of this study it has been concluded that, most farmers lack knowledge on the significance of Sub-tropically beneficial qualitative traits such as lactation performance and milk trait preferences of their goats. Educating farmers on the importance of conserving and inclusion of their flocks possessing such traits in their selection practices is therefore suggested.

Key words: *lactation performance, milk characteristics, extensive conditions, Kilis goats, subtropical climate, small-scale farms*

INTRODUCTION

Goats are also more resilience than other ruminants. Resilience is defined as 'the ability of a species to survive and recover from a perturbation'. Both adaptive capacity and resilience are influenced by species ecology, physiology and genetic diversity. Goats has low body mass, and low metabolic requirements, which is an important asset to them for it minimize their maintenance and water requirements, in areas where water sources are widely distributed and food sources are limited by their quantity and quality. An ability to reduce metabolism allows goats to survive even after prolonged periods of severe limited food availability. A skillful grazing behavior and efficient digestive system enable goats to attain maximal food intake and maximal food utilization in a given feeding situation. An effective urea recycling to the rumen allows goats to effectively digest low-protein feeds. The specious rumen volume of goats plays an important role in the evolved adaptations by serving as a huge fermentation vat and water reservoir. The goat population is 12,35 million heads in Turkey (TUIK,2021). Kilis goat, which is one of the main goat breeds in our country, was formed by crossing Hair goats and Syrian origin Shami goats and constitutes a small part of the total goat population. Kilis goat is widely raised in the Southeastern Anatolia Region of Turkey in the border neighbors of Syria (Adam, 1972; Kaymakçı ve ark., 2005; Ceyhan ve Karadağ, 2009). Especially in the context of goat-forest relations, the gradual decrease of forest areas and the appropriation of this situation to goat breeders necessitate the improvement of the existing goat potential. Efforts in this direction are not new. Studies on the genetic breeding of existing goat breeds in our country first started in 1960, and directing it especially in the context of the development of dairy goats appears as the main goal and studies in this direction are tried to be continued (Kaymakçı ve ark., 2005; Tölü ve ark., 2009). Studies on the genetic improvement of our current goat breeds are a crucial issue to define our domestic gene resources in terms of yield characteristics, to reveal the selection potential in terms of yield characteristics of interest, and to evaluate / carry out together with the awareness (Şengonca ve ark., 2003; Kaymakçı ve ark., 2005; Anonim, 2008).

It is of great importance to establish databases at the point of defining the yield characteristics of our native breeds in extensive conditions of rural areas. Thus the study aims to determine lactation

performances and milk quality characteristics of local Kilis goats, raised in extensive sub-tropical climate conditions.

MATERIALS AND METHODS

The study was conducted in small-scale farms of sub-tropical conditions of Eastern Mediterranean region of Turkey. Kilis goats raised in two different farms in rural areas of Eastern Mediterranean region of Turkey. Lactation performances and dairy traits of 80 heads of Kilis goats were collected during 2nd lactation duration in 2018 production season. The animals were kept in pen shed and grazed almost 9-10 months in natural lands. Only they were fed by low quality grains such as barley and barley hay during cold conditions of winter season. Obtained milk yield values are based on one milking per day basis. Lactation milk yield and duration were calculated according to the ICAR A4 method. Milk samples were collected twice a day in every 2 weeks and Milkoscan FT-120 (Foss, Denmark) device was used to determine milk quality characteristics.

Data was statistically analysed by the SAS (21) package program.

RESULTS AND DISCUSSION

The lactation performances of the Kilis goats were given in Table 1. Accordingly, lactation milk yield and duration in Kilis goats were determined as 201.12±4.26 lt. and 174.65 ± 3.45 days as seen in Table.

Table 1. Lactation performances of Kilis Goats

	n	$\bar{x} \pm S_{\bar{x}}$
Lactation milk yield (lt)	80	201.12±4.26
Lactation lenght (day)	80	174.65±3.45

Dairy Trait preferences were given in Table 2. Accordingly, in Kilis goats, dry matter (%), dry matter free oil (%), oil (%), protein (%), lactose (%), casein (%), urea (%), density (g), pH, free oil acids (%), citric acid (%) and freezing point (°C) in Kilis goats were recorded as, 12.11±0.32, 6.22±0.05, 4.05±0.03, 3.52±0.06, 4.00±0.05, 3.19±0.08,0.08±0.01,1045.68±2.19,6.09±0.23,4.69 ±0.25,0.09±0.01 and 0.52±0.04, respectively.

When dairy characteristics determined for lactation milk yield and length were compared with the values reported for Hair, Kilis and their crosses, Keskin (1995); Hair goats (80–100 kg; 150–200); Hair (90.1 kg; 206 days) and Kilis (206.4 kg; 277.4 days) goats reared in the Dairy Goat Research Farm Unit of Faculty of Agriculture of Çukurova University (Özcan,1989). Several researchers reported dairy performances , lactation milk yield and length of Kilis goats as follows; 204.52 kg; 231.1 days (Özcan et al.,1975); 200–300 kg; 6–8 months (Şengonca,1974), respectively. There is no study on milk quality of Kilis goats in same conditions. But there is some scientific data for other breeds raised in same conditions.

Table 2. Milk characteristics of Kilis goats

	n	$\bar{x} \pm S_{\bar{x}}$
Dry Matter (%)	80	12.11±0.32
Solid Non fat (%)	80	6.22±0.05
Fat (%)	80	4.05±0.03
Total Protein (%)	80	3.52±0.06
Lactose (%)	80	4.00±0.05
Casein (%)	80	3.19±0.08
Ürea (%)	80	0.09±0.01
Specific gravity (g/mL)	80	1045.68±2.19
pH	80	6.09±0.23
Free fatty acids (mmol/10 l milk)	80	4.69±0.25
Citric acid (%)	80	0.09±0.01
Freeezing point (°C)	80	0.52±0.04

Keskin et al. (2004) reported the dry matter, protein, fat, lactose and ash ratios of milk quality characteristics in Damascus (Damascus) and German Goats were determined as 12.2 ± 0.16 and 12.4 ± 0.28 (%), 3.5± 0, respectively. 07 and 3.4 ± 0.11 (%), 4.3 ± 0.12 and 4.1 ± 0.23 (%), 3.6 ± 0.08 and 4.2 ± 0.11 (%) , 0.77 ± 0.02 and 0.72 ± 0.03 (%). the values obtained in terms of dry matter, protein, fat and lactose in milk are similar to the values determined in this study, 12.18±0.13% dry matter, 3.35±0.05% protein, 4.02±0.09% fat and 4.07±0.03% lactose. Similarly, the values of 13.38±0.09 %, 3.98±0.07 %, 0.855±0.004, 1.030±0.001 g and 6.594±0.009 reported by Ata (2007) for dry matter, fat, ash, specific gravity and PH values in milk in Hair goats. These flocks were kept in extensive conditions of sub-tropical region. They were small scale and almost zero input. But these systems have socio-economic importance for small scale production systems.

At the end of this study it has been concluded that, most farmers lack knowledge on the significance of Sub-tropically beneficial qualitative traits such as lactation performance and milk trait preferences of their goats. Educating farmers on the importance of conserving and inclusion of their flocks possessing such traits in their selection practices is therefore suggested.

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APPLICATION OF MARKER GENES IN DOGS

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Abstract

According to the Fédération Cynologique Internationale (FCI), there are currently 336 different dog breeds that have been registered. This amount is said to be significantly higher by a few different sources. Dogs have also changed according to needs, like technology. Formerly, humans used dogs as guards, hunters, and shepherd dogs. For this reason, security dogs, shepherd dogs, and hunting dogs were among the earliest breeds to arise and be utilized. These days, dogs are put to work in a wide variety of contexts, including mine detection, bomb detection, search and rescue operations, and even disease diagnostics. The vast majority of canines employed in these sectors are chosen through behavioral trials based on phenotypic traits. These selection tests differ depending on the trainers´ experience or priorities at the time. Consequently, there is always a chance of making a mistake when doing behavioral assessments. However, in marker-assisted selection studies using genetic markers known as marker genes, it is not necessary to wait for the generation period to develop the desired yield characteristics; progress is made in a shorter time interval, and the difficulties experienced in comprehending the yield level depending on gender are eliminated. The focus of genetic research on dogs worldwide appears to be on degenerative conditions such elbow dysplasia and hip dysplasia as well as behavioral issues including unrestrained aggression. Some firms even claim that taking oral swaps reveals the genetic composition of dogs, including their genetic history, degree of exercise, and even which ailments they are predisposed to. In the scope of this presentation, details concerning the utilization of marker genes in canines will be discussed.

Key words: *Dog, dysplasia, Marker gene, Marker-assisted selection (MAS)*

PREVALENCE OF EXTENDED-SPECTRUM Β-LACTAMASES PRODUCING ENTEROBACTERIACEAE FROM LIVESTOCK

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Abstract

The overuse of antibiotics in production of livestock and poultry beyond therapeutic needs is a potential factor of a very high prevalence of multi-resistant bacteria, in particular to third-generation cephalosporins (3GC). The aim of this study was to estimate the prevalence of 3GC resistant enterobacteriaceae in cattle, sheep and poultry in Batna region and to highlight ESBL-producing strains. A total of 40 rectal samples and 10 environment samples were taken from 4 farms in Batna. The prevalence of 3GC-resistant enterobacteriaceae was 52.63% in cattle, 33.33% in sheep, 66.66% in poultry and 10% in surface samples. The predominant species were Escherichia coli and Enterobacter cloacae (56.52%), followed by Klebsiella oxytoca Citrobacter freundii then Serratia odorifera. Antibiotic sensitivity testing revealed varying resistance rates, the highest being 81.81% towards ampicillin and amoxicillin-clavulanic acid followed by 63.63% for cefotaxime and tetracycline for cattle isolates, 72.42% of strains resistant to amoxicillin-clavulanic acid, 42.83% to cefotaxime and tetracycline for sheep isolates. The avian isolates were 100% resistant to ampicillin, amoxicillin, clavulanic acid, ceftazidime and tetracycline, 75% to cefotaxime and trimethoprim-sulfamethoxazole, and 50% to chloramphenicol. For livestock surfaces, a single isolated strain with multi-resistance pattern. Extended- spectrum β-lactamase (ESBL) isolates was observed in 45.45%, 42.85%, 75% of cattle, sheep and poultry, respectively. In conclusion, the relatively high resistance among these isolates are worrisome and indicate that these animals constitute a reservoir for desimination of ESBL producing strains that can be easily transmitted to humans and threaten public health and the environment.

Key words: *Antibiotic resistance, Enterobacteriaceae, Farm animals, Resistance to third generation cephalosporins, ESBL*

IS CAPONIZATION ONE OF THE SOLUTIONS FOR THE UTILIZATION OF NATIVE CHICKENS?

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Abstract

In the last half century, with the gaining ground of hybrid chickens, the old, indigenous breeds have been pushed into the background and became endangered. The old breeds are not compatible with the modern varieties, they cannot be competitors in the intensive, economical production. Our task is to preserve them and their valuable properties and use them later in breeding works. Beside the gene preservation, we aspire to find the best way for the production-purpose utilisation of our speckled chicken population. In our experiment we wanted to revive an old traditional method, the caponizing, to produce special products for gastronomy. As a result, we can draw the conclusion that the Hungarian indigenous speckled chicken breeds are suitable for caponizing and they can produce special, valuable and marketable rarity products.

Key words: *Caponizing, Indigenous Hungarian chicken, Curiosity products, Gene preservation*

USE OF MEDICINAL AND AROMATIC PLANTS WITH DRINKING WATER IN BROILERS

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Abstract

The prohibition of the use of growth antibiotics in poultry nutrition has gained importance as feed additives, especially since medicinal and aromatic plants and their extracts are natural, environmentally friendly and generally safe products. Medicinal and aromatic plants have been used as preservatives in both folk medicine and food since ancient times. These plants have antimicrobial, antioxidant, antiparasitic, antiprotozoal, antifungal and anti-inflammatory properties and contain many biologically active compounds, especially polyphenolics. Therefore, medicinal and aromatic plants and their extracts have the potential to be new generation substances for animal nutrition and health. In most in vivo studies investigating the usability of medicinal and aromatic plants and their extracts in poultry nutrition, the effects of applying medicinal and aromatic plants and their extracts in feed and throughout the entire rearing period have been investigated, while the effect of incorporating them into drinking water has been less frequently investigated. Providing medicinal and aromatic plants and their extracts with drinking water can also be a valuable way to achieve the expected yield increases from animals. In this review, it has been tried to summarize the researches made in the last ten years when the medicinal and aromatic plants and their extracts were given drinking water in broilers.

Key words: *Broilers, Medicinal and aromatic plants, Drinking water*

HAEMATOLOGICAL AND SEROLOGICAL INDICES OF GROWING PIGS FROM CROSSES OF LARGE WHITE AND DUROC BREEDS FED DIFFERENT UNCONVENTIONAL FEEDS

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Abstract

This experiment was conducted to investigate the blood parameters of growing pigs fed diet containing different unconventional feed stuffs. A total of 45growing pigs were randomly selected from 72pigets obtained in crossbreeding Duroc and Large white parents. The 45 growing pigs were randomly allotted to 5 dietary treatments. Treatment 1 (Control) contained whole maize, Treatment 2-Brewers' dried grain, Treatment 3-Cassava Peel Meal, Treatment 4-Plantain peel meal, and Treatment 5-corn husk meal at 35% inclusion level. Parameters evaluated include: red blood cell counts (RBC), parked cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin count (MCHC) and total leucocytes (WBC). From the serum, total protein, glucose, urea, and cholesterol were evaluated. Data were subjected to analysis of variance in a completely randomized design. Values ranged from 8.87±0.12 (T3) - 12.53±0.22 (T5); 19.20±0.70 (T4) -29.20 (T1); 4.67 (T4) ±0.07-6.50±0.21 (T3); 55.27±0.15 (T4) - 87.37±0.43 (T1); 26.67±0.33 (T3) - 37.33±0.67 (T5); 3.60±0.20 (T1) - 5.20±0.23 (T4) and 33.26±0.37 (T3) - 33.83±0.32 (T4) were obtained for Hb, MCH, WBC, MCV, PCV, RBC and MCHC respectively. Hb, MCH, WBC, PCV, and RBC differed significantly (P<0.05) across treatments while no significant difference (P>0.05) was recorded for MCHC. While values of 5.74 -7.16, 1.97-2.51, 0.90-1.05, 12.13-13.97 were reported for total protein, cholesterol, glucose and urea respectively. The serological parameters studied also differed significantly (P<0.05) across treatments for total protein, cholesterol, glucose and urea. The study shows that plantain peel meal and cassava peel meal negatively affected blood parameters and could pose health challenges to growing pigs when included at higher levels in pig diets.

Key words: *pig, grow, blood parameter*

INTRODUCTION

The physiological statuses of farm animals vary in haematological indices which can be used to assert the pathology of animals when faced with different environmental factors including nutrition. Good haematological and serological indices are indications that the animals are expected to perform excellently well in terms of growth and general performance that is likely to benefit livestock producers (Khan and Zafar 2005, Isaac et al 2013 Olafedehan et al 2010). Most times, stresses posed by the environment, pathology of animals, extent of toxicants in feeds, and the status of the animal's health are basically determined using the changes in haematological parameters (Afolabi et al., 2010; Oyawole and Ogunkunle., 2004). With the rising awareness on the use of unconventional feeds in pig feeding occasioned by the continuous rise in cost of maize and other grains, scientist have commenced experimenting other alternative feed sources (Moemeka et al, 2022). It is also worthy to note that farmers use these unconventional feed ingredients without cognizance to the normal physiological and health functions of the pigs (Etim et al., 2014). It has therefore become paramount that all the health benefits and/or implications of feeding these agro-industrial by-products be properly understood to proffer solutions that may arise from feed stuffs that may be toxic to pigs by negatively impacting their haematological and serological indices. In this sense, when red blood cell count drops, it is an evidence that the haemoglobin and oxygen level of the blood is low and the more difficult it becomes to convey blood to tissues, in the same way the lungs receives less carbon-dioxide (Ugwuene, 2011;

Soetan et al, 2013; Isaac et al, 2013). It is also evident that pigs prone to severe susceptibility to diseases are those reported to have a low level of white blood cells and vice versa, because high white blood cell enables animals to generate more antibodies to fight antigens (Soetan et al., 2013; Isaac et al., 2013). To our knowledge and from available literatures, blood heath indices of weaned pigs have not been properly compared and reported during different unconventional feeding regime. Hence, this study was designed to examine the blood indices of pigs fed diet containing different unconventional feed ingredients that are used especially by local farmers in Pig feeding.

MATERIALS AND METHOD

Experimental site

The experiment was conducted in the teaching and research farm of the Department of Animal Science, Delta State University, Asaba Campus, Asaba. Asaba is located at latitude 6° 14"N and longitude 6° 49"E and is situated in the humid zone of Southern Nigeria.

Experimental Animals and their Management

A total of forty-five (45) growing pigs at ages 3-5 months with body weight range of 9- 14 kg obtained from crosses between Large white and Duroc breeds were used for this study. The animals were raised on a concrete floor. Feed and water were provided ad-libitum throughout the duration of the experiment.

Experimental diets and design

Fresh cassava peels was sourced from cassava farmers while plantain peels were obtained from plantain chip producers. The peels of both plantain and cassava were sundried and milled, while corn husk was obtained from the maize farm of Songhai Delta Amukpe, Delta State Nigeria. The husk was sundried and milled. Whole maize and brewers dried grain was sourced from reputable feed industries. The 45 growing pigs were randomly allotted to five dietary treatments. The treatments were replicated three times with three (3) animals per replicate making nine (9) animals per treatment. Treatment one (T1) contain 35%maize, treatment two (T2) shall contain 35% brewers dried grain (BDG), treatment three (T3)35% cassava peel meal (CPM), treatment four (T4) 35% plantain peel meal (PPM),while treatment five (T5) contained 35% corn husk meal (CHM).

Experimental diet

Table 1. Chemical Composition of the Experimental Diet

Ingredients	T1	T2	T3	T4	T5
Whole maize	35	-	-	-	-
BDG	-	35	-	-	-
CPM	-	-	35	-	-
PPM	-	-	-	35	-
CHM	-	-	-	-	35
PKC	25	25	25	25	25
Wheat offal	10	10	10	10	10
Fish meal (72%)	2	2	2	2	2
Bone meal	4	4	4	4	4
Salt	0.5	0.5	0.5	0.5	0.5
Premix	0.5	0.5	0.5	0.5	0.5
Soya bean meal	23	23	23	23	23
CALCULATED COMPOSITION					
Crude protein (%)	17.52	17.02	16.69	17.21	
Crude fibre(%)	27.00	28.00	27.45	27.10	
ASH (%)	4.00	3.5	4.64	3.85	
TDN	82.31	83.22	85,43	84.57	

CPM- Cassava Peal Meal, PPM-Plantain Peel Meal-BDG-Brewers Dried Grain, CHM- Corn Husk Meal, PKC-Palm Kernel Cake

Blood Sample Collection and Analysis

One pig from each replicate was selected at random for bleeding within the last week of the feeding trial. Water was given on the evening preceding the bleeding. The bleeding was done in the morning before feeding. 10ml of blood was obtained from the jugular vein using a sterilized needle and syringe into a sample bottle. EDTA bottle containing anticoagulant and another set of plain bottle without anticoagulant were used for this exercise. About 4ml of the blood sample was put into the plain sample bottle. The samples in the plain bottle was allowed to cloth so as to obtain the serum that will be used in the determination of some serum metabolites as described by Kaneko (1989).The blood samples were analysed at the Animal science laboratory of the Delta State University, Asaba Campus. Parameters evaluated include: red blood cell counts (RBC), parked cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin (MCHC) and total leucocytes (WBC). From the blood serum, the following parameters were evaluated: total protein, glucose, urea and cholesterol. The Red Blood Cell (RBC) count was determined with a coulter Electronic counter (Model ZF by coulter Electronics Ltd. London) with values displayed as

millions of red blood cells per cubic millilitre of blood ($x10^6/mm^3$). The mean corpuscular haemoglobin (MCH) was computed as: MCH = Hb x 10 divided by the red blood cell and expressed in pg. The mean corpuscular haemoglobin concentration (MCHC) was computed as follows: MCHC = Hb x 100 divided by the pack cell volume and expressed in percentages. The white blood cell (WBC) was determined using standard diluting pipettes with an improved Neubauer haemocytometer. The WBC count was expressed in thousands per cubic millilitre ($x10^3/mm^3$). The serological parameters were determined as follows; Total serum protein by the Buiret technology, Albumin and Globulin by the colorimetric technique, Urea content by the Berthelot (colorimetric) method, while Glucose and Cholesterol was analyzed through the enzymatic colorimetric method.

Statistical Analysis:

All data collected were subjected to analysis of variance (ANOVA) in a complete randomized design using the using the R-Statistical package.

RESULTS

Table 2. Haematological Indices and standard errors (SE) of Growing Pigs Fed Different unconventional feed Sources

Parameters	TREATMENTS T1	T2	T3	T4	T5
HB	10.47± 0.42^{a}	10.20 0.12^{a}	8.87 ±0.12^{b}	9.70± 0.26^{a}	12.53± 0.22^{c}
MCH	29.20± 0.16^{a}	23.60 0.81^{b}	23.97± 0.16^{b}	19.20± 0.70^{c}	26.10± 0.45^{ab}
WBC	6.03 ± 0.38^{ab}	5.70 ± 0.06^{bc}	6.50 ±0.21^{a}	4.67 ±0.07^{d}	5.33± 0.07^{c}
MCV	87.37± 0.43^{a}	70.26± 2.83^{b}	66.53± 0.65^{bc}	55.27± 0.15^{c}	77.67± 0.13^{ab}
PCV	31.33± 1.33^{b}	30.33± 0.33^{bc}	26.67± 0.33^{de}	28.67± 1.16^{cd}	37.33± 0.67^{a}
RBC	3.60 ± 0.20^{d}	4.33 ± 0.18^{bc}	3.73± 0.24^{cd}	5.20± 0.23^{a}	4.80± 0.00^{ab}
MCHC	33.41± 0.18	33.73± 0.21	33.26± 0.37	33.83± 0.32	33.57± 0.08

HB- hemoglobin content, MCH-mean corpuscular hemoglobin, WBC-white blood count, MCV-mean corpuscular volume PCV- pack cell volume, RBC- red blood count, MCHC- mean corpuscular hemoglobin content.

a, b, c.d.e Means within row with different superscript are significantly different (P<0.05).

Table 2; shows the haematological indices of growing pigs fed different unconventional feed sources. The mean haemoglobin content values ranged from 8.87 for T3 to 12.53 for T5. Result of analysis of variance showed there were highly significant differences (P<0.01) among the treatments. Duncan multiple range test showed that T3 is significantly different from T2, T4, T3 while T5 was also significantly different from T1, T2, T3 &T4 respectively. However, T2, T4 and T1 did not differ significantly from one another. The values for Mean Corpuscular Haemoglobin ranged from 19.20 for T2 to 29.20 for T1 with highly significant differences (P<0.01) between treatments. Treatment 2 was significantly different from other treatments while T4, T3 &T5 did not differ significantly but T1 however differed from T4 & T3 respectively. The White Blood Count values ranged from 4.67 for T2 to 6.50 for T3 significant differences among treatments (P<0.05) with observed significant differences between T3 & T1, T4 & T2, and T2 & T5 while other treatment groups were significantly different from each other.

Mean Corpuscular Volume ranged from 55.27 for T2 to 87.37 for T1 with highly significant differences (P<0.01) among the treatment groups but there was no significant differences between T2 & T3, T3, T4 & T5 as well as T5 & T1 while other means were significantly different from each other. Pack Cell Volume showed highly significant (P<0.01) across treatments with significant differences recorded between T5 and the other groups, while T1 & T4, T4 & T2, as well as T2 & T3 did not differ significantly. Red Blood Count showed highly significant difference (p<0.01) among the red blood count in the treatment groups though no significant difference between T1 & T3, T3 & T4, T4 & T5 as well as T5 & T2 while T1 & T3 each differed from T5 & T2, just as T4 differed from T2 & T1. Mean Corpuscular Haemoglobin Content revealed no significant differences (P>0.05) among treatment groups.

Table 3. Serological Indices of Growing Pigs Fed Different Dietary Energy Sources

Parameters	TREATMENTS T1	T2	T3	T4	T5
Total Protein	6.48±0.03[b]	6.26±0.12[c]	5.74±0.03[d]	6.13±0.05[c]	7.16±0.03[a]
Cholesterol	2.28±0.06[b]	2.07±0.15[c]	2.10±0.03[cd]	1.97±0.01[d]	2.51±0.02[a]
Glucose	0.96±0.02[a]	0.96±0.04[a]	1.05±0.01[b]	0.90±0.01[c]	0.95±0.02[a]
Urea	12.53±0.15[d]	13.43±0.12[bc]	12.13±0.07[d]	13.83±0.12[ab]	13.97±0.18[a]

a, b, c. Means within row with different superscript are significantly different (P<0.05).

Table 3 above shows the serum biochemical properties of growing pigs fed different unconventional. The total protein values ranged from 6.13 for T4 to 7.16 for T5 with highly significant differences (P<0.01) among the treatments. There were obvious differences between T3, T1 & T5 and other treatments, while T2 & T4 did not vary significantly from each other. The Blood Cholesterol values revealed a highly significant difference (P<0.01) in the blood cholesterol level with values ranging from 1.97 for T4 to 2.51 for T5. The result showed that T5 and T1 significantly differed from other treatments, while T3 & T4, T2 & T3 did not differ from each other significantly, but T4 however differed from T2. The blood glucose levels also revealed that there were highly significant difference (P<0.01) among treatment groups with values ranging from 0.90 for T4 to 1.05 for T3. However, Duncan multiple range tests revealed differences in the mean of T3 and T4 with other treatments. The Urea values ranged from 12.13 for T3 to 13.97 for T5, with significant differences (P<0.05) between treatments. Furthermore, T2 was significantly different (P<0.05) from other treatments, just as T3 is with T4 & T5.

DISCUSSION OF RESULTS

Haematological indices of growing pig fed different unconventional feed sources

The haematological indices of pigs in the present study was significantly affected by treatment diet as significant differences (P<0.05) were recorded for treatment groups. The Hb range of 8.87-12.53 reported in this study is within range of 10.64, 10.47, 12.53, 12.7,9.25-12,11.30, 10.9, 12.30, and 12.6 reported by Adenkola et al 2009, Irekhore et al 2015, Alagbe et al 2017, Akovbovbo et al 2013, Okah & Ehuriah 2013, Ukpabi et al 2015,Jezek et al 2018,Serem et al 2017, and Fabio et al 2018, but was lesser than 13.56, 13.70, and 15.25-16.30, reported by Nsoh Abora, 2013, Drews et al 2016, and Olajide et al 2021 in growing pigs respectively. The haemoglobin count of treatment five was similar to 12.20 reported by Upkabi et al (2015) for pigs fed tiger nut based diet. The mean corpuscular haemoglobin also differed significantly (P<0.05) among treatment groups with range of 19.20-29.20 for T4 and T1 respectively. This MCH range is higher than 18.26, 17.50, 16.67-17.19,18.67, 17-18, 17.21 in pigs fed 40% cassava peel meal, tigernut meal, and turmeric power supplemented diet (Nsoh Abora, 2013; Irekhore et al 2015; Ukpabi et al, 2015; Alagbe et al 2017; Jezek et al 2018; Serem et al 2017; Abeni et al 2018), but were within range of 19.98 and 29.30 reported by Fabio et al 2018 and Olajide et al 2021 in growing pigs fed different protein levels and benniseed hull as replacement for maize. The MCH values for benniseed hull is close to the value reported for corn husk meal reported for treatment five in this study. This could be attributable to the similar fibre composition of hull and husk from cereals and legumes. The white blood count from treatment animals were significantly different (P<0.05) with values ranging from 4.67 for T4 to 6.50 for T3. These values are far lower than the range of values reported by Ukpabi et al (2015), Irekhore et al 2015, Alagbe et al 2017, Akovbovbo et al 2013, Nsoh Abora, 2013, Okah & Ehuriah 2013, Serem et al 2017 (6.30-16.00, 10.62, 15,09, 16.62, 17.66, 10.20, 16.62). The WBC values are low compared to values found in most of the literatures, however, they are close to the baseline values published for pigs as reported by Etim et al, (2013). The high differences observed could be attributable to the different feed ingredients used in the diets of the pigs. The mean corpuscular volume was significantly different (P<0.05) among the treatment groups with values ranging from 55.27 for T2 to 87.37 for T1. The values for mean corpuscular volume are all higher than the range of 48.84-51.01 obtained by Ukpabi et al (2015), but are comparable to values of 55.09, 54.4, 54.9, 52.30, 56.58, and 88.15 reported by Alagbe et al 2017, Nsoh Abora 2013, Jezek et al 2018, Serem et al 2017, Abeni et al 2018, and Olajide et al 2021. The pack cell volume also differed significantly

(P<0.05) across treatments groups with values ranging from 26.67-37.33(T3-T5) which is within the range of 21.60-36.70, 32, 34.25, 28-37 reported by Ukpabi et al (2015), Adenkola et al 2009, Irekhore et al 2015, okah & Ehuriah 2013, though lesser than reports of Alagbe et al 2017, Akovbovbo et al 2014, and Olajide et al 2021 (42, 38.33 and 45.50), in growing pigs fed diet containing turmeric, water hyacinth and benniseed hull respectively. The red blood count values ranging from 3.60 for T1 to 5.20 for T4 are within range of 3.77, 3.80, and 5.00 reported by Ukpabi et al 2015, Irekhore et al 2015, Okah and Ehuriah 2013 and Olajide et al 2021, but were lower than reports of Alagbe et al 2017, Akovbovbo et al 2014, Nsoh Abora 2013, Jezek et al 2018, Serem et al 2017, Fabio et al, 2018 and Drews et al, 2016. The mean corpuscular haemoglobin content did not differ significantly from each other (P>0.05), which is in line with the report of Ukpabi et al 201, that mean corpuscular haemoglobin content of growing pigs does not differ from each other when fed tiger nut diets at graded level. The range of 33.26 for T3 to 33.83 for T2, is slightly higher than the range of 32.99- 33.16 for MCHC recorded by Ukpabi et al 2015, but were within range of 33.15-33.45 reported by Olajide et al, (2021). Treatment three and treatment four gave values lower than the baseline values for haemoglobin and pack cell volume, while treatment four alone reported lower baseline values for white blood cell count. The reduced PCV and haemoglobin in treatment three and four may be due to available toxins in the feeds, in addition to the presence of some anti-nutritional factors such as trypsin present in plantain peel and cassava peel diets; this suggest that detoxification of these feed will be necessary towards improving its utilization in pig feeding (Oyawale and Ogunkunle, 1998). The lower RBC counts are indications that the haemoglobin and oxygen level of the blood dropped in the affected treatments, and that could conversely affect blood transport to tissues. PCV, MCH, Hb and MCHC are parameters aiding blood levels, transport of blood, transport of oxygen, and therefore act as major indices for the diagnosis of anaemia (Awodi et al, 2005; Chineke et al., 2006). Hence the treatment birds with low values for these parameters may be prone to anaemia and vice versa. It is also evident that pigs prone to severe susceptibility to diseases are those reported to have a low level of white blood cells and vice versa, because high white blood cell enables animals to generate more antibodies to fight antigens (Soetan et al., 2013; Isaac et al., 2013; Aderinola et al., 2012). However, the figures obtained in this study are within range of the acceptable baseline values for normal physiological functioning of the birds (Etim et al. 2014). Hence, the unconventional feed ingredients used in this study could be used conveniently in pig feeding without posing any detrimental health challenges to the animals, but for optimal physiological functioning, ingredients such as cassava peels and plantain peels should be detoxified before inclusion in swine diet.

Serological Indices of Growing Pigs Fed Different unconventional feed Sources

The serological indices of the experimental animals differed significantly (P<0.05) across dietary treatments with values ranging from 5.74 -7.16, 1.97-2.51, 0.90-1.05, and 12.53-13.97 for total protein, cholesterol, glucose and urea respectively. Treatment five recorded the highest values for all the parameters except for glucose value. The values ranging from 5.74-7.16 obtained for total protein are close to 8.03-11.07 values reported by Olufemi, (2014), but far lesser than 67.62, 91.00, and 67.92 reported by Adenkola et al 2009, Nsoh Abora 2013, and Abeni et al, 2018 in growing pigs fed diet containing Ascorbic acid, soya bean milk residues, and different protein levels respectively. Interestingly, the values of total protein in this study are in tandem with range of 5.0-8.7 and 6.66-7.08 (Okah & Ehuriah 2013; Olajide et al, 2021). The cholesterol values reported in this study are far lower than the range of 104-124 reported for growing pigs fed benniseed hull based diet (Olajide et al 2021), but were close to the value of 4.14 reported for growing pigs subjected to ascorbic acid treatment (Adenkola et al 2009), however, the report for cholesterol quite agrees with reports of 2.16-2.24 and 2.47-2.51 reported for growing pigs fed soyabean milk residues and different dietary protein levels respectively(Okah & Ehuriah 2013; Abeni et al, 2018). The glucose values reported in the current study is lower than the range of 10.05-18.30 and 4.19 reported by (Okah & Ehuriah 2013; Fabio et al, 2018). The values reported for cholesterol and glucose in this study are lower than the value reported for growing pigs fed palm kernel cake supplemented with enzyme based diets. Higher glucose means the carbohydrate present in the

feed were readily available for fermentation, but the serum glucose levels in this study were relatively low. The cholesterol values for the treatments is good enough for normal health functioning in growing pigs, because high cholesterol is detrimental to the arteries, since it inhibits blood flow occasioned by fat deposition in the blood vessels. The range of urea, which is not too high, compared to literatures and baseline values, indicates sufficient, standard and efficient utilization of protein in the diets fed to the pigs, since high urea levels connotes otherwise (Oyawale and Ogunkunle 1998). However, the urea values of 12.53-13.97 are lesser than values of 42.25-62.35, but higher than 3.36- 3.89 reported by Okah & Ehuriah 2013; Fabio et al, 2018. High urea levels could also be indications of unnecessary muscular expenditures (Adesehinwa 2007). The variations observed in both blood indices with reports from literatures are a confirmation, and pointer to factors such as breed, age, nutrition and climatic differences.

CONCLUSION

This study concludes that unconventional feeds can be used efficiently in swine feeding provided the level of inclusion does not exceed the range used in our study. Alternatively, bye-products such as plantain peels and cassava peels should undergo detoxification before inclusion in swine diets up to 35%, since they both negatively impacted some of the blood indices.

ACKNOWLEDGEMENTS

The authors wish to recognize the contributions of Songhai-Delta, Amukpe Sapele Delta State Nigeria for providing us with some of the feed materials used for this study.

CONFLICT OF INTEREST

All authors involved in this study from conception to the final draft hereby declare no conflict of interest.

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EFFECTS OF MYCOTOXINS ON FEED CONSUMPTION AND ANIMAL PERFORMANCE

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Abstract

Our study, it was aimed to investigate the effects of mycotoxins in the rations of ruminant animals adjusted according to their needs and environmental conditions. It is known that toxins in feed raw materials, which are undesirable and harmful to animals, negatively affect feed consumption and performance, as well as cause some diseases. Feed raw materials should be evaluated separately from the production stage to the storage, transportation, and consumption stage. At this stage, feed raw materials should be kept in suitable conditions. The effect of feed raw material in animals consuming toxic feed causes heavy losses along with diseases and negatively affects the business economically. Feed raw materials produced and stored in bad conditions cause a decrease in feed consumption in animals due to toxication. The decrease in feed consumption gives us the first information about the status of this feed raw material, and it should be given more carefully by taking precautions or removing from the ration. The rumen plays an important role in the animal for the preferred feed raw material, causing feedback before and after digestion so that the animal can be informed about feed consumption. The animal can recognize and learn the feed raw materials it consumes in a short time. The high moisture content of feed raw materials in the production stage causes a decrease in the storage time and causes mold (mycotoxin) in the feed in a short time. Although mycotoxins seriously threaten animal and human health, they cause economic losses because they cause low yield and quality in animal products. Contamination with mycotoxins in feed raw materials cannot be prevented sufficiently and mycotoxin formation cannot be stopped. Although there are many control methods for mycotoxins, which are an important problem in our country and the world, a permanent solution has not been obtained yet. The toxic effect of mycotoxins can be transmitted to the body not only by ingestion of feed or food, but also by respiration and skin, and it can also be carried from the consumption of these products since it transmits mycotoxin-containing feeds to milk and eggs with the consumption of animals. Similarly, in animals that consume mycotoxin-containing feed, first, a decrease in feed consumption is observed, and if it continues, it causes some health problems to be observed. Mycotoxins mainly affect the digestive system negatively, cause abortion, have a carcinogenic effect, and may ultimately result in death. The number of studies on detoxification and inactivation methods of mycotoxins is increasing day by day because it causes economic losses and health problems and its prevalence are high. The fight against mycotoxins can be divided into three stages the separation of mycotoxins and contaminated raw materials, the fight against mycotoxin in feed, and the elimination of mycotoxin in the digestive system in case of consumption of mycotoxin-contaminated feed.

Key words: *Feed consumption, mycotoxins, Milk yield, Milk composition, Effects on Diseases*

THE EFFECT OF URSOLIC ACID ADDITION INTO HIGH-ENERGY LAYING HEN DIET ON PERFORMANCE, EGG QUALITY PARAMETERS, SERUM LIPID PROFILE AND LIVER FAT RATE

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Abstract

This study was conducted to determine the effect of ursolic acid (UA) at different ratios (0, 0.5, 1 and 1.5%) supplementation into high-energy laying hen diet on performance, egg quality parameters, serum lipid profile, some liver enzymes and liver fat ratio.
A total of 120 Lohman LSL laying hens, 70 weeks old, were used in present study. The animals were divided into 5 groups and each group consisted of six subgroups. In the experiment, the control group was fed with basal feed, and the treatment groups were fed with high-energy (HE) diets including 0, 0.5, 1 and 1.5% UA, respectively. Experiment lasted for 8 weeks. Egg yield decreased in high energy feed groups except HE + 1.5% UA group. Egg weight was found to be highest in the HE + 1.5% UA group. Addition of UA into feed improved the feed conversion ratio (FCR). It was determined that liver fat ratio were higher in the group fed with HE feed (P <0.01) than other groups fed with diets incuding UA, but the addition of UA decreased the liver fat rate significantly. The addition of UA to feed increased blood plasma MDA and NEFA values, and decreased GSH and GPx values (P <0.01). The addition of 1.5% UA to high-energy feed increased ALT and total cholesterol, while lowering glucose. The highest VLDL, TG and LDL values were found for YE + 0% UA and YE + 1.5% UA groups. Conclusion, high-energy feed adversely affected performance values and liver fat ratio, but the addition of ursolic acid improved FCR and decreased liver fat ratio. Positive effects of ursolic acid have been seen, but more studies are needed.

Key words: *Fatty liver, ursolic acid, non esterated fatty acid, feed convertion ratio, hypolipidemic*

INTRODUCTION

In parallel with the rapidly increasing world population, the demand for eggs is increasing day by day. In order to meet this increasing demand, it has been inevitable to make conventional animal production. More production depends on increasing the number of animals as well as good care and feeding. Therefore, it is inevitable to have more animals per unit area. The most advantageous system in laying hen is cage breeding.

However, in laying hens raised in cages, the restriction in the movement area and the high energy value of the feeds may cause fatty liver. Fatty liver syndrome is one of the important causes of death in commercial layers (Leeson, 2007). In many studies conducted in the past years, it has been reported that fatty liver syndrome is detected at a higher rate in animals housed in cages than those raised on the ground (Butler, 1976; Shini et al., 2006; Squires and Leeson, 1988).

Fatty liver is a disease characterized by an accumulation of fat in the abdominal cavity and liver. Excessive fatty liver adversely affects animal health and causes a significant decrease in egg production.

Although there is no known method for the treatment of fatty liver syndrome, its development could be prevented by balanced nutrition techniques. One of them is ursolic acid. Phytosterolic ursolic acid is in the pentacyclic triterpenes group of triterpenes and is found in free or glycoside structure in plants, vegetables and fruits such as apple, basil, olive, oregano, rosemary and thyme (Babalola and Shode, 2013; Mendes Leal, 2012; Ikeda et al., 2008).

In several recent studies on mice and rats, it has been reported that the addition of ursolic acid to feed has a hypolipidemic and hypoglycemic effect. It has been proven by studies that ursolic acid regulates lipid metabolism and has a protective effect on the liver (Azevedo et al., 2010; Liu et al., 1995).

There is no study in laying hens regarding the effect of ursolic acid on fatty liver syndrome. In this study, it was aimed to determine the effect of high energy feed and ursolic acid on performance, egg quality criteria, mortality rate, some antioxidant enzymes and liver fat ratio in laying hens.

MATERIAL AND METHODS

In the study, 120 Lohmann (LSL) white laying hens of 70 weeks were weighed and divided into five groups depending on chance, and each group consisted of six subgroups. Chickens were placed in 4-storey battery-type cages (60 * 59 * 61 cm) with 24 animals in each group, 6 replications and 4 animals in each repetition.

First group (control group) was fed basal diet (Table 1), and the treatment groups were fed with high-energy (3020 kcal / kg ME) diets including 0, 0.5, 1 and 1.5% ursolic acid, respectively. The feed and water were given to animals as ad-libitum during the 8 weeks experimental period. Ursolic acid (99.9% purity) was obtained from a commercial company.

The chemical composition of the diets used in the study was determined according to the Weende analysis method (Kutlu, 2008).

Table 1. Composition of feeds used in the trial (%)

Item	Basal diet (control)	High energy diet
Corn 8.5	63	64.17
Soybean meal 44-46	16.39	12.50
Corn gluten 60	8.48	10.64
Limestone	9.68	7.65
DCP 18	1.44	1.44
Soybean oil	0.17	2.68
Vitamin-Mineral mixture[1]	0.25	0.25
Salt	0.22	0.33
Sodium bicarbonate	0.16	0.16
L-Lysine	0.11	0.10
D-L Methionine %99	0.10	0.08
Calculated composition (%)		
Dry matter	88.41	88.54
Crude protein	17.52	17.20
Ether extracte	2.20	4.84
Crude ash	11.87	10.35
Crude fiber	2.78	2.57
D Methionine	0.38	0.38
Methionine	0.40	0.41
Lysine	0.76	0.70
ME Kkal/Kg	2726	3000
Analysed composition (%)		
Dry matter	88.78	88.37
Crude protein	17.12	16.92
Ether extracte	2.43	5.03
Crude ash	11.24	11.09
Crude fiber	3.18	2.91

[1]Per kg diet added : 12 000 IU vitamin A; 2 500 IU vitamin D3; 30 IU vitamin E; 4 mg vitamin K3; 3 mg vitamin B1; 6 mg vitamin B2; 30 mg niasin; 10 mg calcium D-pantothenate; 5 mg vitamin B6; 0.015 mg vitamin B12; 1 mg folic acid; 0.050 mg D-biotin; 50 mg vitamin C; 300 mg choline chloride; 80 mg manganase; 60 mg iron; 60 mg zinc; 5 mg copper; 0.5 mg cobalt; 0.2 mg iodine; 0.15 mg selenium.

As performance values in the study, daily feed consumption, feed conversion rate (kg feed/ kg egg), egg weight and egg production were determined by measurements made every two weeks. The numbers of the animals that died during the trial were recorded daily.

Egg quality criteria (shell thickness, breaking strength, white ratio, yellow ratio, shell ratio, shape index and Haugh unit) were performed every two weeks on one randomly selected egg sample from each subgroup.

At the end of the experiment, blood samples taken from the sub-wing vein of 6 animals from each group into heparinized tubes were centrifuged at 3000 rpm for 10 minutes and their plasma was extracted and stored at -80 ° C for examination. MDA level in plasma (Yoshioka et al. 1979), SOD activity (Sun et al., 1988), GSH level (Tietze, 1969), GPx activity (Matkovics et al., 1988) CAT activity (Goth, 1991), TP levels (Lowry, 1951) and NEFA levels (Biont Chicken NEFA ELISA Kit, Cat No: YLA0179CH) were measured with Biotek Elisa Reader (Bio Tek µQuant MQX200 Elisa reader / USA). TP levels were used to calculate the SOD and GPx activity. Plasma glucose, cholesterol, VLDL, LDL, HDL, AST, ALT and TG values were analyzed in a special laboratory.

At the end of the experiment, 6 animals from each group were slaughtered and their livers were removed and their wet weights were determined. Then the livers were dried at 105 0C and their dry weight was determined. Later, samples were taken from dried livers and their fat percentage was determined (Kutlu 2008).

Statistical Analyses

Performance values, egg quality criteria, some blood parameters and antioxidant enzyme values variance analysis were performed by the General Linear Model procedure, and the importance controls of the important data were performed using the SPSS 17 package program. Mortality was determined by the X^2 independence test. Differences between groups were foun d by Duncan multiple comparison test (Düzgüneş et al., 1983).

RESULTS AND DISCUSSION

The effect of adding different levels of ursolic acid to high energy feeds on feed consumption, egg production, egg weight and feed convertion ratio is given in Table 2. It was determined that there were no significant differences in feed consumption between the groups. It found that egg production

decreased significantly (P <0.05) in HE + 0% UA, HE + 0.5% UA and HE + 1% UA groups. The highest egg weight was found only in YE + 1.5% UA group. The best feed injury rate was seen in control, HE + 1% UA and HE + 1.5% UA groups. There was no significant difference between the groups in terms of mortality.

Table 2. Effects of high energy feed and ursolic acid supplements on performance values

Groups	Feed Intake (g)	Egg Production (%)	Egg Weight (g)	Feed Convertion Ratio (g:g)	Mortality (%)
Control	111.87	79.80[a]	60.42[b]	2.39[c]	4.2
HE+ 0 % UA	111.18	73.44[b]	61.35[b]	2.56[b]	20.8
HE+ 0.5 % UA	118.11	73.59[b]	61.96[b]	2.90[a]	20.8
HE+1 % UA	110.63	75.26[b]	61.55[b]	2.38[c]	8.3
HE+ 1.5 %UA	113.22	77.32[a]	63.80[a]	2.35[c]	8.3
SE	2.45	1.51	0.34	0.091	X^2=5.22
P	ns	*	*	*	ns

a- b: The averages shown with different letters in the same column are different from each other. HE: high energy, UA: ursolic acid, Control: Basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1% UA: High energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE: Standard error, Ns:Not significant, *:P<0.05

Some researchers observed that the feed consumption of laying hens fed a high energy diet decreased compared to the control group (Harms et al., 2000; Jiang et al., 2013; Valkonen et al., 2008; Yousefi et al., 2005; Zhang et al., 2008). Contrary to these reports, Grobas et al. (1999) found that feed consumption of laying hens fed with feed containing 2680 kcal / kg ME was higher than those containing 2810 kcal / kg ME. Plavnik et al., (1997) reported that as dietary energy increases, feed intake decreases. One of the main reasons for this is that energy content plays a key role in controlling feed intake (McNab and Boorman, 2002).

It has been reported that there are large economic losses due not only to animal deaths but also to reduced egg production due to fatty liver syndrome in caiged-raised chickens (Squires and Leeson, 1988). The metabolic activity of the liver is quite high in poultry, especially during egg production where lipogenesis is stimulated (Nesheim and Ivy, 1970). Butler (1976) reported that animals with fatty liver syndrome may experience sudden decreases in egg production. Smilarly, in many previous studies (Hansen and Walzem, 1993; Julian, 2005; Thomson et al., 2003), it was reported that egg productivity decreased suddenly due to fatty liver syndrome. It has been reported that as dietary energy increases, feed consumption decreases and thus egg production decreases (Plavnik et al., 1997). In this study, it was determined that egg production decreased in high energy groups, but there was no negative change in egg production in the group where 1.5% ursolic acid was added to high energy feed. However, Grobas et al., (1999) reported that there is no significant difference between egg yields of animals fed with feed containing 2680 and 2810 kcal / kg ME. Similarly, Rozenboim et al., (2016) reported that high energy feed does not affect egg yield.

In the present study, it was determined that the addition of 1.5% ursolic acid to a high-energy diet increased egg weight compared to the control group. In previous studies, some of the researchers reported that egg weight was not affected by the energy content of the feed (Summers and Leeson, 1993; Keshavarz and Nakajima, 1995; Grobas et al., 1999; Mathlouthi et al., 2002; Valkonen et al., 2008; Zhang et al., 2008), some reported significant increases in egg weight (Marsden et al., 1987; Peguri and Coon ,1991).

In the current study, it was observed that high energy feed negatively affected the ratio of feed conversion, but the feed conversion ratio values in the groups that added 1% and 1.5% ursolic acid to the feed were similar to the control group.

Unlike this study, Grobas et al., (1999) reported that the group fed with high energy feed had a better feed conversion value. However, in another study conducted on laying hens fed with feed containing different levels of energy, it was reported that there was no significant difference between the groups in terms of feed efficiency (Zhang et al., 2008).

Leeson, (2007) reported that fatty liver syndrome causes significant mortality in commercial layer flocks. Shini et al., (2006) reported that 74% of the cause of death in laying hens in cages in Australia was caused by fatty liver syndrome. Similar to the current study, Valkonen et al., (2008) found that the mortality rate in laying hens fed with high energy feed was higher than the control group,

but the difference was not found to be significant.

The effects of ursolic acid addition to high energy feed on egg quality criteria are given in Table 3. No significant difference was detected between groups in terms of shell breaking strength, shell thickness, ratio of egg shell, yolk, albumen and Hough units values.

Similar to this study, Valkonen et al., (2008) reported that the energy value of the feed does not affect eggshell breaking strength, egg shell ratio, yolk ratio, albumen ratio and Haugh unit.

Table 3. Effects of high energy feed and ursolic acid supplements on egg quality of the laying hens

Groups	Shell breaking strength (kg cm^2)	Shell thickness (µm)	Egg shell (%)	Yolk (%)	Albumen (%)	Hough units
Control	3.27	0.459	12.42	30.22	57.27	81.97
HE+ 0 % UA	3.01	0.414	11.94	31.38	56.63	83.69
HE+ 0.5 % UA	3.16	0.434	12.84	30.34	56.81	78.32
HE+1 % UA	3.01	0.413	12.83	30.99	56.17	82.90
HE+ 1.5 %UA	3.32	0.450	12.94	30.56	56.49	80.95
SE	0.14	0.006	0.13	0.19	0.24	0.64
P	ns	ns	ns	ns	ns	ns

a- b: The averages shown with different letters in the same column are different from each other. HE: high energy, UA: ursolic acid, Control: Basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1% UA: High energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE: Standard error, Ns:Not significant

In many previous studies, it has been reported that high energy feed has no effect on egg shell thickness (Yousefi et al., 2005), yolk weight (Keshavarz and Nakajima, 1995) and albumen weight (Keshavarz ve Nakajima, 1995; Grobas et al., 2001). However, Whitehead et al. (1991) reported that the addition of fat to the ration increases the albumen ratio.

The liver is a central organ for lipid metabolism. The liver synthesizes cholesterol and triglyceride and produces lipoproteins. Generally, the hepatic lipid content is low (wet liver contains less than 5% fat of its weight) and fatty liver syndrome occurs when the liver lipid stores exceed this value.

The results of the wet weight, dry weight and fat ratio of the livers are given in Table 4. When Table 4 was examined, it was determined that using high energy feed had a significant effect ($P < 0.05$) on the wet weight and dry weight of the liver and the lowest wet and dry liver weight was in the control group. However, it was observed that the liver wet weight was lower in the groups that added ursolic acid to the food compared to the YE + 0% UA group. It was determined that there was a significant difference ($P < 0.01$) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Table 4. Wet weight (g), dry weight (g) and fat ratio (%) of liver

Groups	Wet weight of liver (g)	Dry weight og liver (g)	Fat ratio of liver % (DM)
Control	20.32^c	6.05^b	27.43^c
HE+ 0 % UA	39.13^a	14.12^a	48.26^a
HE+ 0.5 % UA	35.27^b	11.60^a	30.49^c
HE+1 % UA	28.18^b	11.78^a	33.03^c
HE+ 1.5 %UA	34.91^b	13.93^a	40.89^b
SE	2.32	1.18	2.62
P	*	*	**

a- b: The averages shown with different letters in the same column are different from each other. HE: high energy, UA: ursolic acid, Control: Basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1% UA: High energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE: Standard error , *: $P < 0.05$, **: $P < 0.01$.

However, it was observed that the liver age weight was lower in the groups in which ursolic acid was added to the high-energy diet than the group without ursolic acid (YE + 0% UA). It was determined that there was a significant difference ($P < 0.01$) between the groups in terms of liver fat ratio on the basis of dry matter, and the group YE + 0% UA had the highest fat ratio.

Ivy and Nesheim, (1973) reported that liver fat ratio exceeds 40% of dry weight and can even reach up to 70% in fatty liver. In the present

study, it was determined that the liver fat ratio was 56.8% higher in the group fed with high energy feed (YE + 0% UA) compared to the control group.

Akkılıç and Tanyolaç, (1975) reported that both liver weight and liver fat ratio increased when feed containing high levels of energy was given to the laying hens raised in the cage system. Similar to this study, many studies conducted in previous years reported that the liver fat ratio increased with the increase in the energy value of the feed (Splitgerber et al., 1969; Jensen et al., 1970).

Rozenboim *et al.*, (2016) reported that the liver fat ratio was not affected by the diet in young animals in laying hens fed a high-fat diet, but the liver fat ratio in older animals was lower in than in the control group.

Jia *et al.*, (2015) investigated the effects of adding 50 and 200 mg / kg ursolic acid to a high-energy diet on the liver in mice, and found that the liver fat ratio decreased significantly in the group that added 200 mg ursolic acid compared to the high-energy feed group without ursolic acid.

Previous studies have reported that ursolic acid has an anti-obesity effect by decreasing lipid accumulation in adipose tissues. According to these studies, ursolic acid acts as a phosphodiesterase inhibitor that increases lipolysis in adipocytes (Jia et al., 2011; Kim et al., 2009; Rao et al., 2011). Jayaprakasam et al., (2006) reported that the addition of ursolic acid to a high-fat diet reduced the amount of liver fat in mice.

In the present study, it was determined that the liver fat ratio in the groups fed rations containing ursolic acid was lower than the group fed ration without ursolic acid. This situation can be explained by the hypolipidemic effect of ursolic acid.

Differences among groups were found to be significant in terms of MDA, GSH, SOD, CAT, GPx and NEFA (Table 5). The highest MDA value was found in HE + 1% UA group. It was determined that the addition of ursolic acid to the ration significantly increased the amount of GSH ($P < 0.01$). Superoxide dismutase (SOD) and catalase (CAT) values were significantly lower in the HE + 1% UA and HE + 1.5% UA groups. The lowest GPx value was detected in the YE + 1% UA group. Non esterated fatty acids (NEFA) concentrations increased significantly ($P < 0.01$) in the high energy feed groups and the highest value was found in the YE + 1% UA group.

Table 5. Non-esterified fatty acids **(**NEFA) and some enzyme activity of liver of laying hen

Groups	MDA (nmol/L)	GSH (nmol/L)	SOD (U/L)	CAT (KU/L)	GPx (U/L)	NEFA (ng/L)
Control	7.79^{d}	2.33^{a}	57.43^{a}	146.65^{a}	1.46^{a}	0.219^{c}
HE+ 0 % UA	7.56^{d}	2.47^{a}	59.05^{a}	151.41^{a}	1.48^{a}	0.299^{b}
HE+ 0.5 % UA	10.15^{c}	1.92^{b}	57.20^{a}	139.21^{a}	1.40^{b}	0.262^{b}
HE+1 % UA	18.37^{a}	1.62^{c}	52.79^{b}	113.20^{b}	1.22^{d}	0.465^{a}
HE+ 1.5 %UA	15.93^{b}	1.77^{bc}	53.07^{b}	120.24^{b}	1.38^{c}	0.306^{b}
SE	1.18	0.09	0.82	4.23	0.02	0.02
P	**	**	*	**	**	**

a- b: The averages shown with different letters in the same column are different from each other. HE: high energy, UA: ursolic acid, Control: Basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1% UA: High energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, SE: Standard error, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, NEFA: Non-esterified fatty acids, **: $P < 0.01$.

Similar to this study, Yang et al., (2017) observed that serum MDA and NEFA levels increased in laying hens fed with high energy and protein feed. Sundaresan et al. (2014), in their study on mice that induced fatty liver syndrome with a high-energy diet, reported that free fatty acid levels increased significantly in mice fed high-energy ration compared to the control group, and the addition of ursolic acid to the diet significantly reduced these values.

Li et al., (2014) found that ursolic acid supplementation significantly lowered serum NEFA levels and increased SOD, MDA, CAT and GSH-PX values in mice with high-fat diet obesity.

Researchers have suggested that the addition of ursolic acid increases the levels of b-hydroxybutyrate in the blood and, based on this result, ursolic acid may increase the oxidation of free fatty acids.

Average values of some plasma parameters are given in Table 6. As can be seen from Table 6, AST and HDL values were not affected by the treatment. Plasma VLDL, triglyceride and LDL values were significantly higher ($P < 0.01$) in the HE + 0% UA and HE + 1.5% UA groups. The highest ALT and total cholesterol values were

found in the HE + 1.5% UA group. Plasma glucose ratio decreased significantly (P <0.05) in the HE + 1.5% UA group.

Table 6. Some blood plasma biochemistry parameters of laying hen

Groups	VLDL mg/dl	ALT U/L	AST U/L	Glucose mg/dl	Total cholesterol mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl
Control	71.66^{b}	3.00^{b}	244.66	260.66^{a}	124.00^{b}	142.50^{b}	46.00	89.00^{b}
HE+ 0 % UA	329.50^{a}	7.00^{b}	265.66	248.00^{a}	135.00^{b}	1567.00^{a}	42.66	206.50^{a}
HE+ 0.5 % UA	52.00^{b}	2.33^{b}	186.50	264.00^{a}	113.00^{b}	258.50^{b}	44.33	93.00^{b}
HE+1 % UA	67.33^{b}	2.00^{b}	252.00	278.66^{a}	115.00^{b}	337.66^{b}	53.66	105.56^{b}
HE+ 1.5 %UA	337.66^{a}	17.50^{a}	236.00	235.66^{b}	278.50^{a}	1687.66^{a}	45.66	183.00^{a}
SE	39.07	1.92	15.53	5.73	19.25	199.98	3.38	24.85
P	**	*	ns	*	**	**	ns	**

a- b: The averages shown with different letters in the same column are different from each other. HE: high energy, UA: ursolic acid, Control: Basal fed group, HE + 0% UA: High energy fed group, HE + 0.5% UA: High energy fed + 0.5% ursolic acid, HE + 1% UA: High energy fed + 1% ursolic acid, HE + 1.5% UA: High energy + 1.5% ursolic acid, VLDL: Very low density lipprotein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TG: Triglyceride, HDL: High density lipoprotein, LD: Low density lipoprotein, SE: Standard error, *:P<0.05, **:P<0.01, Ns: Not significant

In the present study, it was determined that the VLDL, TG and LDL values increased significantly in the high energy feed group, but the addition of 0.5% and 1% ursolic acid in the high energy feed decreased these rates, while the addition of 1.5% ursolic acid did not affect them.

Jia et al., (2015) reported that 200 mg of ursolic acid supplementation decreased plasma triglyceride and VLDL concentrations, increased HDL concentration, and did not affect the total cholesterol ratio in mice fed a high-fat diet. The high estrogen concentration in laying hens also promotes liver-transported TG synthesis in the form of VLDL (Zhu et al., 2013).

High-fat diet increases the amount of free fatty acids in plasma and leads to triglyceride accumulation in the liver (Yki-Järvinen, 2005). The liver plays a central role in maintaining systemic lipid homeostasis. Lipid balance is maintained by the regulation of lipogenesis and lipid oxidation, which are regulated by the cooperative effect of various enzymes and transcription factors found in the liver (Yki-Järvinen, 2005).

Peroxisome proliferator activated receptor (PPAR) is a major regulator of genes involved in fatty acid transport and utilization in the liver and mitochondrial and peroxisomal fatty acid-oxidation (Aoyama et al., 1998; Reddy, 2001). Activation of PPAR with synthetic or natural compounds increases cellular fatty acid uptake and subsequent oxidation rate (Motojima et al.,1998). Jia et al., (2011) reported that ursolic acid activates the nuclear receptor of the PPAR, and there is a decrease in lipid accumulations in hepatocytes through gelation of PPAR-responsive genes in hepatic lipid metabolism. Therefore, administration of PPAR agonists simultaneously improves lipid and glucose metabolism, decreases both plasma and hepatic triglyceride accumulation, increases glucose tolerance, and increases HDL cholesterol concentrations (Harano et al., 2006; Nakajima et al., 2009).

Yang et al., (2017) observed that the serum triglyceride, total cholesterol and LDL-cholesterol ratio increased significantly, while the HDL-cholesterol ratio decreased slightly in laying hens fed with high-energy feed. Jayaprakasam et al., (2006) reported that the addition of ursolic acid to a high-fat diet reduced serum triglyceride levels in mice.

Sundaresan *et al.* (2014) reported that ALT and AST values were significantly higher in mice fed with a high-energy diet with fatty liver syndrome compared to the control group, and ursolic acid added to the diet significantly reduced these values.

In the present study, it was determined that the addition of 1.5% ursolic acid to high-energy food significantly lowered the plasma glucose ratio. Jayaprakasam et al., (2006) reported that a high-fat diet increased glucose levels in mice, while the addition of ursolic acid significantly reduced this value. The same researchers stated that the passage of glucose into the blood is delayed by ursolic acid.

Due to the lack of a study on ursolic acid on laying hens and also the lack of up-to-date studies on fatty liver in laying hens in recent years, this study has not been discussed sufficiently.

CONCLUSION

As a result, it was determined that fatty liver syndrome occurs in laying hens fed with high energy feed. While 1.5% ursolic acid addition to high-energy diet positively affected performance values, it was determined that 0.5 and 1% ursolic acid supplementation significantly reduced liver fat ratio and plasma TG, LDL and VLDL values.

ACKNOWLEDGEMENTS

This study was supported by Atatürk University.- Project No: YL-2019-6936.

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CONTRIBUTION OF AFRICAN TANNIFEROUS BROWSE SPECIES TO RUMINANT NUTRITION AND METHANE MITIGATION: A REVIEW

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Abstract

Methane is a major greenhouse gas (GHG) produced from different sources including ruminants as a result of ruminal fermentation. According to the Food and Agriculture Organization (FAO 2006), livestock contributes globally about 14.5% of GHG emissions, with two-thirds thereof from ruminants. Africa is a world hotspot for livestock GHG emission intensities, primarily due to poor quality feed and low animal productivity. The leaves of many trees and shrubs remain green during the dry season; they thus provide more crude protein and minerals to animals than mature annual grasses and herbaceous species. They grow well even in harsh environments, and their use in animal feeding reduces food-feed competition. Many of these browse feeds contain bioactive compounds such as tannins, saponins, and essential oils, some of which can help in reducing enteric methane emissions. Tannins are polyphenols contained in many African browse species; they can act as rumen modifiers as they have anti- methanogenic properties due to bactericidal and bacteriostatic effects. An extensive literature search in Google Scholar for the keywords; "Africa", "browse species", "methane" and, "in vitro" yielded 139 articles (from 2018 - 2022) on African browse species. From their information, it can be concluded that across Africa, the concentration of crude protein (CP) in browse species is in the range of 109 - 297 g kg-1 DM which is above the minimum requirements (70 - 80 g kg-1 DM) of ruminants. Total and condensed tannins concentration are in the range of 0.15 - 537 g kg-1 DM and 0.08 - 465 g kg-1 DM, respectively. The concentrations of NDF, ADF, and ADL range from 209 - 688 g kg-1 DM, 121 - 380 g kg-1 DM and 77 to 140 g kg-1 DM, respectively. Their digestibility is in the range of 31 - 56% and metabolizable energy values vary between 2.6 and 10.58 MJ kg-1 DM. Meta-analysis studies confirm the negative correlation between tannin concentration and enteric methane emission, with significant CH4 abatement in vitro (-11.5% to -54.5%) upon inclusion of tanniferous browse in test diets at a share of 30 – 100%. However, in vivo studies are also required to explore the potential of individual browse species and their dietary inclusion levels as a strategy to reduce enteric methanogenesis in ruminants; this is a particular challenge in Africa where the availability of suitable instruments such as respiration chambers and sniffer boxes are rare.

Key words: *Africa, Browse species, Enteric methane, Nutritional profile, Rumen, Shrubs, Tannins trees*

USE OF PULSE CROPS IN LIVESTOCK

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Abstract

Pulse crops have a production of 89821452 tons and a yield of 963.9 kg/ha in an area of 93182898 hectares in the world, on the other hand, there is a production of 1296867 tons and a yield of 1488.9 kg/ha on an area of 871009 hectares in Turkey. Pulse crops are plants that are important in animal nutrition as well as human nutrition. Both the seed and stem contain more protein when compared to cereals. It contains 23.0% protein in bean seeds, 23.4% in broad beans, 20.8% in chickpeas, 23.4% in cowpea, 24.2% in lentils and 22.5% in lentil seeds. When the amino acid contents of edible legumes are examined, it is known that amino acids are at a very high level -except methionine. In terms of oil content, chickpea is the richest and contains 5% oil in the seed. On the other hand, the stems are also very rich in terms of nutrients and have an average protein value of 13.74%. Because of the high cost of grain products of pulse crops in animal breeding, stem-straw part is generally used. On the other hand, a type of trypsin inhibitor in the legume, which is an edible legume, has a negative effect on the digestion of ruminants. For this reason, it can be recommended to use broad beans in pig breeding.

Key words: *pulse crops, livestock, animal feed*

INTRODUCTION

The term pulse crops do not include crops such as groundnut (*Arachis hypogaea* L.)) and soybean (*Glycine max* (L.) Merr.) grown for oil and for sowing purposes (e.g., seeds of clover (different species belonging to the genus *Trifolium* L.) and alfalfa (Medicago sativa L.)). Similarly, legume species are not considered as pulses when they are harvested as vegetables (e.g., green beans or green peas) (FAO, 2022). According to the Food and Agriculture Organization, pulse crops have been reported to consist of the following plants; *Phaseolus* spp. (beans), *Vicia faba* (broad beans), *Lens esculenta* (lentils), *Cicer arietinum* (chickpeas), Pisum spp. (peas), *Cajanus* spp. (pigeon peas), *Vigna sinensis* (cow peas), *Vicia sativa* (vetch), *Lupinus* spp. (lupins), *Vigna* spp. (black gram, green gram, mung, etc.) (FAO, 2022).

There are 12.000 annual and perennial species in the legumes family and 200 species are cultivated. Among these species, those used as pulse crops are beans (*Phaseolus vulgaris* L.), chickpeas (*Cicer arietinum* L.), lentils (*Lens culinaris* Medik.), broad bean (*Vicia faba* L.), cowpea (*Vigna sinensis* L.) .) and peas (*Pisum sativum* L.) (Akcin 1988).

Grains take the first place in the areas where field crops are produced in the world, followed by pulse crops (Gulumser, 2016). While cereals have 736.009.199 ha sowing, 2.996.142.289 tons production and 4.070 kg/ha yield value in the world, pulses give 93.182.898 ha sowing 89.821.452 production and 964 kg/ha yield (FAO, 2020). On the other hand, the cultivation area of cereals in Turkey was 11.128.065 hectares, the production was 37.184.688 tons and the yield value was 3.341 kg/ha. The cultivation area, production and yield values of legumes in Turkey are as follows; 871.009 hectares, 1.296.867 tons and 1.489 kg/ha respectively (FAO, 2020).

All of the pulse crops cultivated are annual. While lentils and broad beans can be grown in winter, they are all summer crops. Beans and black-eyed peas have the highest heat demand, followed by chickpeas, peas, broad beans and lentils, respectively. Among these cultivated species, beans, black-eyed peas, broad beans and peas are the ones with high water demand, while lentils and chickpeas are plants that can be grown in arid places (Akcin 1988; Kun ve ark. 2005). *Rhizobium* bacteria, which live in common with legumes, bind the nitrogen in the air to the environment they live in and enrich the soil with organic nitrogen. The amount of nitrogen that edible legumes attach to the soil varies between 50-200 kg/ha per year, depending on the variety and environmental conditions (Sehirali 1988).

In this manuscript, the possibilities of using pulse crops in animal nutrition have been tried to be explained.

PRODUCTION QUANTITY OF LEGUMES

Table 1. Total amount of production area in the world for pulse crops (FAO, 2020)

	Cultivation area (ha)
Beans, dry	34801567
Cow peas, dry	15056435
Chick peas	14841940
Peas, dry	7190442
Pigeon peas	6096038
Pulses nes	5918039
Lentils	5009933
Broad beans, horse beans, dry	2671497
Lupins	888507
Bambara beans	354870
Vetches	353630

The most planted and produced edible legume in the world is beans (Table 1-2). In terms of cultivation area, cowpea and chickpea follow. In terms of total production in the world, chickpea and pea follow it respectively. In terms of yield per hectar, the highest value belongs to broad bean (Table 3). Peas and vetches are high yielding legumes that rank second and third in terms of yield in the world (FAO, 2020).

Table 2. Production amounts in the world for pulse crops

	Production (ton)
Beans, dry	27545942
Chick peas	15083871
Peas, dry	14642466
Cow peas, dry	8901644
Lentils	6537581
Broad beans, horse beans, dry	5669185
Pigeon peas	5012357
Pulses nes	4440414
Lupins	1046170
Vetches	711203
Bambara beans	230619

The legume plant with the highest cultivation area in Turkey is chickpea, followed by lentils and beans, respectively (Table 4). In terms of production, the situation is directly proportional to the cultivation area (Table 5). In terms of yield per hectare, legume plants in Turkey are listed as follows; peas, beans, broad beans (horse beans) (Table 6) (FAO, 2020).

Table 3. World average yield of pulse crops

	Yield (kg/ha)
Broad beans, horse beans, dry	2122,1
Peas, dry	2036,4
Vetches	2011,2
Lentils	1304,9
Lupins	1177,4
Chick peas	1016,3
Pigeon peas	822,2
Beans, dry	791,5
Pulses nes	750,3
Bambara beans	649,9
Cow peas, dry	591,2

Table 4. Total amount of production area in the Turkey for pulse crops (FAO, 2020)

	Cultivation area (ha)
Chick peas	511493
Lentils	247642
Beans, dry	102963
Broad beans, horse beans, dry	3488
Pulses nes	3447
Vetches	1424
Peas, dry	552

Table 5. Production amounts in the Turkey for pulse crops

	Production (ton)
Chick peas	630000
Lentils	370815
Beans, dry	279518
Broad beans, horse beans, dry	9135
Pulses nes	3699
Vetches	2162
Peas, dry	1538

Table 6. Turkey average yield of pulse crops

	Yield (kg/ha)
Peas, dry	2786,2
Beans, dry	2714,7
Broad beans, horse beans, dry	2619,0
Vetches	1518,3
Lentils	1497,4
Chick peas	1231,7
Pulses nes	1073,1

NUTRITIONAL VALUES OF SEED IN PULSE CROPS

Green gram, red gram, bengal gram, horse gram, cluster bean, field bean, cow pea etc. are some of the common types of pulses. Their seeds are legumes with twice the protein content of the seeds of cereals (Table 7).

Pulse crops seeds include both edible and inedible species. Even among edible legumes, toxic principles occur, and it is important to eliminate them to exploit them for edible purposes. Two thermoliable factors play a role in the toxic effects. Inhibitors of the enzymes

trypsin, chymotrypsin and amylase haemagglutinins, which impede the absorption of the products of digestion in the gut. On the other hand, legumes also contain a goitrogen, a toxic saponin, cyanogenic glycosides and alkaloids (Anonymous, 2022c). Soaking, heating, and fermentation are some of the applications that can reduce toxic properties. In addition, cooking is an application that removes some toxic properties if done correctly. Cooking also contributes to the digestibility of pulses. Heat causes the denaturation of the proteins responsible for trypsin inhibition, haemagglutination and the enzyme responsible for the hydrolysis of cyanogenic glycosides. The type of application of heat is important. Autoclaving and soaking followed by heating are effective. Fermentation is another way to eliminate toxic factors (Anonymous, 2022c).

Plants containing various toxic components from legumes and the chemicals they contain are as follows; red gam, pigeon pea, jack bean, sword bean content saponin, bengal gam, chickpea, garbanzo, guarbean, horsegam, mung bean, green bean, lima, horse bean content cyanogenetic glucoside, field or garden pea content goiterogenic factor, fava bean, horse bean, broad bean content favism, common vetch content cyanogenetic glucoside, alkaloid etc. (Liener, 1962).

Table 7. Seed mean protein, Carbohydrate, fat and fiber content of pulse crops seed

Crops	Protein(g/100g)	Carboh.(g/100g)	Fat (g/100g)	Fiber (g/100g)
Bambara bean	18,80	60,80	1,40	10,30
Bean	22,10	38,41	1,40	4,60
Broad beans, horse beans	26,00	21,00	1,50	25,00
Chickpea	20,47	62,95	6,04	12,20
Cowpea	23,53	60,03	1,26	10,60
Faba bean	26,12	58,29	1,53	25,00
Lentil	25,80	60,10	1,10	30,50
Lupins	32,00	28,00	6,90	13,00
Mung bean	22,90	19,15	1,20	7,00
Pea	20,50	22,00	2,00	14,00
Pigeon pea	22,40	48,19	2,74	7,25
Vetches	28,30	43,50	1,50	4,80

(Mateos-Aparicio et al., 2010; Faris et al. 2013; Olaleye et al. 2013; Yao et al. 2015; Wallace et al., 2016; Huang et al. 2017; Talari and Shakappa 2018; Santos et al., 2019; Kose et al., 2019; Kumar and Pandey 2020; Zaheer et al. 2020; Dhull et al. 2021; Anonymous, 2022a; Anonymous, 2022b)

The lectins were not destroyed in the rumen of yearling cattle, and circulating antibodies were developed in response to these toxic materials. Therefore, it is not desirable to use raw kidney beans as a protein supplement in cattle (Williams et al., 1984).

It is reported that raw kidney beans are not suitable for monogastric animals and this is true for ruminants such as young cattle or dairy cows when raw kidney beans are given (Pusztai et al., 1981). Pusztai (1980) toxic factors are defined as bean-lectins. His used rats and pigs in the study. The bean residues can be used to feed the lamb (Eynipour et al., 2019). Pulse crops like as cowpea residues contain more than barley or wheat straw crude protein and metabolizable energy (Singh et al., 2011). On the other hand, it is reported that lectin concentration is important in animal feeding with pulse crops and should be considered (Grant and van Driessche, 1993).

It was reported by Miller and Holmes, (1992) that the use of mung beans in broiler chicken feed has only slight deleterious effects.

On the other hand, It has been reported that lentils are suitable for use in poultry (Anonymous, 2022d).

Chickpea can be used as a high energy and protein feed in dairy cattle and beef cattle, it can also be used in poultry to support egg production (Bampidis and Christodoulou, 2011).

It is recommended to use chickpea, which is better than dry peas in terms of fat, in non-ruminant feeding (Cordesse 1990).

Field peas are a delicious pulse crops for all classes of beef cattle (Anderson et al. 2002).

CONCLUSIONS

Pulse crops are important feed ingredients in animal nutrition, especially ruminant animals, due to their high energy and excess protein content. There are antinutritional factors that restrict the use of pulse crops in large quantities, and the elimination of these factors by various methods or the breeding of cultivars and species free of these factors will increase their use. On the other hand, it would be appropriate to use it in feed rations by taking into consideration the production costs.

ACKNOWLEDGEMENT

This work was supported by the Kahramanmaras Sutcu Imam University Scientific Research

Projects Unit. Project Number: 2022/5-13. The authors are grateful.

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USE OF CHERRY LAUREL FRUIT POWDER (*LAUROCERASUS OFFICINALIS* ROEMER) IN BROILER CHICKEN NUTRITION: INFLUENCE ON PERFORMANCE

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Abstract

To evaluate the effects of Cherry Laurel Fruit (Laurocerasus officinalis Roemer) powder (CLF) supplementation on performance (body weight, BW; body weight gain, BWG; feed intake, FI; feed conversion rate, FCR) of broiler chickens, 600 chicks were randomly allocated into four treatment groups each with six replicates containing 25 birds. Birds were fed dietary treatments for 42 days of age. Treatments consisted of a control diet (C), C diet supplemented with %0.25 CLF (CLF25), %0.50 CLF (CLF50) and %1 CLF (CLF100). The BW, BWG and FI of the CLF birds was higher than the control groups (P<0.05). While FCR was lower in CLF groups, carcass yield was higher in CLF25 and CLF50 groups (P<0.05). These results showed that performance improvement was achieved with the use of CLF in broiler ration due to the bioactive components in its structure.

Key words: *broiler, performance, Laurocerasus officinalis Roemer*

INTRODUCTION

In recent years, there has been an increase in consumer demand for food that is perceived as fresh, healthy, hormone-free, free from antibiotics and harmful chemicals and produced in an environmentally sustainable way. Therefore, a wide variety of natural feed additives are used as an alternative to improve performance in poultry (Windich et al., 2008). For this purpose, various alternative feed additives have been developed recently. These feed additives are used to replace antibiotics that can leave residues in meat and eggs or to limit the current therapeutic effects (Kim et al., 2013). Natural feed additives of plant origin are generally believed to be safer. One of the mechanisms underlying the health benefits of natural feed additives is thought to be related to their antioxidant properties (Kim et al., 2011).

The modern density poultry industry demands faster growth in a closed poultry environment leading to increased susceptibility to stress in broilers. Rapid growth rate in broilers accelerates metabolic rate and makes them vulnerable to oxidative stress due to increased free radical formation (Elizabeth Manju et al., 2011). In many health concerns related to oxidative stress, there is increasing interest in the importance of natural antioxidant compounds in the diet as treatment to prevent damage (Salim et al., 2014; Eken et al., 2016). Natural sources of compounds with antioxidant potential (fruits, vegetables, herbs) can be used as components with phytogenic effects against a number of degenerative diseases partially caused by oxidative stress (Halvorsen et al., 2002). In the last decade, herbal feed additives have attracted the attention of scientists as a useful resource for increasing productivity. In addition, these plants are a natural component and do not have side effects such as residue in meat products (Patel et al., 2016). Many plants and their parts or plant extracts have antimicrobial activities and antioxidant properties that make them useful as natural animal feed additives (Faixova and Faix, 2008).

Various studies have focused on clarifying the biochemical structures and physiological functions of aromatic plants, their extracts and essential oils. Due to the phenolic compounds in the composition of aromatic plants and their extracts, there is increasing interest in including these plants, which have many attractive properties (antioxidant, antistress, cholesterol-lowering, anticarcinogenic, etc.), into the ration (Yıldırım and Gürkan, 2010). Turkey is a country that contains a wide variety of plants as natural vegetation. Researches on the ingredients and effects of various plants in our country are continuing. It has been reported in studies that substances such as antioxidants and antiallergens in the contents of these plants can be natural feed additives that can be used instead of antibiotics when

added to the ration (Ertaş et al., 2005; Buğdaycı, 2008; Alp et al., 2010).

Plant-derived products contain a wide variety of phenolic compounds (such as phenolic acids, flavonoids, anthocyanins, tannins, lignans and catechins) with antioxidant activities. These phenolics protect against harmful free radicals and reduce the risk of certain types of cancer, coronary heart disease, cardiovascular disease, stroke, atherosclerosis and other degenerative diseases associated with oxidative stress (Ness et al., 1997; Mazza et al., 1999; Temple, 2000; Surh, 2003; Watson, 2003; Shahidi and Naczk, 2004). As a matter of fact, it has been reported that Cherry Laurel (Taflan) (Laurocerasus officinalis Roemer) fruit is a rich source of antioxidant substances such as phenolics (chlorogenic acid, phenolic acids, anthocyanins, vanillic acid) and ascorbic acid (Ayaz et al., 1997; Yaylacı-Karahalil et al., 2011). The fruit has anticarcinogenic, antioxidative and antidiabetic properties; makes cherry laurel ingredients attractive to the functional foods and food supplements industries. Although there are studies on the nutritional values of many dried fruits and their effects on poultry performance (Toghyani et al., 2017; Dalal et al., 2018), no study has been found in which cherry laurel fruit is used in broiler feeding. For this reason, due to all these properties, the effect of dried and ground cherry laurel fruit as a feed additive on performance (body weight gain, feed intake, feed conversion ratio) in broilers was investigated in the present study.

MATERIALS AND METHODS

In the current study, approval was obtained from "Eskişehir Osmangazi University Experimental Animals Animal Ethics Committee" to ensure appropriate and ethical animal use (828-2021).The cherry laurel fruit (CLF) to be used in the experiment was obtained from Sakarya, Samsun and Zonguldak. After removing the seeds of the cleaned fruits, they were dried and ground. Cherry laurel fruit was added to the ration at the rate of 0.25%, 0.50% and 1%.Total phenolic content was performed according to Singleton et al., (1999); DPPH (Free Radical Removal Activity) values were analysed according to the procedures described by Blois (1958); ABTS (Radical Eliminating Activity) by the method described by Re et al., (1999) and FRAP (Ferric Ion Reduction Force) values were determined as described Benzie and Strain (1996) in cherry laurel fruit samples. Ascorbic Acid (Vitamin C) values were were performed according to Cemeroğlu (2010). Nutrient components of ground cherry laurel fruit were determined by Weende (crude protein, crude oil, crude ash) and Van Soest analysis method (ADF and NDF).A total of 600 broiler chicks (Ross 308) were randomly allocated into four treatment groups each with six replicates containing 25 birds. Birds were fed dietary treatments for 42 days of age. Treatments consisted of a control diet (C), C diet supplemented with %0.25 CLF (CLF25), %0.50 CLF (CLF50) and %1 CLF (CLF100). In the experiment, corn and soybean based ration was used as feed material. Broiler starter feed (22% HP and 3050 kcal/kg ME) between 0-3 weeks and broiler grower feed between 4-6 weeks (20% HP and 3200 kcal/kg ME) were used. Chickens were fed in accordance with the recommendations of the relevant company during the 42-day treatment period. Feed and water consumption was administered ad libitum.The live weights of the animals were determined at the beginning of the experiment (day 0), on the 21st day after the feed change and at the end of the experiment (42nd day). In addition, feed weighings were made at the same time and feed consumption amounts were calculated. Feed conversion ratios were calculated from both data.

One-way analysis of variance was performed on the obtained data in the SPSS statistical package program. The normal distribution of the data was tested with the Kolmogorov-Smirnov test, and the homogeneity of the variances was evaluated with the Levene test. If the effects were significant, the difference between the treatment groups was determined by the Duncan multiple comparison test. Differences between treatments were considered significant at the $p<0.05$ level.

RESULTS AND DISCUSSION

When the nutrient content of cherry laurel fruit powder (CLF) is examined (Table 1), 4.13% crude protein, 3.49% ash, 0.27% crude oil, 14.04% acid detergent fiber and 6.50% neutral detergent fiber found to contain.

Table 1. *Nutrient content of cherry laurel fruit powder*

Nutrient	***%***
Dry matter	*91,161*
Ash	*3,491*
Crude protein	*4,126*
Crude fat	*0,273*
Acid detergent fibre	*14,048*
Neutral detergent fibre	*6,506*

Bioactive components of KM are presented in Table 2. Total phenolic substance of the components dissolved in water and ethanol medium, respectively, is 327.27 mg GAE/100g,

1046.97 mg GAE/100g; DPPH 5.49 µg TE/mg, 11.56 µg TE/mg; ABTS was 9.27 µg TE/mg, 16.17 µg TE/mg; FRAP was found to be 81.67 µg TE/mg and 331.50 µg TE/mg. Vitamin C was determined as 242.826 mg/kg.

Performance values of broiler chickens fed by adding CLF to the diet at different levels are presented in Table 3. Accordingly, rations supplemented with CLF at different levels had a statistically significant effect on the FI, BW, BWC, FCR and carcass yield of the chickens ($P<0.05$). Feed intake of the treatment groups was higher than the control group ($P<0.05$). Body weight and BWC higher in the other treatment groups compared to the control treatment ($P<0.05$). The FCR was found lower in CLF groups compared to control ($P<0.05$). The carcass yield was the lowest in the control group, and the highest in the CLF25 and CLF50 groups ($P<0.05$).

Table 2. *Bioactive components of cherry laurel fruit powder*

	TPS	DPPH	ABTS	FRAP	C vitamini
	TFM (mg GAE/100g)	TEAC (µg TE/mg)	TEAC (µg TE/mg)	TEAC (µg TE/mg)	AA (mg/kg) Ascorbic acid
Ethanol Extract	1039,39	11,00	16,63	339,92	
	1054,55	12,12	15,70	323,09	
Average	**1046,97**	**11,56**	**16,17**	**331,50**	247,17
Water Extract	342,42	5,81	9,66	78,15	238,48
	312,12	5,17	8,89	85,19	
Average	**327,27**	**5,49**	**9,27**	**81,67**	**242,826**

TPS: total phenolic substance; DPPH: free radical scavenging activity; ABTS: radical scavenging activity; FRAP: ferric-reducing antioxidant power; TEAC: trolox equivalent antioxidant capacity; AA: Ascorbic acid

Table 3. *Feed intake, live weight and feed conversion ratios of broiler chickens added to the diet cherry laurel fruit powder at different ages*

Days	C	CLF25	CLF50	CLF100	P	SEM
Feed intake, g						
21. d	1151.25^{c}	1253.75^{a}	1216.25^{ab}	1181.25^{bc}	0.000	10.090
42. d	4193.75^{b}	4462.50^{a}	4443.75^{a}	4525.00^{a}	0.000	28.376
Body weight, g						
0. d	45.10	45.19	44.94	45.03	0.092	0.037
21. d	684.40^{b}	775.40^{a}	753.65^{a}	740.63^{a}	0.000	8.434
42. d	2382.05^{b}	2633.40^{a}	2603.42^{a}	2677.13^{a}	0.000	25.367
Body weight change, g						
0-21. d	639.29^{b}	730.22^{a}	708.72^{a}	695.60^{a}	0.000	8.428
21-42. d	1697.65^{b}	1858.00^{a}	1849.77^{a}	1936.49^{a}	0.000	22.258
0-42. d	2336.95^{b}	2588.21^{a}	2558.49^{a}	2632.09^{a}	0.000	25.367
Feed conversion ratio, g/g						
21. d	1.68^{a}	1.62^{b}	1.61^{b}	1.60^{b}	0.002	0.009
42. d	1.76^{a}	1.70^{b}	1.71^{b}	1.69^{b}	0.003	0.008
Carcass yield, g/100						
	70.00^{c}	73.00^{a}	72.52^{ab}	70.82^{bc}	0.006	0.370

C: Control diet; CLF25: diet supplemented with %0.25 CLF; CLF50: diet supplemented with %0.50 CLF; CLF100: diet supplemented with %1 CLF; SEM: standard error of the mean; a, b: The averages shown with different letters in each row are statistically different ($p<0.05$).

DISCUSSION

In the current study, it was observed that the addition of cherry laurel fruit to the diet increased FI, BW and BWG, and decreased FCR. The higher FI observed in the mixed-fed groups with CLF supplementation may indicate antioxidant stimulation of the digestive system. It is reported that antioxidants can stimulate the function of pancreatic enzymes (lipases, amylases and proteases). The significant increase in FI may be due to the fact that cherry laurel fruit is a rich source of antioxidants (Vahapoğlu et al., 2018). As a matter of fact, the bioactive component content of the cherry laurel fruit we used in the study supports this finding. Aljumaily et al. (2019), amla powder and Ashour et al. (2020), in their study where they used okra powder addition, the body weight and body weight gain findings showed similar results with our current study. In studies where other fruit powders were used as feed additives, the body weights of the treatment groups were found to be higher than the control group, which was supported by our study (Joshi et al., 2015; Patel et al., 2016; Umativa et al., 2018). Similarly, feed consumption was found to be high in broiler chickens fed with rations containing turmeric and hot pepper powder (Adegoke et al., 2018) and amla fruit powder (Begum et al., 2019a) with high antioxidant content. So much so that the curcumin in turmeric has been shown to

improve feed utilization by stimulating protein synthesis through the enzymatic system of animals, and as a result, improve digestion, increase food metabolism and increase weight gain (Durrani et al., 2006). This may explain the improvement in BW, BWG and FCR in the present study. The phenolic compounds contained in the cherry laurel fruit can limit the metabolic manifestations of stress and alleviate its physiological consequences. In addition, these active compounds may have shown an anabolic effect that contributed to the increase in body weight. As a matter of fact, the fact that FCR is low in CLF groups supports this situation. Significant improvements in FCR were observed in the treatment groups compared to the control group in broiler chickens fed the mix with the addition of amla powder (Aljumaily et al., 2019; Begum et al., 2019b). Adegoke et al., (2018) reported that the addition of turmeric and cayenne pepper powder to the basal diet improves FCR. These studies were found to be compatible with the results of our current study. In the current study, our results on carcass yield can be explained by improvements in FI, BW and FCR. As a matter of fact, the lowest carcass yield was determined in the control group.

CONCLUSIONS

The data obtained as a result of the study showed that the performance improvement was achieved with the use of CLF in broiler ration due to the bioactive components in its structure.

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THE EFFECTS OF IN OVO INJECTION OF THE SELECTED PREBIOTIC, PROBIOTIC AND SYNBIOTIC FORMULATION DELIVERED AT 18.5 ED ON EMBRYONIC DEVELOPMENT, HATCHABILITY AND QUALITY OF DAY OLD CHICKS

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Abstract

Background. In recent years, poultry farming has emerged to be one of the fastest growing industry in the agricultural sector thus playing a pivotal role in world economy. The quality of day-old-chicks has a long-lasting effects on the health and growth performance of the flock. Therefore, in poultry practice, the assessment of day old chicks and the mortality of the first 7 days of live are major productive parameters in the peri-hatch period. Secondly, there are efforts of in ovo technology providers to enable injections of various bioactive compounds using the vaccination timepoints. The aim of the study was to determine the effects of the selected prebiotic, probiotic and synbiotic delivered in ovo on day 18.5 of egg incubation on hatchability and quality of day old chicks using the automated single egg injector machine. Also to confirm that in ovo injection on day 18.5 can be applicable in poultry hatcheries. . Therefore, we hypothesized that the in ovo delivery of the selected prebiotic, probiotic and synbiotic on day 18.5 day of egg incubation using the single egg injector machine do not affect hatchability and quality of day old chicks. and could prove its applicability in the hatchery industry. Methodology. The experimental trial was conducted using 500 Ross 308 fertilized eggs with three repetitions. In total, there were this treatment groups (50 eggs / group). The doses used per egg were synbiotic 0.5mg x 10 ^ 3 CFU, 0.5mg x 10 ^ 6 CFU, 1.0 mg x 10 ^ 3 CFU, 1.0 mg x 10 ^ 6 CFU, prebiotic 0.5mg, 1.0 mg, probiotic 10 ^ 3 CFU, 10 ^ 6CFU, positive control (only vaccine) and control non-injected respectively. During this trial experiment, we optimize the doses of the prebiotic, probiotic and synbiotic which were delivered into the amnion of Ross 308 broiler chicken embryos on day 18.5 of egg incubation using an automated single egg in ovo injector machine. Hatchability was recorded and the chick quality was assessed and measured using Pasgar score, chick length and weight of day old chicks. Hatching debris analysis was performed to examine the possible causes of unhatched eggs. The results were analyzed by one-way analysis of variance using statistical software package SPSS version 16.0 using one-way ANOVA model p. Significant differences among treatment means were determined by performing pos-hoc test, Duncan´s multiple range test and Tukey test (P < 0.05). Results. The results showed that there was no significant difference in hatchability. However, the hatchability was slightly higher in synbiotic 1.0 mg x 10 ^ 3 CFU, prebiotic 0.5mg x 10 ^ 3 CFU and probiotic 10 ^ 3 CFU with 99%, 98.68% 98% respectively. There was no significant differences on the body weight of day old chicks among groups (P <0.05). The Pasgar score used to the quality of chicks. The probiotic group (Pro 10 ^ 3 CFU) with a score of 9.50 (P <0.05) was shown to be highly significant when compared to synbiotic groups (Pre 1.0 mg x 10 ^ 3 CFU and Pre 1.0 mg x 10 ^ 6 CFU) (P <0.05). Conclusions. In conclusion, the selected prebiotic, probiotic and synbiotic delivered in ovo at day 18.5 of egg incubation using the automated single egg injector machine do not affect hatchability and could improve quality of day old chicks. *Acknowledgements. This research was co-financed by the European Union´s Horizon 2020 research and innovation program under grant agreement N ° 955374 MonoGutHealth project, National Science Center UMO-2019/35 / B / NZ9 / 03186, for OVOBIOM project and EcoSET supported by Polish National Agency for Academic Exchange under agreement PPI / APM / 2019/1/00003. I would like to thank Professor Katarzyna Hrynkiewicz from the Departemnt of Microbiology, Nicholaus Copernicus University) and Niloofar Akhavan, MSc, for the preparation of the probiotic doses.*

Key words: *Broiler chickens, Hatchability, in ovo injection, Prebiotic, Probiotic, Synbiotic*

THE EFFECT OF BIODEGRADATION ON SWEET ORANGE PEEL (SOP) AND ITS FEED VALUE IN STARTER BROILER CHICKS DIET

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Abstract

A twenty-eight-day feeding trial was conducted to evaluate maize replacement value of rumen filtrate biodegraded sweet orange peel in the starter broiler chicks diet. Sweet orange fruit peels (SOP) were collected from retailers of peeled sweet orange fruits. F*resh rumen content was collected from a government abattoir, mixed with water at the ratio of 1 kg: 1 litre, and the mixture sieved to obtain rumen filtrate (RF). Rumen filtrate was mixed with sweet orange peels at the ratio of 1 litre : 2.5 kg, poured into polythene bags, tied at the open end, and allowed a 48-hour for biodegradation. The fermented sweet orange peels were sun-dried to about 10% moisture, milled and incorporated into each of five broiler starter diets as a replacement for maize at levels of 0%, 5%, 10%, 15%, and 20% to give diets T_1, T_2, T_3, T_4, and T_5, respectively. Biodegraded SOP contained 8.80% crude protein, 13.25% crude fibre, 8.65% ether extract, 9.90% ash, and 59.40% NFE, and metabolizable energy of 3720.67 Kcal/kg. The experimental diets had significant effect ($p<0.05$) on daily feed intake, final body weight and body weight gain. There was no significant difference ($p>0.05$) among other performance indices measured across the dietary treatments. Dietary incorporation of SOP meal as a replacement for maize did not support the growth of starter broiler chicks, and further studies are necessary to investigate other processing methods that can further reduce its fibre content, to enhance its feed value as a replacement for maize in the diets of broiler chicks.*

Key words: *Sweet orange peel, biodegradation, chick*

INTRODUCTION

The poultry industry offers a quick solution for providing the thronging population with the necessary animal protein. Broiler birds are probably the most universal and important of all poultry as producers of meat for human consumption. Animal protein shortage in the diet of the average Nigerian is shown in the consumption of 3.24g per caput which is far below the 35g daily requirement recommended by FAO (Hon *et al.,* 2009). Energy is key to metabolism and if it is limited, dietary protein will be used inefficiently as another source of energy instead of being converted into body protein, hence, adequate energy must be supplied by the diet to make efficient use of dietary protein. Some agro-industrial by-products like composite mango fruits reject (Orayaga, 2016), palm oil sludge (Famurewa and Olarewaju*,* 2013), citrus by-products like sweet orange peel meal (Oluremi *et al*., 2018) have been used in non-ruminant animals diets to partly replace cereals. Sweet orange (*Citrus sinensis*) fruit peel is an agricultural produce waste in Nigeria and with no cost attached to it, and it is high in energy (Oluremi *et al.,* 2010). Rumen content is another important agricultural by-product, in the abattoir industry in Nigeria (Ahemen and Zahradden, 2010) and can be converted into beneficial use by taking advantage of its microbial population rather than its present status as agricultural waste (Oluremi *et al*., 2010). Its utilisation by taking advantage of its microbial content for the processing of sweet orange fruit (*Citrus sinensis*) peel can result in value addition to the peel to increase its suitability as a dietary energy source for livestock production. The aim of this study was to determine the effect of partial replacement of dietary maize with graded levels of bovine rumen filtrate-treated sweet orange (*Citrus sinensis*) fruit peel meal on the performance response of starter broiler chicks.

MATERIALS AND METHODS

The study was carried out at the Poultry Unit of the Livestock Teaching and Research Farm of the College of Animal Science, Federal University of Agriculture Makurdi, Benue State, Nigeria. Makurdi is situated in the north-central zone of Nigeria with a latitude of 7°.43´N and a longitude of 8°.53´N (Microsoft Encarta 2008).

Sweet orange fruit peels were collected from some sweet orange retail sellers around the Makurdi metropolis. Fresh rumen content was collected from cattle immediately after slaughter at the government-owned Wurukum Abattoir. Rumen content was mixed with water at a ratio of 1 kg : 1 litre, and thereafter sieved to obtain rumen filtrate (RF). The rumen filtrate was mixed with sweet orange peels at the ratio of 1 litre : 2.5 kg, and the mixture put in polythene bags, tied at the open end, allowed a 48-hour biodegradation, and sun-dried to below 10% moisture for safe storage before final use in diet preparation. The sun-dried sweet orange peel material was milled, analyzed for proximate constituent using the standard methods (AOAC, 2015), and used in formulating starter diets replacing maize at levels of 0%, 5%, 10%, 15%, and 20% to give diets T_1, T_2, T_3, T_4, and T_5, respectively.

A hundred and fifty (150) day-old, unsexed broiler chicks were used for this experiment. The birds were weighed and grouped into five (5) of equal number and similar live weight. Each group was randomly assigned to one of the five (5) dietary treatments T_1, T_2, T_3, T_4, and T_5. There were 3 replicates per treatment with 10 birds per replicate. Each treatment replicate was randomly allotted to the experimental pens. The experiment was a completely randomized design. The birds were raised in a deep litter of wood shavings. Feed and drinking water were provided *ad libitum* and standard routine management practices (feeding, watering, and washing of drinkers, cleaning of feeders, and pen passages) were followed. The birds were vaccinated against Newcastle disease (i/o) at day old, infectious bursal disease at day 14, Newcastle disease (Lasota) at day 21, and infectious bursal disease at day 28 as recommended by the manufacturer, National Veterinary Research Institute, Vom - Jos, Nigeria. An anti-stress supplement was administered prior to and after each vaccination, and pre- and post-weekly weighing of the birds. Coccidiostat was administered at alternate weeks to stem occurrence of coccidiosis which is endemic in the study environment, and antibiotics was given if and when necessary as prophylactics. Data collected was used for the evaluation of growth performance.

RESULTS AND DISCUSSION

The proximate composition of biodegraded sweet orange fruit peel meal showed it contained 92.5% DM, 8.80% crude protein, 13.25% crude fibre, 8.65% ether extract, 9.90% ash, and 59.40% NFE. Ojabo *et al.* (2014) reported a DM of 86.20%, 7.40% CP, 8.19% ash, 7.19% EE, 13.50% CF, 62.65% NFE and 3674.44 Kcal/kg ME for sundried sweet orange peel meal while, Agu *et al.* (2010) reported 89.65% DM, 10.74% CP, 7.86% ash, 12.00% EE, 11.90% CF, 56.91% NFE and 3988.70 kcal/kg ME. Also, 7.0 % CP, 12.50% CF, and ME of 3420 kcal/kg were reported by Ashbell and Weinbegger (1999) in Israel for sweet orange peel. The crude fibre level in SOP the test ingredient is high, like what has been reported by some other workers. The nutrient quality of feed ingredients is one of the major prerequisites for the production of good quality feeds. The basic nutrients that cannot be compromised in the choice of ingredients for feed formulation are protein and energy. The dry matter of 92.28% in this study was higher than 87.60% for sweet orange peel (SOP) biodegraded with rumen content for 48 hours reported by Oluremi *et al.* (2008).

The SOP meal with a CP of 8.80% was higher than 7.40% reported by Ojabo *et al.* (2014), and 7.50% by Akpe *et al.* (2019). The disparity in crude protein composition could be attributed to the type of pasture consumed by the cattle which will affect the type and the population of the ruminal microorganism, the ratio of rumen content to sweet orange peel used for processing, and the stage of digesta degradation in the rumen when cattle was slaughtered. The CP is however slightly lower than CP in maize, a conventional energy feedstuff with 9.10% CP (Aduku, 2005), while crude fibre (CF) of 13.25% in the peel was lower than 13.50 % and 14.60% reported by Ojabo *et al.* (2014) and Ani *et al.* (2015), respectively. The slight reduction may be due to the processing method used in this study. The high CF in the peel may reduce its feeding value compared to conventional dietary maize in poultry nutrition even though it has a high metabolizable energy of 3720.67 kcal/kg. The high CF content in the biodegraded SOP in this study most probably caused the reduction of its NFE, the digestible carbohydrate and energy nutrient in feed ingredients. Hence, the energy yield of biodegraded SOP will be of inferior value compared to that of maize in practical broiler chicken feeding.

Table 1. Proximate Composition of Biodegraded Sweet Orange Peel Meal (% DM) [1]Laboratory Analysis; [2]Aduku (2005); [3]Metabolizable energy as determined using Carpenter and Clegg (1956)

Nutrients (%)	Sweet orange peel meal[1]	Maize[2]
Dry matter	92.50	86.50
Crude protein	8.80	9.00
Crude fibre	13.25	1.30
Ether extract	8.65	4.00
Ash	9.90	2.70
Nitrogen free extract	59.40	83.00
[3]Metabolizable energy (Kcal/kg)	3720.67	3432.00

Table 2. Effect of Biodegraded Sweet Orange Peel Meal on the Growth Response of Starter Broiler Chick (Day old – 28 day old)

	Experimental Diets					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
Initial body weight (g/bird)	47.02	47.74	47.29	46.59	46.88	0.42^{ns}
Final body weight (g/bird)	525.37^{a}	468.48^{b}	466.17^{bc}	427.77^{c}	406.10^{c}	14.19*
BWD (g/day/bird)	17.08^{a}	15.03^{ab}	14.96^{ab}	13.62^{b}	12.83^{b}	0.51*
Feed intake (g/bird/day)	36.19^{a}	29.63^{ab}	31.94^{ab}	29.80^{ab}	28.22^{b}	1.07*
Feed conversion ratio	2.12	1.96	2.15	2.19	2.19	0.04^{ns}
Protein intake (g/bird/day)	8.40	6.87	7.39	6.90	6.51	0.25^{ns}
Protein efficiency ratio	2.03	2.20	2.03	1.97	1.98	0.04^{ns}
Mortality rate (%)	0.67	0.00	0.00	0.33	0.00	0.11^{ns}

[a, b, c] Means with different superscripts in the same row are significantly different ($p<0.05$), *($p<0.05$), [ns] Not significantly different ($p>0.05$), SEM = Standard error of mean, SOPM = Sweet orange peel meal, BDG = Body Weight Gain,
T1 = 0% maize replacement with SOPM (Control diet), T2 = 5% maize replacement with SOPM, T3 = 10% maize replacement with SOPM, T4 = 15% maize replacement with SOPM, T5 = 20% maize replacement with SOPM

The ash content of 9.90% obtained in this study was higher than 4.47% (Ani et al., 2015), and 8.19% (Ojabo *et al,* 2014). The implication of the high ash content is that it may lower the dietary caloric yield because of the limitation of mineral elements to yield energy in the metabolic process of oxidation. Therefore, the results of the proximate composition of biodegraded SOP meal showed that, while its high crude protein content can be of nutritional benefit to monogastric animals including broiler chicken, its content of crude fibre and ash can be adverse to the good performance of these farm animals. The experimental diets significantly ($p<0.05$) affected the final weight, body weight gain, and feed intake of starter broiler chicks. The effect of the experimental diets on feed intake by broiler chicks significantly ($p<0.05$) decreased at higher maize replacement with biodegraded SOP. Thus, the lowest mean daily feed intake of 28.22 g was obtained in T5 and the highest feed intake of 36.19 g was obtained in T1. The highest feed intake of 36.19 g was lower than 56.16 g reported by Oluremi *et al.* (2010) who fed fermented sweet orange peel-based diets to broiler chicks and 37 g reported by Aduku (2005) as the mean daily feed intake for starter broiler chicks. This may be attributed to the low fibre content of the control maize-based diet (T1) compared to the high fibre content in the biodegraded SOP meal based diets, the overall feed composition, dietary nature, and strain/breed of broiler chicks used. Abbas *et al.* (2013) also reported dietary fibre effect on broiler chicks fed sweet orange peel-based diet. The body weight gain like feed intake of the chicks significantly ($p<0.05$) decreased as the percent maize replacement with biodegraded SOP increased from 0% to 20%. Consequently, the weight gain was highest in T1 (17.08 g) and lowest in T5 (12.83 g). The range was less than 32.44 g to 43.17 g (Medugu *et al.,* 2010) but comparable with 11.97 g to 21.70 g (Oluremi *et al.,* 2010). Furthermore, feed intake appeared to have a direct effect on body weight gain and thus, a cumulative effect on the final body weight. Feed intake and utilization of the nutrients present are the major factors that influence both body weight gain and feed efficiency in meat-type birds. The final body weight of the chicks was significantly ($p<0.05$) different among the dietary groups decreasing from T1 (525.37 g) to T5 (406.10 g) for the same reason as for body weight gain. The broiler chicks in T2 and T3 had a relatively higher final body weight of 468.48 g and 466.17 g, respectively, which were not significantly ($p>0.05$) different among the biodegraded SOP dietary groups. The experimental diets did not have any significant ($p>0.05$) effect on feed conversion ratio, protein intake, protein efficiency ratio, and mortality rate across the

dietary groups. This showed that the replacement of maize with biodegraded SOP in the range of 0% to 20% did not impact negatively on the quantitative values of all these performance indicators in the starter broiler chicks. The rate of mortality among the experimental birds in this study was less than 5 % regarded as normal for broiler chicks (Oluyemi and Roberts, 2000). Furthermore, since mortality did not show any significant difference among the dietary treatments, biodegraded SOP meal may be a safe ingredient to use in compounding broiler chick diet if its other nutritional limitations can be mitigated.

CONCLUSIONS

From the result obtained in this study, it was concluded that the rumen filtrate biodegraded sweet orange peel meal is comparable with maize in crude protein and higher in metabolizable energy content but inferior in crude fibre, and can thus be transformed from being an agricultural waste into a feed resource in broiler chicken production. The utilization of SOP meal as replacement maize did not support the growth of starter broiler chicks, further studies are therefore necessary to investigate other processing methods that can further reduce the fibre content of sweet orange peel meal to enhance its feed value as a replacement for maize in the diets of broiler chicks.

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BREAST MEAT QUALITY ATTRIBUTES OF BROILERS FED SYNTHETIC AMINO ACIDS BASED DIETS

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Abstract

Synthetic amino acids are used in the buffering and balancing of low protein diets which helps in building of muscles of birds. The aim of this study was to investigate the effect of synthetic amino acids inclusion on the physicochemical attributes of the breast meat of broilers. The experiment was conducted on 210 chicks raised under intensive management. The birds in Treatment 1(T1) having diets of 21.50% crude protein and 19.50 crude protein at the grower and finisher phases respectively. Treatment 2(T2) with less than 1% crude protein of T1 and Treatment 3 (T3) offered less than 2% crude protein of T2 however, with the inclusion of synthetic methionine, lysine and threonine in the diets. The data obtained for color and water holding capacity were not significantly different while that obtained for pH, cooking loss and drip loss were significantly (P<0.05) different across the treatment. The results suggest that birds can be fed low crude protein diets, with synthetic amino acid inclusion without an adverse effect on the breast meat quality.

Key words: *breast meat, synthetic amino acid, physicochemical, broiler chicken, crude protein*

INTRODUCTION

Poultry meat quality is made up of its safety, nutritive value and sensory characteristic (Sokolowicz *et al.,* 2016). Broiler nutrition plays a crucial role as a non-genetic factor in addition to housing system, postmortem processing, cooking and storage. (Nasir Akbar et al., 2007) The choice of feed ingredient, chemical composition, protein and energy values of formulated rations and the degree of nutrient utilization, has a corresponding effect on the chemical composition of muscle tissue (Zafar, 2015).

Diet manipulation, specifically protein, plays a key role in breast meat yield in broilers. Amino acids are known to improve the carcass composition of broilers especially the breast meat yield and has the tendency to reduce abdominal fat deposition (Debut *et al*., 2003).

Amino acids as a major constituent of proteins also affect the production of other muscle constituents and a contributor to the specific flavor of meat (Chen and Liu 2004).

Formulation of diets with low crude protein level is possible with the use of synthetic amino acids because it allows for greater accuracy in diet formulation and ensures that a balanced amino acids profile is instituted to optimize growth performance in birds (Vieira *et al.,* 2004) which improves meat nutritional quality and breast meat yields. It is also beneficial due to its least cost formulation benefits and ability to improve carcass quality of birds

This study evaluated the effect of synthetic amino acid on the physicochemical characteristics of meat obtained from the breast muscle of the broiler chickens.

MATERIALS AND METHODS

The study was conducted on 210 day-old broiler chicks, with the use of completely randomized design the birds were allocated into three treatments with six replicates each. The birds were fed starter diets from day 1 to 10 across all treatments. From day 11 to 25 growers diet and day 26 to 42 finishers diet howbeit, with a modification in the crude protein. Treatment 1: broiler grower offered 21.50% and broiler finisher with 19.50 % (Control), Treatment 2 less than 1%, Treatment 3 less than 2% of the control. The colour evaluation of the breast samples were conducted using Chroma meter which gave CIELAB color evaluation in form of L* (lightness) a* (redness) and b* (yellowness). After slaughtering, the PH meter was inserted into the breast muscle, using a portable pH meter with penetration electrode calibrated with buffer solution at pH 4.0 and 7.0 Drip loss were determined as the broiler breast meat were weighed and placed into a sealed polythene

bags. The samples were stored for 24 hours at temperature range of 1-5C.

% Drip loss = (Initial weight – Weight after 24hour)/ Initial weight X 100

To determine the water holding capacity, 1g of meat sample was pressed between two filter papers with plexi glass for 1 minute using a table device. The amount of juices released from the sample was measured indirectly by measuring the area of the filter paper wetted relative to the area of pressed sample. WHC% = Meat Area/ Water Area X 100

Meat samples in zip-sealed polyethylene bags were boiled in a water bath to 100C to obtain Cooking loss% = (Weight of samples before cooking – weight of samples after cooking)/ Weight of samples before cooking X 100

All data collected were subjected to analysis of variance and the significant differences were separated using Duncan multiple range test.

DISCUSSION

Colour is an important attribute because it informs the consumers on the freshness and wholesomeness of the meat. The value of L∗, a∗ and b∗ is an indication of chicken meat quality. A decreased L∗ value suggests an increase in the water holding capacity and pH which tends to favour product yield and greater moisture retention when meat is subjected to further processing (Barbut, 2008). According to Van Laack *et al*., (2000), the L* values of 55 for chicken meat is considered to be normal while L* values of 60 is considered to be pale. The mean values obtained in this study as shown in Table 1 were not significantly ($p>0.05$) different. The colour values may be influenced by the method of defeathering employed as bleaching of the epidermal layer occurs at temperature above 54^0 C, (cold defeathering was employed in this study), in addition the breed, age and diet of the birds informs the pigmentation of epidermis

Table 1. Colour of Broiler Breast Meat with Supplementation of Synthetic Amino Acid in their Diet

Parameters	T1	T2	T3	SEM±
L*	57.03	58.40	58.44	0.98
a*	17.08	16.90	16.31	0.40
b*	17.75	18.65	19.16	0.67

L* lightness, a* redness, b* yellowness

T1: broiler diet with control crude protein, T2: broiler diet with less than 1% crude protein, T3: broiler diet with less than 2% crude protein

The pH value of meat is significant to quality characteristic. The pH changes is important during rigor mortis because it affects the meat colour, texture and water holding capacity. Increase in pH suggests an increased water-holding capacity and less protein denaturation in the meat, which is probably due to an increased concentration of soluble sarcoplasmic protein found therein. (Guo *et al.,* 2003). This increase in pH may be due to a lower level of glycogen storage in the breast muscle when broiler diets are supplemented with essential amino acids, which would reduce the amount of postmortem acidification in the muscle (Zhai, 2016) as well as the maintenance of cold storage from the farm to the laboratory. The value 5.46 to 5.63 is within the range of normal meat as reported by (Woelfel 2002) indicating a property of a good quality meat (Table 2)

Cooking loss is a measurement of water percentage that is lost upon cooking due to meat shrinkage. The degree of meat shrinkage when cooked has a direct correspondence with loss of juiciness which in result influences the taste (Khan *et al.,* 2015). Water loss in meat is correlated with decline in the nutritional value of the meat because some nutrients inherent in the meat are lost, which is associated with reduction in nutritional value (Jung *et al.,* 2011) which results to a less tender meat. Water holding capacity of meat describes the ability of muscles to bind water under certain conditions. Generally, it increases with age of the birds, as the muscle fat content increases which leads to a greater water holding capacity and a decrease in cooking loss percentage (Corzo *et al.,* 2000).

Table 2. Effect of feed supplemented with Synthetic Amino Acids on the physical parameters of the Broiler breast meat

Parameters	T1	T2	T3	SEM±
pH	5.63^a	5.55^b	5.46^b	0.03
Cooking Loss (%)	39.71^a	36.42^b	39.30^{ab}	0.64
Water Holding Capacity (%)	59.86	60.28	64.34	1.93
Drip Loss (%)	7.47^{ab}	10.34^a	6.48^c	0.71

abc:means along the row with different superscripts are significantly($P<0.05$) different

T1: broiler diet with control crude protein, T2: broiler diet with less than 1% crude protein, T3: broiler diet with less than 2% crude protein.

CONCLUSION

The results of the findings suggests that, the inclusion of synthetic amino acid in low protein diet imposes a positive index on the colour, pH,

cooking loss and water holding capacity of the meat obtained from the birds. This is an indication that such meat will have improved nutritional and better keeping qualities.

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EVALUATION OF THE EFFECT OF ADDING DIFFERENT LEVELS OF ISOCHRYSIS GALBANA MICROALGAE AS A FEED SUPPLEMENT TO THE DIET ON RUMINAL MICROBIAL POPULATION BY IN VITRO METHOD

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Abstract

The aim of this study was to investigate the effect of adding Isochrysis galbana (I. galbana) microalgae at levels of 1 and 4% of the diet as feed supplement on the rumen microbial population. For this purpose, purified ruminal fluid, three Holstein castrated calves were used for genomic extraction. The experiment was performed in a randomized design with three replicated runs. Data were analyzed using SAS statistical software (9.4). The results showed that the use of 1% I. galbana in the diet reduced lipolytica, R. flavefaciens, F. succinogenes and protozoa by 6%, 15%, 18%, and 40%, respectively, and the 4% level reduced the population by 12%, 32%, 18%, and 60%, respectively. Also in 1% treatment the population of R. albus, Butyrivibrio fibrisolvens, S. ruminantium and fungi increased by 10%, 10%, 4%, and 4%, respectively, and in 4% treatment by 18%, 18%, 24%, and 20%, respectively. Microbial population changes were significant between 1 and 4% levels (p <0.05). The results showed that increasing the level of microalgae in the diet increased the microbial population changes by in vitro method.

Key words: *Microalgae, Cellulolytic bacteria, Methanogenesis*

THE EFFECT OF NANOCHLOROPSIS OCULATA MICROALGAE SUPPLEMENTATION ON RUMINAL METHANE PRODUCTION IN VITRO

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Abstract

The aim of this experiment was to investigate the effect of adding Nanochloropsis oculata (N. oculata) microalgae at levels of 1, 2, 3, 4, and 5% and compare it with the control treatment (without microalgae) on methane production by in vitro method. This experiment was performed in a completely randomized design. Data were analyzed using SAS statistical software (9.4). For this purpose, the ruminal fluid of three castrated fistulated Holstein male calves with an average weight of 430 ± 20 kg was used. The results showed that the addition of N. oculata microalgae at levels of 1 to 5% to the diet reduced the amount of methane and the ratio of methane to gas 24 h after incubation (Table 1). The amount of methane and ratio of methane to gas 24 h in the control treatment was the highest and in the treatment containing 5% of N. oculata microalgae was the lowest. The results showed that N. oculata can be used as an animal feed supplement to methane reducing in ruminant diets.

Key words: *Microalgae, Cellulolytic bacteria, Methanogenesis*

STUDY OF THE RELATIONSHIP BETWEEN SIZE AND WEIGHT OF LITTER, PLACENTA WEIGHT AND WEIGHT OF RABBIT'S AT PARTURITION

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Abstract

The objective of our work is to determine the relationship between the size and weight of the litters born, the weight of the placentas and the weight of the rabbits at birth. The study was carried out on 22 rabbits distributed in the field. All the rabbits were followed from insemination to kindling. At parturition, the weights of the rabbits, the sizes and weights of the litters born were recorded. Placentas were recovered (not all) and identified per doe, weighed and fixed in formalin for histological study. An analysis of variance was performed with JASP (2000) software, considering the factors AI weight class (3 levels), AI weight class (3 levels). The average weights of female rabbits of the synthetic strain were 4682.5±422.2g at insemination and 3985.0±400.0g at kindling. The average litter sizes born are 10.08 NT & 6.55 NV, with average litter weights of about 395.0g. The mortality rate is very high (33%). The average placenta weight was 6.19±2.0g. The analysis of variance revealed significant differences in litter size in terms of total and live births according to the weight of the rabbits at insemination and at parturition. Placenta weight is affected by this factor. The relationship (correlation) between the variables of live weight of rabbits with litter size and weight was positive and highly significant (P<001).

Key words: *Rabbit doe, alive born, total born, placenta, weight*

MITIGATION OF GREENHOUSE GAS EMISSIONS FROM POULTRY HOUSE THROUGH FEED AND ENVIRONMENTAL MODULATIONS

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Abstract

Currently, consumers of animal products are well aware of the impact of the footprint of different food items in the form of greenhouse gas emissions (GHG) on the environment, so the use of sustainable foodstuff and feed supplements in animal feed is of prime importance. Agriculture sector accounts for 10–12% of the World's total GHG emissions. Animal manure alone is responsible for 13% of GHG emissions. GHG emissions are a global challenge, and it is a significant threat to life on earth in the form of global warming. N_2O is the primary GHG representing 57% of total GHG emissions, CO_2 represents 38%, and CH_4 represents 5% of total emissions. There are five main contributors to this situation; one of them is agriculture (poultry industry-based). The poultry industry's GHG emission is about 40-50%. There are various types of fermented products from poultry birds' gastrointestinal (GIT) leading to GHG emissions. The primary GHG emissions are NH_3, CH_4, H_2S, N_2O, and CO_2. While others include biogenic amines, phenols, indole, skatole, branch-chain fatty acids. The fermentation of undigested protein mainly produces these products in the hindgut by pathogenic microbes. So, the wise and judicious use of protein is essential. Various studies have reported that smart use of protein is helpful in both saving economy and birds' health and minimizing GHG emissions. Diet formulation based on low CP and dAA:ME basis will lower the feed cost and hindgut fermentation and will lower the impact on the environment and birds' health. Using alternative protein sources (non-soy; single-cell protein, insect-based meal), enzymes, phytobiotics, and pro/prebiotics (feed additives) may help devise least-cost feed formulation and increase protein digestion. Poultry farms' managemental aspect are also important, the studies have showed that poultry housing is complex interaction of biological and physical factors which include birds, husbandry systems, social interatction, lighting, temperature and ventilations systems. Research results showed that emissions of propane and diesel contributed a lot in polluting the anvironment. Failure to manage the poultry housing operations efficiently may lead to have negative impact on the ecological systems and may result from the direct release of harmful constituents into the atmosphere or indirect deposition of these elements into groundwater. Now a days, to evacuate the poultry house's gases, air cleaners are being installed in poultry houses. The air cleaners have plastic filters that are sprinkled with liquid that captures ammonia and other gases. Furthermore, droplet separators are being used to segregate the fluid droplets from the airstream. About 58% of the total amount of emissions that is normally discharged through the chimney is collected through this method. In poultry houses houses, efficient fans can be used to evacuate polluted air in a fast manner through ventilation to minimize the impact of gases on birds' health and perofromance. Poultry house's gas removal techniques also use some impact curtains or biomass stack-wall. Moreover, Vegetative environmental buffers like planted trees at downstream of the exhaust air have also been used. Recently, wet scrubbers have been investigated to precipitate NH_3, dust and odor from the exhausted air. However, the biggest challenge for poultry housing is the obstruction of the filtration system by feathers. The system is also relatively energy-intensive, using extra energy to overcome the resistance to the airflow.To reduce the impact of GHG emissions on the environment, both nutritional strategies and poultry house's management are essential. So, reducing dietary CP, dietary supplementation of pre-and probiotics and organic acids, or feeding diets with larger particle sizes may increase protein digestibility, reduce undigested protein fermentation in the large intestine, and lower the environmental impact. Besides this, proper disposal of poultry wastes and poultry house gases is alo highly important. Hence, intense studies are required to achieve these targets and to curtail the impact of poor dietary combinations and housing anomalies.

Key words: *Poultry, greenhouse gases, feedstuffs, protein sources, feed supplements, environment*

RED PARTIDGE PRODUCTION EXAMPLE OF YOZGAT ÇALATLI WILD ANIMAL PRODUCTION STATION

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Abstract

Red partridge (Alectoris chukar), which has a socio-cultural feature in hunting and wildlife especially in Turkey, is a hunting bird belonging to the pheasant family (Phasianidae).
The gradual decrease in the number of red Partridges has led to the extinction of wild red partridge populations, especially with pesticides used in agricultural lands, unconscious hunting. The population continued to decrease as technological developments accelerated industrialization.
One of the studies carried out to increase the red partridge population in Turkey is the Çalatlı Poultry Wild Animal Production Center.
A wild animal production center was established on 27.04.2007 on an area of 17.8 hectares in the Çalatlı village of the central district of Yozgat, and the target species is red partridge.
In 2007-2020, 155.524 partridges with henna were produced and released into the environment in various regions of Turkiye. In this study, the amount of red partridge produced between 2019-2021 was compared.

Key words: *red partridge, production, wild animal*

INTRODUCTION

In order to protect the ecological balance, keep endangered animals present, and promote ethical hunting and wildlife protection, T.C. The Ministry of Agriculture and Forestry raises wild animals. Red partridges is only one among them.
Production centers were opened in many parts of Turkey. These; It is located in the provinces of Kahramanmaras, Yozgat, Gaziantep, Malatya, Gümüshane and Afyon. Yozgat production facility, registered 128 and approved by the Ministry on April 27, 2007, has a 10,000 wild animal production capability. (Alkan et al, 2008, Özkan et al.,, 2013, Özkan, 2020)
Between 2007 and 2021, 172.649 red partridges produced at the production facility were released into the environment. Red partridges released in the wild play a role in maintaining the ecological balance while also participating in ecological conflict. (Anonymous, 2022a., Anonymous, 2022b)

MATERIALS AND METHODS

It is the second isatson established by the Ministry of Agriculture and Forestry of the Republic of Turkey. It was established in 2007 within the body of Yozgat Nature Conservation and National Parks Provincial Branch Office.It was established to contribute to sustainable hunting wildlife activities and to support natural life.

After this station, Gaziantep, Afyon, Malatya and Gümüşhane Production Stations were established by the relevant ministry. At the listed Stations, an average of 150,000 years/pieces of red partridge/pheasant are produced. In accordance with the allocations made by the General Directorate of Nature Conservation and National Parks, it is released into the natural environment in the relevant regions of Turkey. (Anonymous, 2022b)
Yozgat Çalatlı Wild Animal Breeding Station was established on 27.04.2007 on an area of 17.8 hectares. Production station layout is given in the figure 1.

Figure 1.: Location plan of Yozgat Çalatlı Wıld Anımal Productıon Statıon (Anonim., 2022)

It consists of 1 administrative building, 2 hatcheries, 7 nature adaptation cages and feed storage, water treatment section and heating

unit sections. The target species is the red partridge. Guinea hen has been produced since 2012. The annual average production amount of the station is 10,000 units. (Anonymous, 2022a)

RESULTS AND DISCUSSION

In 2007-2020, 155.524 red partridges and 29,847 Guinea hen were produced.

It has been released all over Türkiye.

Guinea hen production was terminated by the end of 2020. Yozgat Çalatlı Wild Animal Breeding Station is an extremely modern production station. Activities at the production station start in January.

The first chicks of the year are taken in February. 46.715 red partridges were produced between 2019 and 2021, as shown in Table 1, and they were transported to various Turkish districts where they were released loose in the wild. At the production station, semi-intensive production is carried out. (Anonymous, 2022a., Anonymous, 2022b)

The animal has unlimited access to water and food. The Nature Conservation and National Parks Department of the Ministry of Forestry and Water Affairs is in charge of enforcing Land Hunting Law No. 4915 in Türkiye.

Based on Articles 18, 19, and 20 of the Land Hunting Law No. 4915 and Article 10 of the Animal Protection Law No. 5199, both dated 24/6/2004, the "Hunting and Wild Animals and Products Obtained from Them" published in the Official Gazette on June 16, 2005, and numbered 25847. "The Possession, Production, and Trade Regulation" was issued. Hunting and Wild Animal Breeding Stations were established under the purview of this regulation. The Ministry raises pheasants and partridges from game birds at these stations. Although the poultry houses used for other poultry are not suitable for these animals, some special production facilities are required. These requirements can be classified as breeding cages, main machines, rearing cages, incubators, and cages for natural adaptation.

Türkiye's partridge production is one of the ornamental birds with significant commercial potential and socio-cultural characteristics. In both domestic and wild life, there are 14 subspecies of partridge.

Partridges are raised for hunting, meat production, and egg production. Red partridge lays 40-100 eggs per year on average. The hatching power of the chicks at the stations ranges from 59 to 73%. It is a very difficult animal to feed and raise. (Anonymous, 2022a).

Table 1. Yozgat Red Partridge Production Station production data in 2019, 2020 and 2021 and the provinces sent

Production Station	Years	Production Amount	Provinces sent in Türkiye
Yozgat Production Station	2019	11.800	Balıkesir, Çanakkale, Karabük, Kastamonu, Tokat, Yozgat ve Zonguldak
Yozgat Production Station	2020	14.000	Yozgat, Çorum, Kırşehir, Kırıkkale, Nevşehir. Tokat, Aksaray, Ankara, Karabük, Konya, Bolu, Eskişehir, Kütahya ve Bilecik
Yozgat Production Station	2021	12875	Eskişehir, Kütahya, Bilecik, Bolu, Ankara, Kırıkkale, Kırşehir, Balıkesir, Çanakkale, Yalova, Denizli, Uşak ve Yozgat

It is made available to hunting grounds after a 12- to 16-week fattening program in production stations affiliated with the Ministry of Forestry. (Anonymous, 2022a., Anonymous, 2022b)

In 2006, breeding red partridges were obtained from the Kapçam red partridge production station. Yozgat Wild Animal Production Station began operations as the Ministry of Forestry and Water Affairs' 2nd Station. Table 1 shows Yozgat red partridges Production Station data by year.

CONCLUSIONS

The most important purpose in the production of Red Partridge from wild animals; is to release it to nature in order to increase the population in nature.

To achieve these objectives, wild animal breeding stations have been established in many Turkish provinces, and my production is still active.

It is also used as a biological weapon in the battle against adults of the sunn pest, which is one of our country's grain pests that is suitable for agriculture and to protect the natural balance. (Anonymous., 2021b).

In Turkey, the number of facilities active in production by the General Directorate of Nature Conservation and National Park is 6 to produce game birds. The production capacity of these stations is increasing day by day, and more wild animals are produced by improving the nature adaptation units.Further research results in animals that are more compatible with nature.

ACKNOWLEDGEMENTS

We would like to thank the Republic of Turkey Ministry of Agriculture and Forestry, General

Directorate of Nature Conservation and National Parks, and the Yozgat and Kahramanmaras Provincial Branch Offices for their support with this research.

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CLIMATE CHANGE IMPACTS ON LIVESTOCK PRODUCTION

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Abstract

Livestock activities have a major effect on almost every aspect of the environment, especially to climate change. Climate change and its impacts has lately become a widely accepted reality by scholars, and its impact on the environment can already be seen. While the effects of global warming will not be felt evenly over the world, the faster changes occur, the grater the harm that exceeds our ability to deal with the consequences. Temperature increases have a negative impact on animal production, including growth, meat and milk yield and quality, performance, reproductive performance, health status, and immune response. This article reviews, the effects of climate change on livestock production and provides so suggestions for productions systems.

Key words: *climate change, livestock production, animal disease, heat stress, mortality, reproduction*

INTRODUCTION

Global warming is described as the intense increase in greenhouse gas emissions such as CO_2, CH_4, and N_2O in the atmosphere as a result of human activities while artificially increasing the earth's climate with its layers close to the earth. The change of other climatic factors such as precipitation, humidity, air movements, and drought due to global warming is also described as "global climate change" (Dogan, 2005).

Extreme events and seasonal changes will negatively impact animal welfare and produce a decrease in yield and reproductive performance (Sejian et al., 2013). Therefore, climate change is a major threat to the sustainability of livestock production systems the worldwide. For instance, pasture-based livestock systems will be more affected by global warming than industrial livestock systems. The harsh weather conditions that animals are exposed to (intense heat waves, floods, and drought) can cause animal death in extreme cases, in addition to production losses (Gaughan and Cawdell-Smith, 2015).

Climate change is seen as a major threat to the survival of many species, ecosystems, and the sustainability of livestock production systems in many parts of the world (Moss et al., 2000). In this context, increasing temperatures cause reduced feed consumption, reproduction, and yield levels in different animal species. Furthermore, increased disease susceptibility in animals will result in mutations in disease and parasitic agents, an increase in zoonotic diseases, and the creation of some new diseases.

Impact of climate change on livestock

Animal disease

The effects of climate change on animal diseases depend on the geographical region, land use type, disease characteristics, and animal susceptibility (Thornton et al., 2009). Climate change has direct and indirect impacts on animal health. According to Grace et al. (2015), the direct effects of climate on animal disease are most likely to be significant for disease that are vector-borne, soil-associated, water- or flood-associated, rodent-associated, or air temperature, humidity- associated and climate-sensitive. According to Yatoo et al. (2012), direct impacts fallow more complex ways and include those resulting from animals attempts to adapt to thermal climate environments or from the influence of climate on microbial populations, vector-borne disease distribution, and host resistance to infectious agents, feed and water shortages, or food-borne diseases.

Climate change might impact disease vectors through a variety of methods and also affect the location and number of arthropod vector interaction. It can also increase animal interaction, improving the possibility of survival of its intermediate host and the rate of infectious disease transmission. However, they have a significant impact on the distribution and prevalence of vector-borne diseases.

Temperature and humidity limit the distributions of vectors. As a result of climate change, cooler regions previously too cold for specific vectors may begin to allow them to flourish. Warmer regions may become even warmer while remaining permissive to vectors if precipitation

or humidity levels rise; conversely, these regions may become less permissive to vectors if moisture levels remain constant or fall, resulting in an increase in moisture stress (Baylis and Githeko, 2006). For example, studies have shown that the hot-humid weather conditions were found to aggravate the infestation of cattle ticks like Boophilus microplus, Haemaphysals bispinosa, and Hyalomma anatolicum (kumar et al. 2004).

Higher temperatures and humidity encourage the growth of parasites and pathogens that spend part of their life cycle outside the host. Impact of climate change on the wind can affect the spread of pathogens. Besides that, flooding that fallow extreme climate events provides suitable conditions for many water-borne pathogens. Drought and desiccation, according to Grace et al. (2015), are damaging to most pathogens.

Some authors believe that when some livestock increase their range, they will be exposed to new pathogens and vectors, the results of which can be severe. Climate change, according to Thornton et al. (2009), may cause significant shifts in disease distribution, and outbreaks of severe disease may occur in previously unexposed animal populations (possibly with the breakdown of endemic stability).

Heat stress

Climate change is associated with increasing global temperatures. According to Nardone et al. (2010), the mean global temperature by the year 2100 may be 1.1- 6.4 °C warmer than in 2010. All livestock animals need a thermal comfort zone to provide for their physiological needs and throughout the day maintain a body temperature in the ±5 °C range (Henry et al., 2012). Lower and upper critical temperatures (-18 to +27) are the temperature levels at which livestock cannot compensate for the reduction in productivity and begin to be damaged. When temperature range is above the critical temperature (depending on the species), the livestock begin to suffer from heat stress (FAO, 1986). Heat stress depends on temperature, humidity, species, genetic potential, life stage, and nutritional status in livestock (Thornton et al., 2009). According to many authors, heat stress as part of the adaption process to climate change causes behavioral and metabolic changes in livestock, such as a decrease in feed consumption, deterioration in health, a decrease in reproductive efficiency and productivity, changes in many physiological functions, and susceptibility to disease (Lacetera et al., 2003; Nardone et al., 2010). Livestock animals are expected to be able to adapt to increasing temperatures as long as fundamental requirements like feed and water are supplied. Warm and humid conditions cause heat stress in livestock animals, which affects behavioral and metabolic variations and even mortality. Livestock in higher than latitudes will be more affected by the increase in temperatures than livestock located in lower latitudes are usually better adapt to high temperatures and droughts (Thorn et al., 2009). In addition, according to Rotter and Van de Geijn (1999), confined livestock production systems that have more control over climate exposure will be less affected by climate change.

Feed intake

Forage is a significant part of ruminants' diets and is essential for weight increase, production, and reproduction. Under heat stress, ruminants exhibit decreased feed intake, gut mortality, and rumination. Some studies have found that when ambient temperatures exceed 25-26°C, lactating dairy cows' feed intake decreases and this decrease accelerates above 30°C (Kadzere et al., 2002). Relative to other ruminants, goats are less resistant to heat stress. However, when the ambient temperature is more than 10 °C above their thermal comfort zone, their voluntary feed intake decreases (Lu, 1989). When exposed to extreme temperatures, poultry animals show lower feed consumption. When the ambient temperature increased from 21.1 to 32.2 °C from the post-hatch period to six weeks of age, poultry feed intake decreased by 9.5% (Syafwan et al., 2011).

Generally, lower feed intake caused by heat stress among all livestock species result in decreased milk, meat, and egg production, which in turn causes additional economic losses.

Water

Global agriculture uses 70% of freshwater resources, making it the world largest consumer. The livestock sector uses water for consumption by animals, growing feed, crops, and product processing (Thornton et al., 2009). Climate change is projected to change water availability (Barros et al., 2014; Masson et al., 2021) and water usage in animal production (Rojas et al., 2017). However, water shortages will probably become a bigger challenge for agriculture as a result of population growth and the consequent rise in demand for livestock products. Water availability issues will influence the livestock sector, which uses water for animal drinking, feed crops, and product processes (Thornton et al., 2009). Consequently, global water demand is moving towards increased competition due to water scarcity and depletion, where 64% of the world's population may live under water-stressful conditions by 2025 (Rosegrant et al., 2002).

Research on the effects of decreased water availability for land-based livestock systems due

to climate change is lacking (Thornton et al., 2009). Therefore, in the perspective of sustainable livestock production, it is important to take into account water availability and appropriate management strategies.

Animal reproduction and production

Extreme temperatures caused by climate change may have a negative impact on livestock production performance, reproductive, physiology, and metabolism, especially growth, meat, milk, and egg production in both sexes. Heat stress decreases the estrous period and fertility in female animals while raising the risk of anestrous and embryonic mortality (Cheng et al., 2021). Semen quality, testicular volume, and the number of viable sperm are all decreasing in male animals (Cheng et al., 2021). According to Nienaber et al. (1999), climate change, particularly global warming, can have an impact on livestock productivity and livestock production worldwide. More than half of the world's cattle population lives in the tropics, and heat stress can cause) economic losses in almost 60% of the world's dairy farm (Wolfenson et al., 2000).

According to the findings of numerous reproductive research; decreased fertility is because by the inability to recognize estrus, the initial insemination period is prolonged and the pregnancy rate decreases, due to the extreme temperatures, an increase in body temperature occurs, and accordingly the amount of blood coming to uterus decreases and the intrauterine temperature increases, decreased fertilization rate, slowed embryonic development, and increases early embryonic death (Lacetera et al., 2003).

Animal mortality

The mortality rate is one of the negative effects of heat stress that causes economic loss. It has been found by researchers on dairy cows and pigs that increased heat causes stress and mortality (Vitali et al., 2015; Ross et et al., 2015). Hot and humid weather has been found to be more life threatening to cows and hogs compared to hot but dry conditions, and a temperature higher than 37.7 °C with over 50% humidity was shown to be detrimental (Jeffrey et al., 2021). For poultry, they function normally up to an ambient temperature of 27°C or a body temperature of 41°C, but an increase of 4°C in body temperature would be lethal to them (Saeed et al., 2019). As a result, there is a need for integration in terms of developing genotypes with high adaptability and the ability to overcome stress, as well as improving soil and water management to reduce the potential effects of climate change (Thorne, 2007).

CONCLUSIONS

Climate change has many effects on livestock production systems. As a result, converting livestock production systems to sustainable systems will be one of the most essential solutions for mitigating the effects of climate change in the future. The study's overall results show that climate changes will have a negative impact on livestock production in terms of quality and quantity, resulting in economic losses (Klinedinst et al., 1993; St-Pierre et al., 2003; Seo and Mendelsohn, 2008; Mauge et al., 2015). Climate change will result in significant losses for producers at the micro level in the future. The livestock sector is faced with the challenge of providing enough animal protein to meet the needs of a growing global population while minimizing environmental impact. Because the energy needs of the rising population will increase, local and regional policies will be required to ensure food security in livestock production. The interaction of the components involved in these processes will be crucial in preventing the possible future consequences of climate change on livestock systems. To mitigate all of these negative effects, livestock production must be adapted to climate change. In this context, it is considered that, as in many other sectors, clear solutions and policies are required.

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HARNESSING PHYTOBIOTIC QUALITIES OF SCENT LEAF (OCIMUM GRATISSIMUM) MEAL AS SUBSTITUTE FOR IN-FEED ANTIBIOTICS IN BROILER PRODUCTION: IMPLICATION ON ECONOMIC TRAITS AND IMMUNE RESPONSES

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Abstract

Total of 150 unsexed day old broiler chicks comprising of 75 Arbor acre and 75 Cobb strains were used in an eight weeks experiment to determine the effects of Scent leaf (Ocimum gratissimum L) meal on growth performance and expression of immune genes in broiler chickens. Five experimental diets were formulated to include 0.00%, 0.50%, 1.00%, 1.50% and 2.00% of SLM, respectively referred to as treatments labeled T1, T2, T3, T4 and T5. The birds were randomly allotted to treatment groups of 15 chicks each in a completely randomized design (CRD). Data on feed intake and weekly weight were collected and used to calculate performance indices. Spleen tissues were collected from three birds per treatment from each strain for Interleukin1ß, Interluekin10, and MHC2 gene expression analysis using the Delta-Delta CT method. The results revealed significant differences (P<0.05) in the final body weight, weekly feed intake, weekly body weight gain and feed conversion ratio (FCR). The weekly body weight gain and FCR mean values ranged from 225.67g (T1)-262.39g (T3) and 2.15 (T3)-2.59 (T1) respectively. The birds fed 1.0% Ocimum gratissimum diet had the best average weekly weight gain and FCR. Gene expression on broiler spleen tissues were significantly affected by strain at both time points. Increased inclusion rate of the test ingredients significantly reduced IL1B, while increasing IL10. Conversely the MHC2-gene expression was more expressed on Abor acre spleen tissues than Cobb at 56 days. However, strain had no significant effects on growth performance characteristics except on percentage livability where Cobb was significantly better (99.62%) than Abor acre (98.52%). The result showed that the broilers fed experimental diet up to 1.50% performed significantly better than the control. Scent leaf meal diet had no detrimental effects on the broilers fed treated diet.

Key words: *Ocimum gratissimum, leaf meal, broiler chicks, performance characteristics, Gene Expression*

A HOLISTIC APPROACH TO THE EFFECTS OF INTRAUTERINE ANTIMICROBIAL THERAPY ON PREGNANCY RATE AFTER ARTIFICIAL INSEMINATION IN DAIRY COWS

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Abstract

Post-partum (pp) infertility following artificial insemination (AI) can be a very common (around 50%) phenomenon in high-yielding dairy cows kept under poor management and feeding shelters in particular. Even in clinically healthy females, intrauterine antimicrobial therapy (Lugol, Gentamicin, Rifaximin, etc.) may increase the fertility rate, especially in cows with latent (sub-clinical) intrauterine infection. Undoubtedly, modern animal husbandry requires reducing possible calving losses with a microbial origin that can prevent conception to occur and/or even terminate the ongoing pregnancy. In livestock farming/breeding, numerous obstacles (related to either the animals, humans, or else) would prevent achieving ultimate goals (regular reproductive cycle, insemination, pregnancy, calving, milking, and dry period) that allow the acceptable or minimum level of income (profit). For sustainable herd management, individual females have to be in good health that would be achieved by strict rules to provide optimum animal productivity at the animal welfare level. In this sense, a physiological heavy load of candidate mothers and their sustainable reproduction and milking requires at first good management and feeding practices. Beyond that, regarding the routine health services of subfertile dairy cows, a holistic approach is needed for efficient therapy and nutritional recovery during lactation. Otherwise, over this open period (until the new conception during the ongoing lactation), non-pregnant cows may not conceive and thus peculiar delays in conception would become inevitable. Undoubtedly, a holistic approach in the modern practice of farming/breeding animals requires effective management and feeding along with the provision of appropriate health services towards meeting animal welfare levels (sustainable high milk yield and regular calving annually). These ultimate sectoral targets would be easily achieved by providing optimum feeding, choosing the right individuals (age and breed) and working with dedicated care-takers and experienced Veterinarians. For the latter, the provision of health services should cover comprehensive factors including reproductive hormones, major vitamins-minerals, and efficient antimicrobials (systemic and/or local), as needed. Finally, numerous profit-limiting factors (climate changes, heat-stress, water and food scarcity, market prices, and residual problems) should also be dealt with carefully. Otherwise, undesirable outcomes (ovarian, uterine and mammary disorders) in dairy farming would become inevitable as commonly faced worldwide.

Key words: *Cow, Artificial insemination, Intrauterine treatment, Antimicrobial, Fertility*

SENSITIVE LIVESTOCK AND INFORMATION COMMUNICATION TECHNOLOGY APPLICATIONS TO PREVENT THE SPREAD OF COVID-19

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Abstract

The epidemic disease called COVID-19 (SARS-CoV-2) has affected the whole world. With the spread of the epidemic, various measures such as distance education, home-office, especially movement restrictions, have been tried to be taken. These measures have increased people´s demand for healthy food. The formation of food safety awareness in consumers has revealed the necessity of control of the food chain (production, storage, transportation of products, etc.). In this process, animal products gained importance, especially as people paid more attention to their nutrition compared to previous years. Especially in this process, animal production should be systematically sustainable in order to meet the increasing animal protein needs of people. In this review, it aims to compile sensitive livestock systems in order to ensure the sustainability of animal production, the production of healthier animals and the production of the obtained products within the framework of food safety rules, with the cessation of mobility due to the measures taken under quarantine and social distance in the COVID-19 epidemic. Thus, in addition to reducing the human workforce during the epidemic process, the data collected with modern animal husbandry will prevent diseases, and the diagnosis and treatment process in case of disease will be facilitated. With the use of information and communication technologies (ICT), which have an important place in this system, the data obtained through the modern livestock system can be easily processed, managed, and shared, thus reducing the possibility of disease transmission during the pandemic process.

Key words: *Animal production, COVID-19, Data, Artificial Intelligence, Sensitive livestock, Information communication technologies*

PERFORMANCE RESULTS OF 12-24 MONTH OLD IMPORTED BULLOCKS IN DIFFERENT HERD MANAGEMENT CONDITIONS

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Abstract

In Turkey, the red meat and milk demand are increasing day by day with the increasing human population. However, supply and demand projections show that will not meet the demand. In Turkey, the problem was tried to be solved through imports by reducing the customs duties, to meet the need for red meat and especially against the price increase problem that emerged in 2010. This study, it was aimed to reveal the farm fattening performances of crossbred cattle between the ages of 12-24 months imported in 2017. Within the scope of the study, businesses working with imported cattle were interviewed and the data of 5 businesses that agreed to share the data were evaluated. The ADWG values of imported crossbreeds obtained in many different places vary between 900 grams and 1600 grams on average. It has been determined that the enterprises that monitor, weigh animals, and ration arrangements among the enterprises included in the evaluation have achieved the highest daily live weight gain and can complete the fattening in a short time. The results of this study show that more efficient and more profitable fattening can be made with herd management practices that take into account issues such as weighing, grouping, and optimum fattening time of imported crossbreeds to meet the country's needs.

Key words: *Imported crossbreeds, fattening, farms, herd management, performance*

INTRODUCTION

Animal production is the most important source of adequate and balanced nutrition. Especially red meat is strategically important in a healthy diet due to its nutritional content. The global red meat industry is a developed industry. Countries meat trade; different resource structure of each country, meat consumer preferences and domestic industry structure determines. Low-cost meat producing countries has a competitive advantage in world trade. The high rate of price increase in red meat prices in Turkey, especially in 2010, has been tried to be reduced through imports. However, the imports did not yield the expected results. Beef carcass price in 2020, increased by 19.89% compared to 2019 The average was 35.75 TL in Turkey, the. In addition, beef consumer price 2019 while it was 45.64 TL/kg on average in 2020, It increased by 12.58 percent to an average of 51.38 TL/kg (TUIK, 2021). In 2019 17 million 872 thousand 331 cattle in the first six months of 2020 number of cattle increased by 4.2% to 18 reached a million 614 thousand 990 heads. For fattening, especially male animals that are in the appropriate growth period are preferred. However, there are differences between breeds as well as among male animals (Arpacık et al., 1994, Göncü, 2018; Göncü ve ark. 2019; 2018;Duru & Sak, 2017). Chambaz et al. (2003) reported that the differences in intramuscular adiposity at the end of fattening period with Sharole, Simmental and Limousin. Thonney (1987) compared the dry matter consumption of individually fed Angus, Hereford and Holstein castrated cattle, and found the highest dry matter consumption in Holsteins. In the same study, it is reported that Holsteins provide GCAAs on average 11% faster. According to TUIK data, the average carcass weight of cattle slaughtered in slaughterhouses, which was 269.5 kg in 2015, increased by 9.83% to 296 kg in 2019. In addition, the number of slaughtered cattle in 2019 increased by 6.1% compared to the previous year and reached 3,633,730 heads (TUIK, 2021).

However, the yield per animal is as critical as the number of animals. The breed, age, weight, size and health status of the animal affect the purchase and sale price of the Beside material in the market. There are no domestic beef cattle breed in Turkey. A significant part of the red meat produced is obtained from dairy or combined breeds. Animal production depends on environmental and genotype (Düzgüneş, 1976). Although the genotypic improvement is long-term, it gives permanent and continuous results (Foley et al., 1973; Düzgüneş, 1976). Crossbreeding studies for meat breeds in the world still continue intensively. Crossbreeding can be done to take advantage of the desired

characteristics and hybrid vigor of breeds. Genetic evaluations are made according to the performance results of the obtained crossbreeds all over the world. Many researcher reported that the breeds, diet, days on feed, environment and housing condition may affect the results obtained (Foley et al., 1973; Düzgüneş, 1976; Thonney, 1987; Chambaz et al. 2003; Barton et al, 2006; Cuvelier et al. 2006; Rich, 2016; Duru and Sak, 2017; Göncü ve ark. 2020a,b)

This study was carried out in Adana province in order to reveal performance results of imported crossbreeds between 12-24 months of age imported in 2017 at the herd management and animal tracking practices

MATERIALS AND METHODS

Within the scope of this study, interviews were conducted with 15 enterprises that were fattening imported bullocks in the province of Adana and the data of 5 enterprises that agreed to share the data were evaluated. Adana province with its climate characteristics, it has a hot and humid climate.

Adana province is located between 35°-38' latitudes and 34°- 46' east longitudes in the Mediterranean Region. It is one of the provinces with the highest average temperature in Turkey. The average annual temperature is 24°C in Adana. The hottest month of the year is August, with an average temperature: 32°C (Figure 1). These regions create stress conditions for animal husbandry. Stress factors also affect hormone levels and cause changes in yield levels. Temperature and humidity index (THI) values of lactating cow are reported as 72 (Armstrong, 1994) and as 84 for cattle in fattening (Göncü ve Özkütük, 2003).

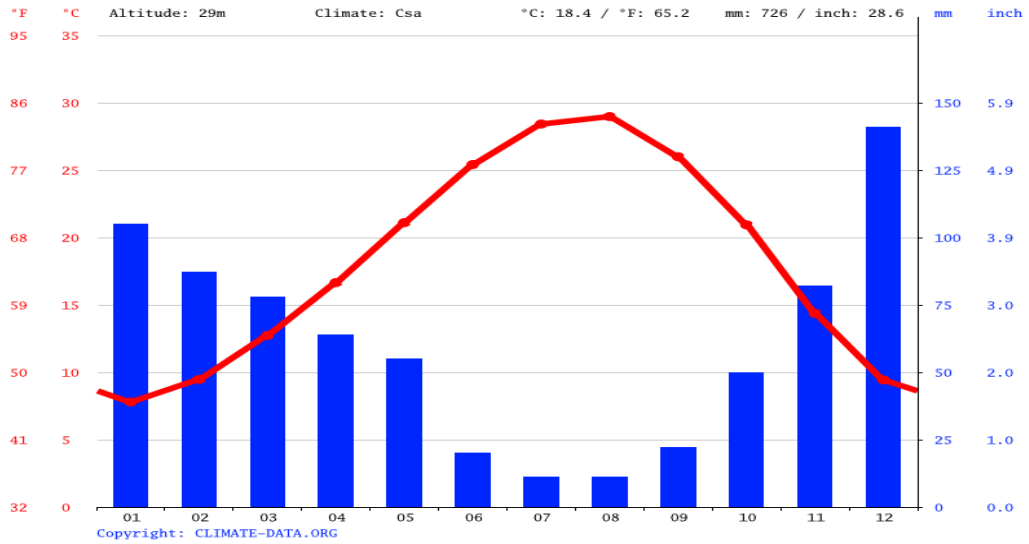

Figure 1. Temperature and humidity values of Adana according to months (anonymous, 2021)

Within the scope of this study, the information about the general conditions of the enterprises engaged in fattening with imported animals is summarized in Table 1.

Table 1. General characteristics of beef farms

Farms	1	2	3	4	5
Semi open barn	X	X	X	X	X
21 days quarantine	X	X	X	X	X
Vaccines	X	X	X	X	X
Parasite spraying	X	X	X	X	X
Forage and fattening feed separately	X		X		
TMR		X		X	X
Ration formulation		X	X		
Monthly weighing		X	X	X	
Carcass weight	X	X	X	X	X

Animals with livestock came to the farms in January and after 21 days of quarantine, they were fed with smallpox and foot-and-mouth vaccines and parasites. The herd management practices of the beef farms are summarized in Table 1. The average values of the beginning and the end of the fattening were calculated with the Excel program by taking the weighing results of the monthly weighing enterprises. On the other hand, in the farms that do not weigh monthly,

the weight per fattening was calculated from the farm weights and the weights at the end of the fattening and slaughterhouses. Daily live weight values were calculated by taking the sales body weight data of the enterprises at the beginning and end of the fattening. The obtained figures were analyzed using Excel and SPSS package program.

RESULTS AND DISCUSSION

The main purpose in cattle breeding is to make profit, as in all branches of production. However, a profitable and efficient cattle breeding requires knowing and managing the cost and cost elements that make up the cost in the business. In this sense, some values such as body weights at the beginning and end of fattening, daily body weight gain are important indicators. The findings that allow general evaluation of the enterprises included in the research are given in Table 2.

In the study, data of 478 animals from 5 farms were evaluated. In the farms, the live weight per fattening was 149 kg to 519 kg, the body weight at the end of the fattening was 362 kg to 745 kg, and the fattening period was 94-302 days. During this period, the average daily live weight gain was between 0.350 kg and 2.28 kg. Daily CAA remained between 0.752 and 1.581 kg for businesses. There are differences between businesses in terms of performance. The high difference in the weight of the animals at the beginning of the fattening, the grouping capacity of the holdings, the animal weighing and tracking levels changed the effect of these differences on the later performance.

Table 2. Performance data of 12-24 months old imported crossbred bulls in different farms

Beef farms	Animal number (head)	Fattening period (days)	Initial body weight (kg)	Final body weight (kg)	Daily weight gain (kg)
1	124	160	233,79 ± 60,60 (149-369)	486,81± 69,70 (362- 639)	1,581±0,080 (1,28-2,28)
2	70	94	393,91±4,43 (261-519)	484,80±5.00 (365-639)	1,011±26,76 (0,693-2,000)
3	94	124	281,69± 40,12 (242,31-322,51)	464,39±43,42 (423,58-510,02)	1,475±0,32 (1,155- 1,795)
4	68	250	332.94±8.06 (265.00-436.00)	566.64±10.11 (479.00 -745.00)	0.982±0.20 (0.70- 1.30)
5	112	302	308,50±27,98 (274,00-340,00)	535,04±47,38 (488,58 -582,02)	0.752±0.40 (0.350- 1.15)

It is understood that while the imported animals should be in the range of 200-300 kg on average, they lost weight due to unknown reasons during transportation and dropped to 149 kg. Findings showing that the transportation welfare criteria are not taken into consideration in the general structure of the animals coming to the enterprises have been determined. These problems both create stress and cause yield losses (Tarrant 1990). Heitschmidt (1982) determined that the transport shrinkage decreased to 0.77% in the first 3 hours of transportation and to 0.35% in the next 21 hours. Warriss et al. (1995) reported a loss of 3 to 11% in body weight of cattle in the first 24 hours of transplantation. It has been reported that in cattle breeding, animals with a lower body weight per fattening and/or younger have a higher feed efficiency and an increase in profitability in the enterprise due to the decrease in the amount of dry matter consumed for 1 kg of daily gain (Tüzmen 1995, İmik ve ark 2000, Cevger ve ark 2003).

In this study, the GCAA values of the 1st and 3rd enterprises were determined by Chambaz et al. (2003) reported 1,300 kg; Duru and Sak, (2017) reported 1,275 kg; Hollo et al. (2012)'s 1,240 kg and Barton et al (2006) 1,170 grams, Schoeaman, (1996) 1,629 kg and Cuvelier et al. (2006) was found to be less than 1.66 kg. The 2, 4 and 5 enterprises were lower than the reports of the researchers. Göncü et al (2019) reported that imported cross-bred bulls showed a daily live weight gain of 1,474, 1,583, 1,666 and 1,604 kg between January and July. Göncü ve ark. (2020a) breeds affected daily gain, final body weight and feed to gain ratio significantly ($P < 0.01$). Angus (1.5kg d-1) and Hereford 1.51kg d-1) had higher daily gain than Brangus (1.41kg d-1).

In general, it is understood that enterprises that can make ration regulation, weighing and grouping have better performance than others. It is an expected result that the fattening period of the farms with low GCAA is long and the fattening period of the farms with high GCAA is short. In addition, businesses that put their animals up for sale in the early period gain time for the fattening period to prepare for the next season and to use their resources for red meat production in the same year, to the extent that they can obtain animal material by the end of the year. The prolongation of the fattening period affects the fattening performance and thus the profitability in many respects. In the same period,

when the fattening period of the enterprises that buy fattening animals is extended

1. The extension of the food to the stressful summer months creates heat stress and the yields decrease
2. As the fattening gets longer, the expenses of the enterprise such as labor, electricity, water and fuel are added to the costs.
3. The number of fattening periods in a year is decreasing
4. The amount of carcass to be produced in a year will decrease
5. With the extended fattening period of the animal, the feed efficiency ratio will also deteriorate.

For this reason, it is very important for profitability to take into account the optimum times in fattening enterprises. The optimum end-of-fat weight is the point at which marginal revenue equals marginal expense. The economic optimum point is calculated as total income, total expense, total profit, marginal profit and average profit per animal at the end of each period. Making these calculations depends on the use of the weighing results in herd management. However, the weighing process, which will provide very important information, is not done for many reasons. Weighing is delayed due to reasons such as

1. Difficulty of the job
2. Employee injuries
3. Inappropriate weighing place
4. Taking a long time.

CONCLUSIONS

Within the scope of this study, it has been understood that it is higher by 1.475- 1.581 kg, compared to approximately 0.752-1.011 kg, in enterprises that keep records by weighing regularly by grouping, use the records in herd management decisions and make ration manipulations depending on the fattening period requirements. For this reason, it is clear that the use of technical knowledge by enterprises will make a significant difference in profitability in order to achieve the expected result in meeting the country's red meat demand.

ACKNOWLEDGEMENTS

This work was supported by the Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: ZRT.E2.16.002

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ECONOMIC EVALUATION OF MOHAIR PRODUCTION IN ANKARA PROVINCE

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Abstract

Angora goat is the most important goat breed that spread from Central Asia to Anatolia and became a part of Turkish culture. Angora goat, which is thought to have been brought to Anatolia in the 13th century, is intensively raised in the Central Anatolian region, especially in Ankara and its surroundings. In this study, it is aimed to give information about the distribution of the goat population according to Ankara province and its districts, the amount and price of mohair purchase by years, and the fiber quality of Angora goats raised in the region. According to the findings, it has been observed that there is an increase in the number of Angora goats in Ankara parallel with the total number of small ruminant in Turkey. When the farms that are members of Ankara Sheep and Goat Breeders' Association are examined, Ankara goat breeding is carried out in almost all districts of Ankara. Furthermore, when mohair prices are analyzed in dollars, it is determined that the highest price was in 2019, and the amount of subsidy given by the State decreased over the years. In terms of literature, we can say that there are not enough new studies on Angora goats and that up-to-date studies are needed. The fact that Ankara is suitable for goat breeding due to its geography and that goat breeding is important for those living in rural areas in cultural terms has ensured the continuity of Ankara Goat production. Although the goat population seems to be increasing in the last 10 years, there has been a serious decrease compared to the beginning of the 1900s. Necessary studies should be done properly in order to increase the Angora goat population.

Key words: *Ankara, mohair, Angora goat, price, quality*

INTRODUCTION

Angora goat is the most important goat breed that spread from Central Asia to Anatolia and became a part of Turkish culture. Angora goat, which is thought to have been brought to Anatolia in the 13th century, is intensively raised in the Central Anatolian region, especially in Ankara and its surroundings (Yanar and Akpınarlı, 2016).

Ankara, which is an important commercial transit point due to its geography, had an important place in the export income obtained from Angora goats, especially in the Ottoman period (Sen et al., 2015). However, in the last 50 years, the increase in migration from rural areas to the city, low quality mohair production and decrease in mohair income have caused a decrease in the interest in Angora goat breeding. Especially in recent years, crossing with Hair Goat, which has been done intensively and unconsciously, has adversely affected the production and quality of Mohair (Daskıran and Koluman, 2015).

The number of goats in our country has a share of approximately 20% in the sheep and goat population and has reached approximately 12 million with an increase of 47.67% in the last 10 years. The number of Angora goats has doubled in the last 10 years and is approximately 289 000. (TUIK 2021).

The Comminute-Based National Animal Breeding Project, carried out by the Ministry of Agriculture and Forestry and the Ankara Sheep and Goat Breeders' Association, had started breeding studies and financial support for dirty lint played an important role in this increase since 2005.

In this study, it is aimed to give information about the number of Angora goats in Ankara and its surrounding, the amount and price of mohair, and the fiber quality of Angora goats raised in Ankara.

MATERIALS AND METHODS

Ankara has 25 districts namely Altındağ, Çankaya, Mamak, Keçiören, Sincan, Yenimahalle, Akyurt, Beypazarı, Çamlıdere, Çubuk, Elmadağ, Etimesgut, Evren, Kazan, Gölbaşı, Bala, Ayaş, Güdül, Haymana, Kalecik, Kızılcahamam, Nallıhan, Polatlı, Pursaklar and Şereflikoçhisar. It is the second largest city of Turkey due to being the capital city and the immigration from nearby cities. The coordinates of Ankara are 39.57 N latitude and 32.53 E longitude. It has an area of

26,897 km2 and its altitude is approximately 890 meters (m).
Ankara has a mainly continental climate and has a large territory, so different climate characteristics can be seen. The steppe flora can be seen the north part of the city due to the climate effect of the Black Sea. But usually it has cold winters and dry summer. The annual temperature ranging from -25˚C to 40˚ C. Precipitation is between 300 mm and 540 mm and humidity is between 40-79%. (Anonymous a, 2020).

In the study, using the data of the herds registered to Ankara Sheep and Goat Breeders' Association, determinations were made regarding the number of Ankara Goats, the status of breeding by districts, the amount of mohair collected, the price of mohair and the quality of mohair. The dollar-based annual subsidy amount in the tables has been calculated according to the T.C. Central Bank's annual dollar rate.

RESULTS AND DISCUSSION

The number of Small Ruminants in Turkey

Table 1. Sheep and goat numbers by year (TURKEY)

	Domestic Sheep	Merino Crossbreed Sheep	Hair Goats	Angora Goats	Total
			(heads)		
2012	25 892 582	1 532 651	8 199 184	158 102	35 782 519
2013	27 485 166	1 799 081	9 059 259	166 289	38 509 795
2014	29 033 981	2 106 263	10 167 125	177 811	41 485 180
2015	29 302 358	2 205 576	10 210 338	205 828	41 924 100
2016	28 832 669	2 151 264	10 137 534	207 765	41 329 232
2017	31 257 408	2 420 228	10 419 027	215 645	44 312 308
2018	32 513 293	2 681 679	10 698 553	223 874	46 117 399
2019	34 199 467	3 076 583	10 964 374	241 055	48 481 479
2020	38 579 748	3 547 033	11 698 825	287 020	54 112 626
2021	41 182 899	3 994 791	12 051 957	289 557	57 519 204

(TUIK, 2020).
When the numbers of sheep and goats in Turkey are examined in Table 1, it is observed that the number of domestic sheep, Merino cross, Hair goat, Angora goat and total small ruminants increased by 78%, 59%, 160%, 46% and 83%, respectively.

Ankara Sheep and Goat Breeders' Association and The Number of Members

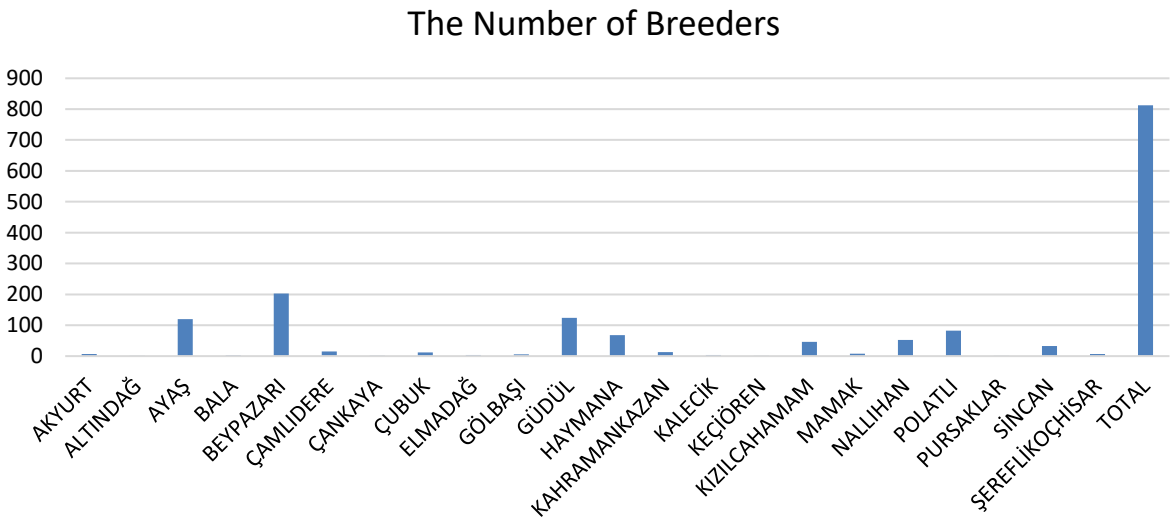

Figure 1: The number of members registered in Ankara Sheep and Goat Breeders' Association by regarding districts of Ankara (2021).

The districts with the highest number of breeders registered in Angora goat breeding in Ankara are Beypazarı (203), Güdül (124) and Ayaş (120), respectively. There are no registered breeders in

Etimesgut, Evren and Yenimahalle districts. The total number of herds registered to Ankara Sheep and Goat Breeders' Association is 812.

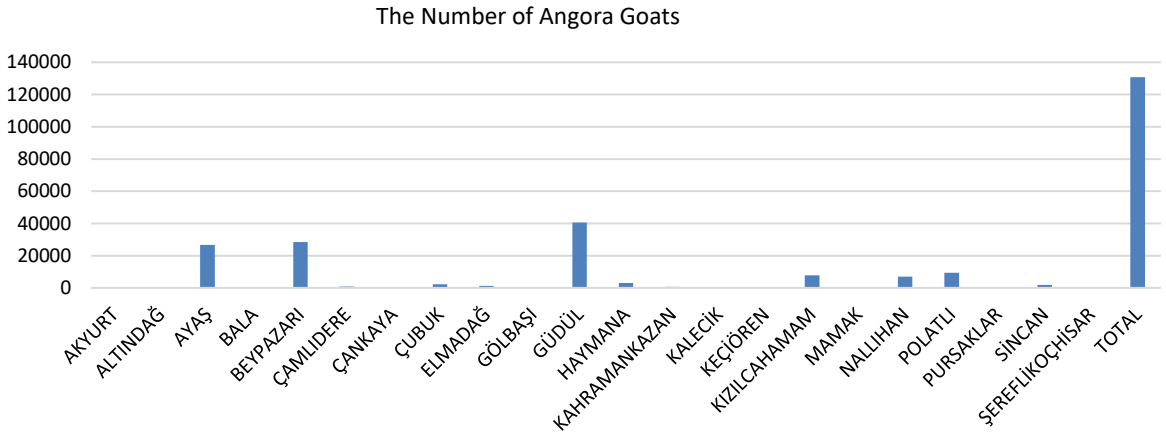

Figure 2: The number of district-based animals belonging to herds registered to Ankara Sheep and Goat Breeders Association (2021).

When we examine the districts in terms of the number of Ankara Goats, Güdül, Beypazarı and Ayaş districts are in the first three places with 40,615, 28,359 and 26,745 head animals, respectively. As seen in Figure 2, Ankara Angora Goat is raised in almost all districts.

Table 2: Kids, adults and un-classified dirty mohair prices of Ankara Sheep and Goat Breeders Association (ASGBA) and Mohair and Wool Agricultural Sales Cooperatives Union (MWASCU) by years.

	Kids kg/$		Adults kg/$		Unclassified Mohair kg/$	
Years	**ASGBA**	**MWASCU**	**ASGBA**	**MWASCU**	**ASGBA**	**MWASCU**
2015	None	3.73	None	2.98	None	0.74
2016	5.08	4.07	4.41	3.39	1.02	1.02
2017	5.10	4.25	4.25	3.68	0.85	0.85
2018	4.36	3.70	3.70	3.27	0.65	0.65
2019	8.91	6.00	7.54	5.14	0.86	0.86
2020	6.61	6.61	5.88	5.58	0.73	0.88
2021	5.88	5.88	5.29	5.29	0.59	0.71

(Anonymous b, 2021)

Until 2015, Mohair and Wool Agricultural Sales Cooperatives Union (MWASCU) was the only organization in our country for purchasing mohair. However, after 2015, Ankara Sheep and Goat Breeders Association (ASGBA) started to purchase mohair as of 2016, with the authorization given by the Ministry of Agriculture and Forestry. In our country, mohair subsidy is given if mohair is sold to the institutions authorized by the Ministry of Agriculture and Forestry. In addition, according to Article 4 of the Presidential Decree dated 5.11.2020, wool processing factories registered with the Ministry are allowed to collect mohair with receipt. Starting from 2020, farmers selling mohair to these factories are also included in the support.

It is seen that there has been an increase in mohair prices since 2016, with ASGBA starting to purchase mohair and also depending on the world markets. Table 2 shows that prices were the lowest in 2018 and the highest in 2019. Moreover, Table 2 shows that Kids mohair is a more preferred product due to its smaller micron diameter and accordingly, its price is higher than that of 2 years old Goat mohair. Unclassified mohair, on the other hand, is defined as dirty mohair with faces remaining on the underbelly and rear parts and finds buyers at a very low price.

Table 3: The amount of mohair collected by Ankara Sheep and Goat Breeders Association (ASGBA) by years

Year	**Kids (kg)**	**Adults (kg)**	**Unclassified Mohair (kg)**	**Total (kg)**
2016	4.483.86	12.333.70	430.20	17.247.76
2017	7.734.10	17.451.30	364.40	25.549.80
2018	14.328.80	29.478.00	946.4	44.753.20
2019	17.354.90	29.475.40	529.8	47.360.10
2020	13.440.50	56.006.00	1.845.50	71.292.00
2021	11.709.00	36.542.00	563.00	48.814.00

(Anonymous b, 2021)

The amount of mohair collected by Ankara Sheep and Goat Breeders Association (ASGBA) is presented in Table. According to this table, it is seen that the highest mohair was collected in 2020. All mohair (ASGBA) use OFDA 2000 device to sort them according to micron diameter and make them ready for sale.

Table 4: The amount of subsidy provided by the Republic of Turkey Ministry of Agriculture and Forestry for kids, adults and unclassified mohair.

Year	Kids kg/$	Adults kg/$	Unclassified mohair kg/$
2016	7.46	7.46	7.46
2017	7.65	7.65	7.65
2018	6.53	6.53	6.53
2019	5.14	4.80	3.43
2020	4.41	4.11	2.94
2021	4.11	3.52	2.58

(Anonymous b, 2021)

The amount of subsidy given by the Republic of Turkey Ministry of Agriculture and Forestry has decreased in dollar terms over the years. The subsidy model is as follows: Ankara Sheep and Goat Breeders Association (ASGBA) or Mohair and Wool Agricultural Sales Cooperatives Union (MWASCU) collects and invoices mohair from breeders. Farmers receive subsidy over the amount they produce according to these invoices.

According to previous studies, the average weight of dirty mohair for kids and adults are 1.42 kg and 3.62 kg, respectively. As can be seen in Table 5, according to previous studies, the average weight of dirty mohair is 1.42 kg and 3.62 kg, respectively, according to different age groups in kids and matriarch goats. Again, for the kids and adult groups, the mean fibre diameter was 25.36 μ and 39.81 μ, the mean elasticity was 27.35% and the mean strength was 45.26%, 10.16 g and 23.74 g, the mean medulla fibre ratio was 0.29%, 6.89%, the mean length was 62.9 mm and 176.3 mm, respectively. (Şen 2015, Öztürk and Goncagül 1994, Öztürk and Örkiz 1994, Bilgen, et al 2008, Erol, et al 2017 Vatansever and Akçapınar 2006). It is seen that the dirty mohair weight is the lowest in kids and the dirty mohair yield increases as the age increases. When we examine the studies, thinness increases as age increases.

The results vary depending on the age groups, elasticity, strength, medulla fibre ratio and length. Considering these differences between studies, it can be thought that the wide variation in Angora goats causes differences in quality characteristics.

Table 5: Previous studies on Mohair characteristics.

Year	Sex	Dirty Mohair (kg)	Diameter (μ)	Elasticity (%)	Strength (g)	Medulla fibre ratio (%)	Length (mm)	Reference
Kid	Male	-	25.36	43.25	12.27	6.89		Şen (2015)
	Female		26.55	45.26	14.31	5.82		
1,2,3 year	Male	1.62, 2.98, 3.13	30.03, 32.34, 34.72	29.25, 27.50, 27.35	14.12, 23.45, 23.74	0.39, 0.65, 2.58		Öztürk and Goncagül (1994)
1,2,3 year	Female	1.42, 2.55, 2.69	30.10, 31.34, 34.21	30.56, 31.34, 34.21	14.38, 23.63,22.68	0.29, 0.48, 0.28		
>2 year	Female	3.67, 3.41	38.41, 39.81	29.68, 29.98	20.82,21.78	0.50, 0.53	176.3, 175.5	Öztürk and Örkiz (1994)
1,2,3,4,5,6 X±	Female	2,67	34.64	38.92	10.16	-	62.9	Bilgen, et al (2008)
2,3,4,5,6 X±	Female	3.11	37.98	37.78	10.89	1.50	64.9	Vatansever and Akçapınar (2006)
1,2,3,4,5 X±	Female	2.26, 2.18, 1.81	37.15	40.15	20.24	-	73.5, 111.2	Erol, et al (2017)

CONCLUSIONS

The fact that Ankara is suitable for goat breeding due to its geographical structure and the cultural importance of goat breeding for those living in rural areas has ensured the continuation of Ankara goat production. Although the goat population seems to be increasing in the last 10 years, there has been a serious decrease compared to the beginning of the 1900s. It is essential that the necessary

studies be done consciously in order to increase the current number. It is thought that the price of mohair and subsidy, which has decreased in dollar over the years, will weaken the production. For this reason, the Ministry of Agriculture and Forestry, the Associations and the Cooperatives should determine the improvement and subsidy policy of the next 10 years and carry out studies that will make Angora goats more attractive for breeding.

ACKNOWLEDGEMENTS

I would like to thank the Ankara Sheep and Goat Breeders Association for their contribution to this study.

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ADVANCES IN SMALL RUMINANT GENETICS AND THEIR IMPLEMENTATION INTO FRENCH MEAT AND DAIRY SELECTION SCHEMES

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Abstract

The implementation of new tools and in particular the genomics ones will be illustrated by some examples in the dairy goats, dairy sheep and meat sheep French industries. Prolificacy genes, muscularity genes, disease resistance genes (scrapie, mastitis, gastro-intestinal parasitism) as well as pangenomics chips or livestock precision tools will be highlighted. Impacts on different kind of selection schemes (parental testing, individual testing, progeny testing) will be reviewed.

Key words: *Animal genetics, small ruminant, sheep, goats, selection, genomics*

STUDY OF EXTERNAL AND INTERNAL PARASITIC INFESTATION IN ORNAMENTAL FISH FARMS IN ALBORZ PROVINCE, IRAN

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Abstract

The global ornamental fish trade is a rapidly growing industry. Ornamental fish constitute an extremely large segment of the pet animal industry. Although this worldwide interest in ornamental fish has led to developments in their culture techniques there are still many difficult-to-culture species with high demand. Cultivation and propagation of ornamental fishes have been increasing in the last 30 years in Iran. Alborz province is one of the main sources of culture and propagation of ornamental fish in Iran. The aim of the present study is an investigation of parasitic infestation of ornamental fish farms that have reported deaths in different fish species. The research was conducted for twelve months from July 2021 to July 2022 on 6 farms and 7 species of ornamental fish referred. Fish were packed in water-filled plastic bags, supplied with oxygen, and transported to the Ornamental Fish Clinic, Faculty of Veterinary Medicine, University of Tehran. 7 ornamental fish species including, Poecilia reticulata, Carassius auratus, Pterophyllum scalare, Danio rerio, Cyprinus carpio koi, Andinoacara rivulatus and astronotus ocellatus were sampled. Wet smears of skin and gills were prepared and observed by light microscopy (E600, Nikon). Then necropsy was performed and fish were examined for internal. In the results, external parasites including Trichodina sp., Gyrodactylus sp, Lernaea sp, Ichthyobodo sp., Dactylogyrus sp., Ichthyophthirius multifiliis and internal parasites including Capillaria sp. and Hexamita sp. were detected. The highest prevalence of parasites was related to Capillaria sp. (40%). and the lowest prevalence was related to Lernaea (5%).

Key words: *Internal parasite, External parasite, ornamental fish*

INTRODUCTION

The global ornamental fish trade is a rapidly growing industry. Aquarium fish constitute an extremely large segment of the pet animal industry (Winfree, 1989; Noga, 2010). Although this worldwide interest in ornamental fish has led to developments in their culture techniques (Wilson et al., 2001) there are still many difficult-to-culture species with high demand. Cultivation and propagation of ornamental fishes have been increasing in the last 20 years in Iran. More than 150 species of fresh water ornamental fishes are farmed in Iran.

The estimated annual income from the sale of ornamental fish in the world was US$ 900 million. In the past few years farming of ornamental fish has been well developed in Iran. Some cases of propagation have been reported in Iran, but some of them have been imported to the country. To the best knowledge of the authors there are few studies on diseases of ornamental fish and causes of fatality that were reported in Iran and very little research has been done on the helminth parasites of freshwater ornamental fish in Iran. The objective of this study was to survey the infestation of ornamental fish with internal and external parasites in Alborz province of Iran.

MATERIALS AND METHODS

The research was conducted for twelve months from July 2021 to July 2022 on 6 farms and 7 species of ornamental fish from alborborz province, Iran. Fish were selected randomly. Fish were packed in water-filled plastic bags, supplied with oxygen, and transported to the Ornamental Fish Clinic, Faculty of Veterinary Medicine, University of Tehran. 7 ornamental fish species including*, Poecilia reticulata*, *Carassius auratus*, *Pterophyllum scalare*, *Danio rerio*, *Cyprinus carpio koi, Andinoacara rivulatus* and *astronotus ocellatus* were sampled. Then fish were sampled. In this atudy wet smears of skin and gills were prepared and observed by light microscopy (E600, Nikon). Then necropsy was performed and fish were examined for internal parasites. Whole gastrointestinal tract were disected and studied under light microscopy (E600, Nikon). Photograpgy was performed by UI2250 IDS imaging microscope camera, when parasites observed.

RESULTS AND DISCUSSION

140 fish from 7 species were studied. In total, 87 out of 100 examined fishes (equivalent to 87%) were infected with parasites and the rest had no parasite infestation. External parasites including *Trichodina sp. (6%)*, *Gyrodactylus sp. (7%)*, *Lernaea sp. (5%)*, *Ichthyobodo sp. (6%)*, *Dactylogyrus sp. (6%)*, *Ichthyophthirius multifiliis* (10%) and internal parasites including *Capillaria sp. (40%)* and *Hexamita sp*. (7%) were detected. The highest prevalence of parasites was related to *Capillaria sp.* (40%). and the lowest prevalence was related to *Lernaea* (5%). Parasitic diseases cause economic losses to the ornamental fish industry by affecting physiological and biological characteristics and causing mechanical damage (Jalali, 1997 and 1998, Adel et al., 2015).

In this study, we found a total of 8 parasite species among 7 ornamental fish species.

A significant part of the world trade of aquatic animals is the trade of tropical aquarium fish (Evans and Lester, 2001). Therefore, Quarantine measures should be implemented during the trade of live animals, including aquatic animals, to prevent the transmission of pathogens, which lead to disease outbreaks and economic losses The risk of parasites entering other countries and their further spread in the future is minimized by treating infected fish before export or after entering the importing country (Koyuncu, 2009).

In this study, the prevalence of crustaceans was low, which could be since the life cycle of these parasites can reach up to 3 months depending on the water temperature and the duration of keeping most of the common species of commercial ornamental fish on farms, before selling, it is not more than 2 to 3 months and therefore they have less opportunity to be contaminated with crustaceans (Rahmati-Holasoo et al., 2022).

Some nematodes, such as *Capillaria sp.*, have a direct life cycle and do not require an intermediate host that can be problematic (Wildgoose 2011), as in this study *Capillaria sp.* accounted for the highest rate of parasitic involvement. *Capillaria* sp. has been reported previously by Rahmati-holasoo et al., 2010) that cause severe mortality in ornamental fish. Severe infestiaon with C*apillaria* sp. in this study shows that C*apillaria* sp. can be a major risk for culture of ortnamental fish.

ACKNOWLEDGEMENTS

Mr. Mahmoodi is thanked for his assistance.

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THE ZOOTECHNICAL PERFORMANCE OF THE SEVENTH GENERATION BLACK SEA SALMON IN RECIRCULATING AQUACULTURE SYSTEM

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Abstract

This study was conducted to determine the zootechnical performance of seventh generation (F7) of Black Sea salmon (Salmo labrax PALLAS, 1814) as latest culture line produced at freshwater recirculating aquaculture system. The fish used in the study were about one month old and weighed an average of 0.14 g. After larvae consumed their yolk sac, the study was started. The trial study lasted for 13 months. Fish were fed by hand with commercial rainbow trout feeds at different sizes up to apparent satiation. As the fish grew, the condition factor and feed intake increased. The growth rate increased progressively in the first 6 months, and then gradually decreased in the following months. The feed conversion ratio varied between 0.77-1.08 during the trial. At the end of trial, survival rate was about 84.12%. The results showed that the seventh generation (F7) of Black Sea salmon, including the incubation stage, could be successfully reared up to smolt stage at freshwater RAS. However, for a profitable and sustainable aquaculture, transferring Black Sea salmon to sea cages at the smolt stage will provide an important advantage especially in portion size production.

Key words: *Aquaculture, fish, RAS, nutrition, growth*

INTRODUCTION

Black Sea salmon (*Salmo labrax* PALLAS, 1814) from the brown trout family is anadromous characteristics and an endemic species of Türkiye. Its natural distribution area is the Black Sea and the rivers pouring into it. This species are preferred in aquaculture due to their beautiful appearance, taste and high economic value in our country. Also, Black Sea salmon is listed as an endangered species according to several local Black Sea countries' databases (Çakmak et al. 2022a; Çakmak et al. 2022b). Black Sea salmon is a species that shows carnivorous feeding habits. The food groups of this species in nature consist of 6 taxonomic living groups: aquatic insects, mollusks, crustaceans, maggots, spiders and fish. Molluscs and maggots are the dominant food groups in the lake environment, aquatic insects in the river environment, and crustaceans and fish in the marine environment (Tabak et al. 2001). Black Sea salmon are grown to different sizes in culture conditions by using three different systems including stream, dam lake and marine cages. Production in stream covers production from egg stage to smolt length (12 cm), portion weight (200-300g) or pre-fillet weight (>600g). In dam lake production, fish reached 2-30g in stream production are grown to portion weight, pre-fillet weight or fillet weight (>3000g) by transferring to dam lake net cage systems. In the production of marine cages, fish grown from egg stage to smolt size, portion weight or pre-fillet weight in stream or dam lake production systems are transported to sea net cage systems and grown to portion weight or fillet weight (Özel et al. 2022).

In addition to the suitable ground for cultured fish, the amount and quality of water is considered one of the most important problems faced by aquaculture (Al-Dubakel et al. 2011). Recirculating aquaculture systems (RAS) are land-based aquatic systems that allow reuse water after mechanical and biological treatment in order to decrease water and energy consumption and release of nutrients to the environment (Zhang et al. 2011). Water recirculated in RAS remove or convert to non-toxic forms of toxic nitrogenous metabolites via bioreactors (Prabhu et al. 2017). This system, which is used in the presence of limited water and ensures that 90-99% of the water is recycled, are a technological application developed for intensive fish farming (Badiola et al. 2012). RAS compared to flow through systems has some advantages such as minimal exposure to challenging conditions, better control of the growing medium and less water consumption (Timmerhus et al. 2021).

This study aimed to monitor the one-year growth course of the 7th generation Black Sea salmon obtained by selective breeding studies in RAS.

MATERIALS AND METHODS

This study was used seventh generation (F7) of Black Sea salmon as latest culture line produced by Central Fisheries Research Institute in 2019-2020 breeding season. After an incubation period of approximately 40 days at average 10 °C at freshwater recirculating aquaculture systems (RAS), larvae emerged from eggs obtained from stripping broodstocks. Trial study was started in the 3rd week of January after larvae consumed their yolk sac. In this study, fish with initial weights of 0.14±0.00g obtained by culture activities at RAS was used. Fish were placed randomly in 400 L (100x100 cm square with depths 40 cm) fiberglass tanks. During the trial study, the stock density was adjusted to be 15kg/m^3. The study was performed as triplicate, and lasted for 13 months. Water temperature, pH (7.42±029) and oxygen (8.55±0.38) were recorded three times a day. Ammonia (0.06±0.05) was measured weekly. Since egg incubation continued in the RAS during trial study, the water temperature was kept at 10.12±1.28 °C until the end of March. Then it was increased gradually over one month and fixed at 15.45±0.99 °C. Water change in tanks was 20 times in a day. Trial tanks were cleaned by siphoning daily.

Feeding procedure was begin with 300-500 µ granule feed. Pellet-size progressively increased to 500-800 µ 800-1200 µ, 1.5 mm, 2 mm, 3 mm, 4 mm and 5 mm as the fish grew. The fish were initially fed five times a day for the first 4 months, and then the number of meals per day was fixed at 3 until the end of trial. Commercial rainbow trout feeds were used in this study. Fish were fed up to apparent satiation during the trial period.

For performance evaluation, fish were measured monthly. For this, fish were starved for 24 hr, slightly anesthetised with benzocaine (50 ppm). Length measurement was performed using Von-Bayer scale and weight was measured using digital scales with an accuracy of 0.01g (Holden and Reitt, 1974). Fish performance was determined using equations shown below.

Condution factor (CF) = 100 × [(weight ÷ (lenght3)]

Weight gain (WG) = (final weight – initial weight)

Spesific growth rate (SGR) = 100 × [(ln final weight – ln initial weight) ÷ days]

Feed conversion ratio (FCR) = (feed intake ÷ weight gain)

Survival rate (SR) = 100 × [(final number of fish ÷ initial number of fish]

Data were analyzed by one-way analysis of variance (ANOVA) procedure of SPSS 14.0. Statistical differences were determined by Duncan's multiple comparison test. Results are presented as means ± standard errors. Probability levels of $p<0.05$ were chosen for statistical significance.

RESULTS AND DISCUSSION

Black Sea salmon larvae completely consumes their yolk sac until 30th day after the hatching (Cankırılıgil et al. 2016). A similar trend was observed in this study, which showed that this consumption lasted for up to 32 days. However, the first feeding after the hatching in Black Sea salmon can be make when the larvae consume 60-70% of the egg yolk (SUMAE, 2010). In this study, the first feeding to fish was carried out on the 25th day after hatching. After the larvae consumed their yolk sac, growth and development began increasing with the first feed intake. As the fish grew, the required feed consumption increased in quantity. While the specific growth rate increased progressively in the first 6 months of the experimental, it gradually decreased in the following months. The fish reached the necessary weight for transfer to marine cages about 9 months after emerged from eggs. The condition factor, which was 0.87 at the beginning, reached 1.30 at the end of the experimental. Besides survival rate changed between 97.26% and 84.12% (Table 1). Daylight has a significant effect on the nutritional intake of Black Sea salmon in the natural environment. This species enters an intense feeding period with sunrise and feeding continues throughout the day (Tabak et al. 2001). In the cultivation of this species, the first feeding can be done when the daylight becomes evident, or it can be done after a few hours. This situation is closely related to the experience of the feeding staff. The first feeding of the day is an important indicator for subsequent meals. Since the effect of social interaction on the feed intake of Black Sea salmon is very important, the behavior of the fish should be followed while being feed. Since it is not possible to address the need for feed of each fish as they occur, the feeding program should be regular and stable. Tabak et al. (2001) reported that Black Sea salmon, which can adapt to the Black Sea salinity, should be at least 12 cm in length and 15 g in weight. Çakmak et al. (2018) reported that the fifth culture generation of Black Sea salmon transferred to the marine cages in the 10th month (12.67±0.69 cm and 19.84±3.16 g) after the hatching. In this study, the transfer of Black Sea salmon (F7) to seawater occured at 9th month after the hatching. Atlantic salmon smolt can be reared in the RAS for longer periods prior to marine cages transfer (Timmerhus et al. 2021). However, Atlantic salmon raised in RAS have a lower weight compared to those raised in marine cage

(Yanfeng et al. 2019). In a previous study, Çakmak et al. (2018) reported that Black Sea Salmon transferred to marine cages at weight of 19.84 g reached an average of 196.63 g after 4 months. In this study, Black Sea salmon smolts reared in RAS remained at an average of 104.05 g the end of same rearing time. Besides, Martinez et al. (2020) reported that Atlantic salmon had a slow growth in the first 5 months of rearing in the RAS, but after 5th months, the fish began to grow. Çakmak et al. (2018) found that the fifth culture generation of Black Sea salmon (about one month old) with an initial weight of 0.11 g reared at freshwater RAS reached a weight of 1.09 g after 4 months. A similar result was seen in this study. In addition, unlike this study, Kolarevic et al. (2014) also reported that Atlantic salmon reared in freshwater RAS reached from 7.1 g to 93.9 g in about 3 months, and had average 2.41 SGR. This may be due to environmental factors, feeding programme or breeding practices. Besides, FCR in Altantic salmon grown in different temperature in freshwater recirculating aquaculture system was reported as average 1.11-1.15 and 0.81-1.01 by Crouse et al. (2022) and Ignatz et al. (2020). Çakmak et al. (2018) found that SGR and FCR in Black Sea salmon (F5 generation) in freshwater recirculating aquaculture system were determined as between 0.98-2.70 and 1.02-1.30 until smolt stage. This study was demonstrated that SGR and FCR were 1.15-2.44 and 0.73-1.08. This difference in FCR and SGR may be due to water quality of RAS, fillial generation, nutrient content and composition of the feed, feeding programme and stock density.

Table 1. The growth performance of Black Sea salmon

Months*	BL (cm)	BW (g)	CF	FI (g)	FCR	WG (g)	SGR (%)	SR (%)
Initial-January	2.50±0.06^{o}	0.14±0.00^{k}	0.87±0.07^{d}	--	--	--	--	--
1st – February	3.03±0.03^{n}	0.25±0.00^{k}	0.91±0.01^{d}	0.09±0.00^{k}	0.98±0.02^{b}	0.11±0.00^{k}	1.99±0.03^{d}	97.26±0.16^{a}
2nd – March	3.53±0.03^{m}	0.45±0.00^{k}	0.90±0.02^{d}	0.28±0.00^{k}	1.08±0.03^{a}	0.32±0.00^{k}	2.01±0.03^{d}	93.10±0.02^{b}
3th – April	4.47±0.03^{l}	0.86±0.01^{k}	0.95±0.01^{d}	0.67±0.00^{k}	1.03±0.03^{ab}	0.72±0.02^{k}	2.05±0.04^{cd}	91.55±0.14^{c}
4th – May	5.23±0.01^{k}	1.70±0.02^{k}	1.16±0.02^{c}	1.47±0.00^{k}	0.99±0.02^{b}	1.56±0.02^{k}	2.11±0.03^{c}	89.69±0.08^{d}
5th – June	6.85±0.00^{i}	3.92±0.01^{i}	1.18±0.01^{bc}	2.96±0.01^{i}	0.80±0.00^{de}	3.79±0.00^{i}	2.25±0.01^{b}	89.16±0.20^{e}
6th – July	8.82±0.03^{h}	8.19±0.01^{h}	1.17±0.01^{bc}	5.14±0.06^{h}	0.77±0.04^{ef}	8.05±0.01^{h}	2.44±0.01^{a}	88.72±0.19^{e}
7th – August	10.34±0.04^{g}	12.84±0.56^{g}	1.16±0.02^{bc}	9.59±0.05^{g}	0.82±0.03^{de}	12.70±0.57^{g}	1.96±0.07^{d}	88.16±0.21^{f}
8th – September	11.60±0.01^{f}	18.72±0.10^{f}	1.17±0.01^{bc}	13.23±0.12^{f}	0.73±0.00^{f}	18.58±0.10^{f}	1.73±0.01^{e}	87.37±0.06^{g}
9th – October	13.57±0.09^{e}	30.17±0.46^{e}	1.28±0.01^{a}	25.82±1.00^{e}	0.88±0.05^{cd}	30.04±0.45^{e}	1.56±0.05^{f}	87.22±0.08^{g}
10th – November	15.20±0.05^{d}	40.24±0.14^{d}	1.16±0.00^{bc}	36.76±0.29^{d}	0.94±0.01^{bc}	40.11±0.14^{d}	1.26±0.00^{g}	86.91±0.12^{g}
11th – December	16.43±0.04^{c}	58.11±0.58^{c}	1.21±0.02^{b}	52.62±0.52^{c}	0.97±0.00^{b}	57.98±0.59^{c}	1.25±0.01^{g}	85.05±0.10^{h}
12th – January	18.53±0.08^{b}	80.08±0.61^{b}	1.26±0.02^{a}	77.64±0.92^{b}	1.03±0.03^{ab}	79.83±0.62^{b}	1.21±0.01^{gh}	84.66±0.15^{h}
13th – February	20.06±0.06^{a}	105.04±1.42^{a}	1.30±0.03^{a}	102.34±0.97^{a}	0.95±0.03^{bc}	104.59±1.42^{a}	1.15±0.01^{gh}	84.12±0.27^{i}
P values	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Mean values in a column with different superscripts were significantly different. Values are given as means with standard errors (n=50). BL: Body length, BW: Body weight, CF: Condition factor, FI: Feed intake, FCR: Feed conversion ratio, WG: Weight gain, SGR: Spesific growth rate, SR: Survival rate.
*Measurements were made on the 22nd days of each month

CONCLUSIONS

According to the results of this study, RAS can provide an important advantages in growth up to smolt size if the target in business management is portion size fish production. Black Sea has suitable aquaculture conditions for Black Sea salmon only in October-June period. In addition, Black Sea salmon at RAS conditions (including the incubation stage) exceeds smolt size until October. After this stage, the fish transferred to the marine cage units can be successfully grown up to portion size in the same production period.

ACKNOWLEDGEMENTS

The present work, supported by General Directorate of Agricultural Research and Policies (TAGEM/HAYSUD/2017/A11/P-01/3), was performed within the project named "Investigation of possibilities of using some phytobiotic added feeds in feeding of Black Sea salmon"

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THE EFFECTS OF PARTIALLY SLATTED FLOOR DESIGNS ON SOME EARLY BEHAVIORAL TRAITS IN BROILER CHICKS

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Abstract

Chicken meat is an essential source of animal protein in the adequate, balanced, and healthy diet of humans. An intensive system is widely used to meet increasing consumer demands, and broiler chickens are generally reared on litter material. In broiler farming, interest in alternative floor systems has increased as a solution to the problems related to animal health and welfare that arise when litter management is not sufficient. Although cage and slatted floor applications have been known for many years, they have not become widespread due to their undesirable effects on welfare, foot-leg health, and some behavioral characteristics. This study was carried out to determine the effects of different levels of slatted floor applications on some early behavioral characteristics of broiler chickens. Thus, it is aimed to minimize the negativities arising from both systems by combining the littered and slatted floor systems and to reveal the advantages of these systems by using them together. In this study, male-female mixed 600 fast-growing broiler chicks (Ross308) at d-old age were used. The experiment consisted of five treatment groups: fully littered, fully slatted, ½ littered+½ slatted, 1/3 littered+2/3 slatted, 2/3 littered+1/3 slatted. A total of 120 d-old chicks were weighed and randomly distributed to each treatment group, and 24 chicks (6 chicks/m2) were placed in each pen. Each treatment consisted of 5 replicates (5 pens; 25 pens in total) and wire mesh pens with a floor area of 4 m2 in each replicate. In the study, the feeding, drinking, resting, other behaviors, aggregation behaviors, and slatted floor preferences were evaluated three times a day (at 9.00, 13.00, and 17.00) of the chicks in each treatment group at the 2, 5, 9 and 11 days of age. Each behavioral trait was expressed as a percentage of the total number of chicks showing the relevant behavior at the pen level. After arcsin square root transformation to percentage data, statistical analysis was done with SPSS 21.0 statistical software. However, actual averages were used to interpret the traits. Different floor design practices significantly affected the chicks´ feeding, resting, aggregation behavior, and preference for being on the slatted floor (P<0.05). Feeding behavior was higher in chicks reared on the fully slatted floor than in the other groups (P<0.001). The percentage of chicks showing resting behavior was highest in the 2/3 littered+1/3 slatted floor application (P=0.001). Aggregation behavior was higher in chicks reared on a fully litter and 1/3 slatted floor (P=0.024). The 64.42% of the chicks reared in 2/3 slatted, 47.53% of those reared in ½ slatted, and 36.38% of those raised in 1/3 slatted preferred the use of the slatted floor. In addition, it was determined that age significantly affected the behavioral characteristics of chicks in the early period (P<0.05). The percentage of chicks showing feeding behavior was highest at 5 (16.12%) and 2 d-old (15.73%) (P=0.001). The drinking behavior was found lowest at 2 (4.70%) and 5 (6.95%) days of age (P<0.001). Resting behavior was highest at 2 (78.72%), 5 (76.89%), and 9 (72.82%) days of age (P<0.001). Aggregation behavior was the highest at 2 (18.35%) and lowest at 11 days of age (2.56%). In conclusion, this study revealed that different floor designs affect some behavioral characteristics in the early chick period. Since it is known that early rearing conditions affect later performance in broilers, slatted floor systems with higher feeding behavior can be an effective tool for better performance in broiler production.

Key words: *Broiler chick, Slatted floor, Litter, Behavior, Feeding*

HAPLOTYPE DIVERSITY OF PARTIAL 16s-rRNA GENE IN AFGHAN TORRENT (Amolops afghanus) AND GREEN CASCADE (Odorrana livida) FROGS AT INDO-CHINA REGIONS: A META-ANALYSIS STUDY

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Abstract

Afghan Torrent (Amolops afghanus) and Green Cascade (Odorrana livida) frogs are endemic species at Indo-China region. This study was aimed to observe the haplotype diversity of partial 16s-rRNA gene in Afghan Torrent (AT) and Green Cascade (GC) frogs with a meta-analysis study. A total of 36 16s-rRNA gene sequences (23 AT and 13 GC) were collected from GenBank database for the sequence analysis. Thus, along 691 bp (AT) and 956 bp (GC) of 16s-rRNA sequence were observed in this study to detect the point mutations. Four molecular softwares of BioEdit, MEGA-X DNAsp and ARLEQUINE were used for the sequence analysis. Research showed that two point mutations (AT frog) and seven point mutations (GC frog) were occured in this study. Three haplotypes (Hap.) of 16s-rRNA gene were detected with the Hap.1 as the common haplotype in both frogs species. The haplotype diversity (Hd) in both frogs species were included of moderate category (0.30 - 0.50). The neutrality test (Fu's Fs statistic and Tajima's D value) revealed that the species expasion was occured in AT frogs frog. In addition, about 9% of the geographical factor was influenced to the sequence variation in AT frogs. It can be concluded that AT and GC frogs can be characterized based on 16s-rRNA gene sequence variation.

Key words: *Amolops afghanus, Odorrana livida, 16s-rRNA gene, Mitochondria, Haplotype*

FACTORS AFFECTING FARMERS PREFERENCES WHILE BUYING TRACTORS IN DETERMINED NEIGHBORHOODS IN DULKADİROĞLU DISTRICT OF KAHRAMANMARAŞ PROVINCE.

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Abstract

This study was carried out to determine the factors affecting the tractor selection and purchasing behavior of farmers in Kahramanmaraş region. In the study, pre-prepared questionnaire forms were filled in by face-to-face interviews with randomly selected farmers in the districts determined from the central district, Dulkadiroğlu district, and according to the results of the study; KMO=0.748 and 3 factors were determined. These factors were collected at 96,148 of the variance. In addition, since the Bartlett test result was p= 0.000<0.05, it was determined that there was a relationship between the factors determined with the help of the questionnaire while the farmers were purchasing tractors (H0 hypothesis was rejected).
As a result of the answers given by the farmers who were included in the survey, there are 73,33% single-wheel drive tractors and 26,67% double-wheel drive tractors in the neighborhoods, and 98,66% of the lands that these tractors plow are irrigated land and 1,34% are dry land. . The average annual working hours of the tractors are 299, the average age is 27,86, and the average tractor horsepower is 74,18. While Ford, Steyr, Başak, Erkunt and Massey Ferguson were the top 5 tractor brands most used in the neighborhoods, 75,33% of the tractors were purchased in cash, 18,79% with bank credit or deferred payment and 5,88% with government grant support.
Factors affected by farmers in neighborhoods when purchasing tractors; 24,52% fuel economy, 21,36% endurance, 15,36% horsepower, 14,07% competition, 8,21% comfort and convenience, 7,69% influence from neighbors It was determined that 2,60% of the PTO and pulley connection, 2,09% of the wheels, 2,05% of the cabin and 2,05% of it is widely used in the region.

Key words: *Factor analysis, survey, tractor selection and purchasing behavior.*

INTRODUCTION

With the development of the agricultural sector depending on the technology, the importance of mechanization tools in the studies related to agriculture and the increase in their place in the production area have contributed to the reduction of the need for human labor and to obtaining more efficiency in the agricultural sector (Demir et al., 2011). Agricultural mechanization level; It is the technology it incorporates for the development of agricultural production in the agricultural production field, which is a complementary element that is made to increase the efficiency of the inputs in the agricultural production field, to minimize the disruptions in the quality of the workforce in the agricultural field and to provide sufficient economic freedom to the agricultural field (Zeren et al., 1995). The fact that the choice of tractor and tractor equipment in the field of agricultural management has criteria compatible with the production in the region has an important place in terms of optimization and planning. Land size, crop pattern and land type are the most important factors in these preferences. Making a choice in the field of agricultural production in accordance with the goal and importance of the work to be done will increase the amount of agricultural productivity, quality and efficiency. In addition, while purchasing tractors, which is known as an important investment in the agricultural production field, it is possible to purchase new or second-hand tractors. While the agricultural enterprises with good financial situation buy new tractors, the remaining business owners tend to buy second-hand tractors (Sümer et al., 2008). The tractor is the most important tool used by the enterprises in the agricultural production field among the agricultural machinery. For this reason, the choice of tractors with features that will meet the needs of the producers is the

main factor for the enterprises to produce in order to contribute to the economy. Tractor preference; The production method is associated with the size of the enterprise, the land structure, the soil characteristics of the land, the climatic conditions and the sufficient time for the agricultural operations to take place (Demirci, 1986; Işık, 1988).

Various studies have been carried out on the factors affecting the purchase of tractors by farmers in Turkey (Aytuğ et al., 1998; Silent et al., 2006; Cankurt et al., 2009; Altıkat et al., 2011; Sağlam et al., 2012). ; Korucu et al., 2015; Sağlam et al., 2016; Unakıtan et al., 2020). According to these studies, it is to determine the factors that affect the tractor preferences of the farmers with scientific methods.

MATERIALS AND METHODS

In this study, a face-to-face interview was conducted with the farmers in order to determine the factors affecting the purchase of tractors in 3 neighborhoods (Kapicam, Tevekkeli and Yeniyurt) determined in the Dulkadiroğlu district of Kahramanmaraş province. The names and numbers of the tractor brands used by the farmers are given in Table 1.

Table 1. Tractor brands and numbers.

	Districts		
Tractor Brand	Kapıçam	Tevekkeli	Yeniyurt
Ford	17	25	5
Steyr	11	13	5
Başak	9	1	3
Erkunt	3	2	6
Hatat	1	1	1
John Deree	2	1	0
Case(i)	1	1	1
MasseyFerguson	3	3	4
Tümosan	2	3	2
Deutzh	0	1	0
Fiat	0	1	9
New Holland	0	3	4
Valtra	0	1	0
David Brown	0	1	0

In line with the information obtained from the survey conducted with the farmers, a survey was conducted with 49 people in Kapıçam neighborhood. As a result of the answers given by the farmers, there is a total of 77,55% single-wheel drive tractors, 22,45% double-wheel drive tractors, and 95,91% of the lands that these tractors plow are irrigated lands and 4,09% are without water. The average annual working hours of the tractors are 331,02 , the average age is 28,91 and the average tractor horsepower is 75,08. Ford, Steyr, Başak, Erkunt and Massey Ferguson are the top 5 most used tractor brands in the neighborhood, while 63.26% cash, 26,53% bank credit or deferred and 10,21% are used in purchasing tractors. received with government grant support.

Factors affected by farmers in the neighborhood when purchasing tractors; 30,61% fuel economy, 20,43% horsepower, 14,28% competition, 10,20% durability, 8,16% comfort and convenience, 8,16% influence from neighbors , 2,04% tail shaft and pulley connection, 2,04% wheels, 2,04% cabin and 2,04% widespread use in the region.

A survey was conducted with 61 people in the Tevekkeli neighborhood. As a result of the answers given by the farmers, there is a total of 73,77% single-wheel drive tractors, 26,23% double-wheel drive tractors and 100% of the lands that these tractors plow is irrigated land and 0% is dry land. The average annual working hours of the tractors are 214,75, the average age is 31,47, and the average tractor horsepower is 73,91. Ford, Steyr, Başak, New Holland and Massey Ferguson are the top 5 most used tractor brands in the neighborhood, respectively, while 85,24% cash, 9,83% bank loan or deferred and 4,93% in the purchase of tractors. received with government grant support.

Factors that affect farmers while purchasing tractors in the neighborhood; 22,95% fuel economy, 22,95% competition, 16,39% durability, 13,17% horsepower, 11,47% comfort and convenience, 4,91% being affected by neighbors, 3,27% PTO and pulley connection, 1,63% wheels, 1,63% cabin and 1,63% being widely used in the region.

A survey was conducted with 40 people in Yeniyurt neighborhood. As a result of the answers given by the farmers, there is a total of 67,50% single-wheel drive tractors, 32,50% double-wheel drive tractors and 100% of the lands where these tractors plow is irrigated land and 0% is dry land. The average annual working hours of the tractors are 351,25, the average age is 23,22, and the average tractor horsepower is 73,55. Fiat, Erkunt, Ford, Steyr and New Holland are the top 5 most used tractor brands in the neighborhood, while 77,50% cash, 20% bank loan or time deposit and 2,50% government grant in purchasing tractors. received with support.

Factors that affect farmers while purchasing tractors in the neighborhood; 37,5% endurance, 20% fuel economy, 12,50% horsepower, 10% influence from neighbors, 5% competition, 5% comfort and convenience, 2,50%' PTO and pulley connection, 2,50% wheels, 2,50% cabin and 2,50% widespread use in the region.

In this study, factor analysis was used in the data obtained with the help of a questionnaire. Factor analysis is a multivariate statistical method that provides a small number of unrelated variables by classifying the values that are related to each other on many data. Since the large number of variables observed in the factor analysis is tried to be explained by fewer factors, it pays attention to the correlation between the variables at first (Johnson and Wichern 1992). Along with achieving the goal of eliminating the dependency and dimension reduction structure, it aims to bring together the variables that are related to each other in a p-variable situation and to create a small number of unrelated variables (Tatlıdil, 2002).

Factor analysis consists of 4 main stages: obtaining factors, evaluating the appropriateness of factor analysis, factor naming and factor rotation. Whether the obtained data set is suitable for analysis is determined by the Bartlett test, correlation matrix and Kaiser-Meyer-Olkin (KMO) test (Akgül et al., 2003).

Kaiser-Mayer-Olkin (KMO) test,

$$KMO = \frac{\sum_{i \neq j} \sum_{ij} r^2}{\sum_{i \neq j} \sum r_{ij}^2 + \sum_{i \neq j} \sum a_{ij}^2}$$

It is formed by comparing the simple correlation coefficients calculated as shown in the formula with the partial correlation coefficients. The value of the analysis varies between 0 and 1 (Norusis and SPSS Inc 1994).

If the value found in the Kaiser-Mayer-Olkin (KMO) test is below 0,50, there is an error in the data set and it is not suitable for factor analysis. If it is above 0,50, the data set is suitable for analysis (Sharma, 1996).

The Bartlett Test of Sphericity tests whether the correlation matrix is a unit matrix whose diagonal terms are 1 and non-diagonal terms are 0. For the application of this test, the data must show multiple normal distribution (Hair et al., 1998).

In factor analysis, vertical transformations such as Varimax, Orthomax, Biwuartimax and oblique transformations such as Oblimin, Oblimax are used for better interpretation of the results (Özdamar 1999).

In this study, Kaiser-Mayer-Olkin (KMO) test and Bartlett Test of Sphericity were used in factor analysis, and Varimax transformation in the interpretation part was used to find out what factors affect the farmers' choice while purchasing tractors.

RESULTS AND DISCUSSION

In this study, first of all, by using the survey conducted in 3 selected neighborhoods in Kahramanmaraş Dulkadiroğlu district, to determine the factors that farmers are affected by when purchasing tractors;

H_0: There is no relationship between the factors determined with the help of the questionnaire while the farmers were purchasing tractors.

H_1: There is a relationship between the factors determined with the help of the questionnaire while the farmers buy tractors.

hypotheses were determined and

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_{7it} + \beta_8 X_8 + \beta_9 X_9 + \beta_{10} X_{10} + u_i$$

Yi= Farmers' preferences when purchasing tractors.

X_1= The preference of the farmers participating in the survey to be fuel saving in tractor selection.

X_2= Preference of the farmers participating in the survey to have more horsepower in tractor selection.

X_3= The preference of the surveyed farmers to be durable in tractor selection.

X_4= The preference of the farmers participating in the survey to be widely used in the selection of tractors in the region.

X_5= The preference of the farmers participating in the survey to be comfortable and comfortable in tractor selection.

X_6= The preference of the farmers participating in the survey to be affected by the neighbor's tractor in the tractor selection.

X_7= The preference of the farmers participating in the survey to be a tractor with a cabin in their tractor selection.

X_8= The preference of the farmers participating in the survey to be competitive in the selection of tractors.

X_9= Tractor wheels preference of the farmers participating in the survey in tractor selection.

X_{10}= The preference of the farmers participating in the survey to have PTO and pulley connection in tractor selection.

model was established.

The model established with the help of factor analysis was tested.

Table 2. Kaiser-Meyer-Olkin(KMO) test result.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy
,748

Table 3. Barlett test result.

Bartlett's Test of Sphericity	Approx. Chi-Square	181,296
	df	3
	Sig.	,000

KMO= 0,748 was calculated as a result of the test performed to determine whether the data obtained in the survey study were appropriate and sufficient for factor analysis. This value was determined as a very good value (Kalaycı, 2010). Since KMO= 0,748>0,60, it was determined that the data obtained from the survey study were appropriate and sufficient for factor analysis. In factor analysis, the varimax method was chosen to ensure that there was no change in the structure of the variables. As a result of the factor analysis, since the Bartlett test result was p= 0.000<0,05, it was found that there was a relationship between the factors determined by the help of the questionnaire while the farmers were purchasing tractors.
(H_0 hypothesis was rejected).
In Table 4. A. and table 4. B. , 3 factors were determined as a result of the factor analysis for the factors affecting the farmers' purchase of tractors and these factors were collected at 96,148 of the variance. The explained variance value shows that it is valid and safe.

Table 4. A. The result of explaining the total variance and factors.

Total Variance Explained			
Initial Eigenvalues			
Component	Total	% of Variance	Cumulative %
1	2,884	96,148	96,148
2	,084	2,807	98,955
3	,031	1,045	100,000

Table 4. B. The result of explaining the total variance and factors.

Total Variance Explained		
Extraction Sums of Squared Loadings		
Total	% of Variance	Cumulative %
2,884	96,148	96,148

As a result of a study conducted in the Pasinler district of Erzurum province, it was determined that the main factors affecting the tractor selection of the farmers were the horsepower of the tractor and the fuel savings of the tractor (Erkmen et al., 2001). As a result of a study conducted in Kahramanmaraş, it was determined that the main factors affecting the tractor selection of the farmers were the fuel savings of the tractor and the horsepower of the tractor (Aybek, 2002). As a result of a study conducted in Aydın province, the factors that farmers are affected by when purchasing a tractor were determined as information about the tractor, recommendation to the tractor, the brand of the tractor, the age of the tractor, experience, the number of days the farmer and family members worked with the tractor and the size of the cultivated area (Cankurt et al., 2010). .
As a result of a study conducted in the Harran district of Şanlıurfa province, the annual average working hours of tractors in the region is 570 hours, while the factors affecting the tractor preferences of the enterprises are 35% tractor brand, 33% ease of service, 16% tractor cabin, 15% economic suitability of the tractor price and 1%. It has been determined that there is also influence from the neighbor (Aybek, 2016). As a result of a study conducted in Kayseri province, while the average annual tractor working hour is 273,4 hours, it has been determined that the main factors that affect farmers in purchasing tractors are the price of the tractor 15%, the horsepower of the tractor 14,40% and the brand of the tractor 8,70% (Sağlam et al. , 2017). As a result of a study conducted in Çumra district of Konya province, it has been determined that new generation tractors are preferred and the working hours of the annual tractor are 545,4 hours, while the tractor brand selection is mainly 36,80% Tümosan, 23.90% Massey Ferguson and 16,20% Fiat (Berk et al., 2020).

CONCLUSIONS

KMO = 0,748 and 3 factors were determined as a result of the factor analysis applied to the data obtained with the help of the questionnaire, which was arranged face-to-face with the farmers, as well as the accessibility in the Dulkadiroğlu district of Kahramanmaraş province. These factors were collected at 96,148 of the variance. In addition, since the Bartlett test result was p= 0.000<0,05, it was determined that there was a relationship between the factors determined with the help of the questionnaire while the farmers were purchasing tractors (H0 hypothesis was rejected).
As a result of the answers given by the farmers who were included in the survey, there are 73,33% single-wheel drive tractors and 26,67% double-wheel drive tractors in the neighborhoods, and 98,66% of the lands that these tractors plow are irrigated land and 1,34% are dry land. . The average annual working hours of the tractors are 299, the average age is 27,86, and the average tractor horsepower is 74,18. While Ford, Steyr, Başak, Erkunt and Massey Ferguson were the top 5 tractor brands most used in the neighborhoods, 75,33% of the tractors were purchased in cash, 18,79% with bank credit or deferred payment and 5,88% with government grant support.

Factors affected by farmers in neighborhoods when purchasing tractors; 24,52% fuel economy, 21,36% endurance, 15,36% horsepower, 14,07% competition, 8,21% comfort and convenience, 7,69% influence from neighbors It was determined that 2,60% of the PTO and pulley connection, 2,09% of the wheels, 2,05% of the cabin and 2,05% of it is widely used in the region.

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DETERMINING THE REASONS OF HIGH OR LOW EFFICIENCY IN WHEAT PRODUCTION BY THE CHI-SQUARE METHOD

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Abstract

The aim of this research is to use the Chi-square method among the predicted reasons for the landowners to have high or low productivity in wheat production, in line with the answers given by the landowners as a result of the data obtained from the survey, which has a single participation condition in 3 neighborhoods determined in the Dulkadiroğlu district of Kahramanmaraş province. The aim is to determine whether there is a relationship between the factors affecting high or low productivity with the help of the help of The sample of the research was obtained with the help of the questionnaire, which had produced wheat in the agricultural land in the year 2021, in which the only participation condition was foreseen in 3 neighborhoods in the Dulkadiroğlu district of Kahramanmaraş province. As a result of the data obtained from the survey, the Chi-square method was used to indicate the degree of influence of the factors affecting low or high productivity. According to the estimation results obtained in the research, as a result of the answers given by the land owners who were thought to be under the influence of low or high wheat productivity in the specified year and who participated in the survey in Kapıçam District, respectively, the first three reasons for the high productivity of wheat are proper timed maintenance, farmer. information and fertilizer amount, the first three reasons for low productivity, respectively, were irrigation status, land ploughing and wheat production in the field for a long time. As a result of the answers given by the land owners who participated in the survey in Tevekkeli District, the first three reasons for the high productivity of wheat were proper timely maintenance, amount of fertilizer and farmer information, while the first three reasons for low productivity were respectively long-term wheat production in the field, land ploughing and irrigation status. has been. As a result of the answers given by the land owners who participated in the survey in Çiğli District, the first three reasons for high productivity of wheat were proper timely maintenance, fertilizer amount and certified seed planting, while the first three reasons for low productivity were irrigation status, land ploughing status and long-term wheat production in the field, respectively. determined to be done. According to the estimation results, as a result of the Chi-square goodness of fit analysis, Y (low or high productivity in wheat compared to the previous year) 14,041 and X (Projected low productivity (change in input prices, irrigation situation, wrong spraying, climatic change, land ploughing, tractor and equipment) shortage and long-term wheat production in the field) and high productivity (reasons for timely maintenance, fertilizer amount, farmer information, use of seeds suitable for the region, certified seed sowing and sprinkling or machine planting) 16,410 and according to the result of Pearson Chi-square independence analysis, 271.719 'type.

Key words: *Wheat, productivity, land, land care*

INTRODUCTION

One of the auxiliary areas related to agriculture is the grain area within the plant production areas. In Turkey, the grain production area covers an important area in protecting the integrity of the economy, as well as the agricultural area. Grain has an important place in terms of providing income to the farmers engaged in agriculture, being the raw material of various industries, and food used by people (Kızılaslan, 2004; Demir and Yavuz 2014). In the world, it meets the nutritional and energy needs of individuals with a ratio of more than 60% with cereals and wheat covers the most important cereal area (Yıldız et al., 2013). Grain products such as wheat, corn and rice meet the nutritional needs of humanity to a large extent (Estes et al., 2013; Hokazono and Hayashi, 2012; Lobell et al., 2013). While bulgur, pasta, flour and starch obtained from wheat product, which is at the forefront of human nutrition resources, constitute the nutritional resources

of people, the stems of the wheat plant are also used in animal nutrition. In order for the farmers to continue their wheat production, it is also important to obtain sufficient yield in the unit area together with the necessary conditions and conditions (Birinci and Akın, 2008).

The amount of input used in wheat production, the cost of production and the calculation of the profitability to be obtained, together with the planning to be made for the region and the great benefits of such studies to be carried out in the future, the farmers of the region express their opinions to compare the profitability conditions between wheat and other crops planted in the field and to determine the production models. is foreseen.

Various studies have been conducted in wheat production with low or high productivity (Vuruş Akçaöz et al., 2002; Kızılaslan, 2004; i Konyalı, 2008; Bayramoğlu, 2013; Bulut et al., 2013; Kızıloğlu et al., 2016; Karadaş, 2016; Oztekin, 2017). According to these studies, in order to determine the reasons for low or high productivity in wheat production, it is to determine the extent and size of the effects of the factors affecting the low or high productivity of the landowners in wheat production within the specified period, by scientific methods.

MATERIALS AND METHODS

Based on the wheat production in 2021, this research has been referred to the survey study, which was arranged with the interviews with the land owners in 3 selected neighborhoods in the Dulkadiroğlu district of Kahramanmaraş province. The most important factor in determining the places where the research is applied is the thought that the general data will be met in the agricultural lands in the region.

In this research, a single participation condition was determined in 3 neighborhoods and a questionnaire was applied with a total of 195 participants who had produced wheat in agricultural land during the year. In the survey conducted with the land owners in question, the total wheat production was done.

It has been determined that there is a land of 5853,000 m^2. The total land parcel sizes of the neighborhoods are given in Table 1.

Table 1. Total parcel size of wheat planted lands in the determined neighborhoods

District	Total parcel size
Kapıçam	1679.000 m^2
Tevekkeli	1907.000 m^2
Çiğli	2267.000 m^2
Total	5853.000 m^2

A total of 195 people were surveyed by reaching as many people as they could reach, and as a result of the answers given by our farmers participating in the survey, 114 people in 3 neighborhoods were found to have higher wheat productivity on their land compared to the previous year, while 81 people were found to have low productivity on their land compared to the previous year.

In addition, in accordance with the answers given by the land owners who participated in the survey in Kapıçam neighborhood, 50.76% of them had higher productivity compared to the previous year, while 49.24% had low productivity compared to the previous year, and 68.75% of them were higher than the previous year, according to the answers given by the landowners who participated in the survey in Tevekkeli neighborhood. While productivity was higher at 31.25% compared to the previous year, 56.92% had higher productivity compared to the previous year, and 43.08% had lower productivity compared to the previous year, in line with the answers given by the landowners who participated in the survey in Çiğli district.

As a result of the interviews made with the land owners in the determined neighborhoods, it was determined as a result of the answers given by the land owners that the wheat production in 1 decare was 472.92 kg in Kapıçam district, 524.61 kg in Tevekkeli district and 562.92 kg in Çiğli district.

Goodness of fit analysis in the chi-square test analyzes whether a investigated variable fits an expected distribution or whether the two investigated variables have a similar distribution. If the H_0 hypothesis is accepted, it is predicted that it is suitable for the investigated distribution, and it is not suitable for the investigated distribution in H_A (Masoom et al., 1992).

In goodness-of-fit analyses, G's are given as a single column or a single row composed of k clusters. The B's corresponding to the G's consist of a different column or row containing k clusters in nature. Therefore, values consist of k elements in a single column or a single row, and

$$\chi^2_{hes} = \sum_{j=1}^{c}\sum_{i=1}^{r}\frac{(G_{ij}-B_{ij})^2}{B_{ij}} = \sum_{j=1}^{c}\sum_{i=1}^{r}\frac{G_{ij}^2}{B_{ij}} - n$$

expression,

$$\chi^2_{hes} = \sum_{j=1}^{k}\frac{(G_j-B_j)^2}{B_j} = \sum_{j=1}^{k}\frac{G_j^2}{B_j} - n$$

is written as.

The Bjs found here are the values belonging to the predicted distribution in H0. Since column or row sum creates a constraint in this analysis, the degree of freedom is
sd = k-1
is expressed as.
Also, if there is an estimation of some parameters from the sample, a new constraint will occur in each parameter estimation, and if m parameter estimations are in question, the degrees of freedom are,
sd= k-m-1
It is expressed as (Hogg et al., 1978).
The independence test, on the other hand, can be tested by chi-square analysis whether there is a significant relationship between the two qualitative variables in the statistical domain. In H_0, the existence of any relationship is not observed and it is independent; It is suggested that there is a relationship in H_A and it is not independent from each other. In order to find the test statistic, it is necessary to find B's. Test statistic

$$\chi^2_{hes} = \sum_{j=1}^{c}\sum_{i=1}^{r}\frac{(G_{ij}-B_{ij})^2}{B_{ij}} = \sum_{j=1}^{c}\sum_{i=1}^{r}\frac{G_{ij}^2}{B_{ij}} - n$$ and

sd = (r-1)(c-1)
is calculated as. If

$\chi^2_{hes} \geq \chi^2_{tab}$ If the condition is met, the alternative hypothesis (HA) is accepted. (Çelik Y, 1999).

RESULTS AND DISCUSSION

In this research, first of all, the only condition of participation in the 3 determined neighborhoods in the Dulkadiroğlu district of Kahramanmaraş province is to determine the reasons for the high or low productivity in wheat production as a result of the data obtained from the land owners who have produced wheat in the agricultural land in 2021. For this, first of all, by using Chi-square analysis:
H_A: There is no interaction between the predicted causes of high or low productivity of wheat production in the survey study.
H_1: There is an interaction between the predicted causes of high or low productivity of wheat production in the survey study.
hypotheses have been established and
A: Low or high productivity in wheat compared to the previous year.
X: Projected low productivity (change in input prices, irrigation situation, wrong spraying, climatic change, land ploughing, tractor and equipment limitations, and long-term wheat production in the field) and high productivity (appropriate timely maintenance, fertilizer amount, farmer information, suitable for the region) seed use, certified seed planting and spreading or machine planting).
The dependent and independent variables of the model were determined and the result of Chi-square conformity analysis is given in Table 2.

Table 2. Chi-square goodness of fit analysis result.

	Y	X
Chi-square	14.041	16.410
Degrees of Freedom	1	1
Probability Value	.000	.000

As a result of the analysis in Table 2, the Chi-square probability value at 95% confidence level is less than 0.05 and the H0 hypothesis is not accepted. In other words, there is an interaction between the predicted causes of high or low productivity of wheat production in the survey study, and it has been determined that there is a difference between the observed values and the expected values.
In addition, whether there is a relationship between low productivity and high productivity predicted according to low or high productivity in the previous year was examined by using the Chi-square independence test and the result is given in Table 3.

Table 3. Chi-square independence analysis result.

	Values	Degrees of Freedom	Probability
Pearson Chi-square	271.719	1	.000
Continuity Correction	268.255	1	.000
Probability Ratio	310.474	1	.000
Linear Association	271.022	1	.000

As a result of the analysis in Table 3, the Pearson Chi-square probability value is less than 0.05 at the 95% confidence level and the H_0 hypothesis is not accepted. In other words, there is an interaction between the predicted causes of high or low productivity of wheat production in the survey study, and there is a relationship between low productivity and high productivity predicted according to low or high productivity in the previous year, and it has been determined that the data are not independent from each other.
As a result of the survey, in line with the answers given by the landowners in the fields where wheat production was made, the wheat

that was predicted to cause low or high productivity in wheat production and took place in the survey is in high productivity; timely maintenance, fertilizer amount, farmer information, use of seeds suitable for the region, certified seed sowing and spreading or machine planting reasons and low productivity; As a result of the answers given by the land owners who participated in the survey in Kapicam District, the first three reasons for the high productivity of wheat are respectively, among the reasons for the changes in input prices, irrigation situation, wrong spraying, climatic changes, land ploughing, tractor and equipment limitations, and long-term wheat production in the field. , farmer information and fertilizer amount, the first three reasons for low productivity, respectively, were irrigation status, land ploughing status and long-term wheat production in the field. As a result of the answers given by the land owners who participated in the survey in Tevekkeli District, the first three reasons for the high productivity of wheat were proper timely maintenance, amount of fertilizer and farmer information, while the first three reasons for low productivity were respectively long-term wheat production in the field, land ploughing and irrigation status. has been. As a result of the answers given by the land owners who participated in the survey in Çiğli District, the first three reasons for high productivity of wheat were proper timely maintenance, fertilizer amount and certified seed planting, while the first three reasons for low productivity were irrigation status, land ploughing status and long-term wheat production in the field, respectively. has been done.

Studies have been carried out on many influencing factors in wheat production and productivity, and indeed, as a result of a study conducted in Erzurum province, it was determined that 33% higher yield was obtained in the field sowing in dry farming conditions, possibly with a pressurized seeder (Öztürk et al., 2001). As a result of another study conducted in Erzurum, it was determined that winter wheat sowing yields 57% more than summer wheat planting and 14% more grain yield than ice cream sowing in wheat planting (Bulut, 2005). As a result of a study conducted in Mexico, the effects of recent climatic changes on wheat productivity in Mexico were examined. It has been determined that there has been an increase in wheat yield by 25% in the last 10 years and this increase is due to climatic changes in the Northwest regions (Lobell et al., 2005). As a result of a study conducted in Muş, the effect of rotation systems on yield was examined and the highest yield was Vetch-Wheat (338 kg/da), Vetch-Fallow-Wheat (336.6 kg/da), and Chickpea- Fallow-Wheat (325.4 kg/da).) while sorted as ; the least productivity was determined as Wheat-Wheat (236.3 kg/da) (Partigöç et al., 2007). As a result of a study conducted in Hatay and Şanlıurfa provinces, it was determined that while irrigation and fertilizer amount affected the productivity in Hatay, irrigation, fertilizer amount and the age of the producer were also effective in Şanlıurfa (Tiryakioğlu et al., 2017). As a result of a research conducted in Kayseri province, grain productivity and production have been negatively affected economically due to the lack of sufficient capital and technical knowledge in agricultural enterprises, the elderly and insufficient education level of the producers, the lack of sufficient technical staff, the insufficiency of activity of agricultural establishments and the problems experienced due to climatic conditions. It has been determined that high rate of fallow application, lack of certified seed use, mistakes made in soil cultivation, delay in winter planting or excessive summer planting, lack of fertilizer use due to high input, lack of scientific research, delay in combating weeds significantly affect grain yield (Bulut, 2017).). As a result of a study conducted in our country, in the comparison of new wheat varieties and regional population, local varieties; It has been determined that it is risky to prefer organic farming in areas where the demand for wheat is high in the regions and in low yield conditions despite the presence of a traditional production area (Kaplan et al., 2019). As a result of a study carried out in Muş province, it is important to develop compatible wheat varieties, to determine the cultivation opportunities compatible with the application and to foresee them to the producers, to review the relationship conditions between producer communities and growers and to establish a structure for creating a dynamic, continuous and solid communication environment. determined (Ozturk, 2020).

CONCLUSIONS

According to the estimation results obtained in the research, as a result of the answers given by the land owners who were thought to be under the influence of low or high wheat productivity in the specified year and who participated in the survey in Kapıçam District, respectively, the first three reasons for the high productivity of wheat are proper timed

maintenance, farmer. information and fertilizer amount, the first three reasons for low productivity, respectively, were irrigation status, land ploughing and wheat production in the field for a long time. As a result of the answers given by the land owners who participated in the survey in Tevekkeli District, the first three reasons for the high productivity of wheat were proper timely maintenance, amount of fertilizer and farmer information, while the first three reasons for low productivity were respectively long-term wheat production in the field, land ploughing and irrigation status. has been. As a result of the answers given by the land owners who participated in the survey in Çiğli District, the first three reasons for high productivity of wheat were proper timely maintenance, fertilizer amount and certified seed planting, while the first three reasons for low productivity were irrigation status, land ploughing status and long-term wheat production in the field, respectively. determined to be done.

According to the estimation results, as a result of the Chi-square goodness of fit analysis, the Chi-square probability value is less than 0.05 at the 95% confidence level and the H0 hypothesis is not accepted. In other words, there is an interaction between the predicted causes of high or low productivity of wheat production in the survey study, and it has been determined that there is a difference between the observed values and the expected values. In addition, according to the result of the Chi-square independence analysis, the Pearson Chi-square probability value is less than 0.05 at the 95% confidence level and the H0 hypothesis is not accepted. In other words, there is an interaction between the predicted causes of high or low productivity of wheat production in the survey study, and there is a relationship between low productivity and high productivity predicted according to low or high productivity in the previous year, and it has been determined that the data are not independent from each other. It has been determined by scientific analysis that the factors obtained with the help of the questionnaire, which had a single participation condition in the year 2021, had an effect on the reasons of low or high productivity in wheat production of the land owners.

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INVESTIGATION OF GROWTH IN HOLSTEINS BY REPEATED MEASUREMENT EXPERIMENTAL DESIGN

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Abstract

Repeated measurements occur when a feature under investigation on the same experimental unit is measured repeatedly over time. In this research, body measurements of Holstein cattle were taken from a total of 44 cattle, periodically bull and cow at the age of weaning, three months, six months, twelve months, eighteen months, twenty-four months, thirty-six months, and forty-eight months. analyzed. In the study, the analyzes of whether there is a statistically significant difference between body measurements (age) and gender were performed with repeated measurements two-way ANOVA and IBM SPSS v25 program. In the study, in which the normality assumption, which is one of the prerequisites of repeated measurement, is provided, another important assumption, the sphericity assumption; It was examined by Mauchly´s Test of Sphericity, and it was found to be statistically significant ($p<0.01$). According to the analysis using Greenhouse-Geisser, Huynh-Feldt statistics, it was determined that the effect of age on body measurements and the interaction of gender x age were statistically significant ($p<0.01$). Changes in body weight between males and females from 12 months of age were found to be significant ($p<0.05$). As a result, it is recommended to use the repeated measure analysis of variance technique in studies that require time-dependent data in terms of small experimental error.

Key words: *Repeated experiments, Qubic test, Wilks´ Lambda, Hotelling´s trace*

USE OF BAYESIAN PRINCIPAL COMPONENT ANALYSIS ON ANIMAL DATA

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Abstract

Dimension reduction is an important aim for multivariate studies in animal science as in other scientific areas. It is essential to reduce the dimension because for the multivariate statistics such as clustering unimportant variables reduces the reliability of the model. To avoid the erroneous results variable selection or dimension reduction should be done. There are many methods improved and introduced to the literature for this aim. One of the most general known method is classic Principal Component Analysis. In this study, Bayesian Principal Component Analysis was examined and compared with classic Principal Component Analysis using placental characteristics of Bafra sheep genotype. Analysis were done by using R package with Rdimtools library. Results showed that Bayesian Principal Component Analysis was superior to classic Principal Component Analysis.

Key words: *Dimension reduction, PCA, Bayesian PCA, Placental characteristics*

INTRODUCTION

Principal Component Analysis is a multivariate and useful statistical method used in image compression fields that provides recognition, classification, size reduction and interpretation. This approach tries to find the strongest pattern in the data. Therefore, it can also be used as a pattern finding technique. Its main purpose is to keep the data set with the highest variance in high dimensional data and while doing this, it is a technique that aims to reduce the size. By finding the general features in the over-dimensional data, it reduces the number of dimensions and compresses the data. It is certain that some features will be lost with size reduction; but the intent is that these disappearing traits contain little information about the population. This method combines highly correlated variables to create a smaller set of artificial variables, called "principal components," that make up the most variation in the data. PCA is a very effective method to reveal the necessary information in the data. The basic logic behind PCA is to represent a multidimensional data with fewer variables by capturing the key features in the data.
PCA has three main purposes:
Reducing the size of the data
Guessing
View the dataset for some analysis

MATERIAL AND METHODS

Materials

Presental characteristics of Bafra sheep were used. Properties taken as variables: Large cotyledon width, Medium cotyledon width, Small cotyledon width, Large cotyledon length, Medium cotyledon length, Small cotyledon length, Large cotyledon thickness, Medium cotyledon thickness, Small cotyledon thickness, Total cotyledon surface area, Cotyledon activity, Cotyledon density, placental activity, cotyledon volume, Volumetric cotyledon efficiency used as the explanatory variable.

Methods

Principal Component Analysis – PCA

PCA is the most popular orthogonal linear transform. PCA is the representation of the data with the largest variance in low-dimensional space. High variance features are preferred over low variance. It finds the linear mapping M, which increases the cost function by calculating the covariance matrix of the samples of the X data matrix. Extracts the eigenvectors of the largest eigenvalue. PCA uses Euclidean distance between data points xi and xj.
PCA conversion;

$$\mu^T = X^T W$$

is in the form.
W ortogonal matris,
μ^T linear transform,
W shows the eigenvectors corresponding to the covariance matrix.
PCA is a linear representation of data. The data is parsed with PCA as follows:

$$x_i = \sum_{j=1}^{p} w_{ij} Q_j$$

It is a method of finding the projection of a data in a multidimensional space to a lower dimensional space in a way that maximizes the variance. For a set of points in space, the "best fit line" with the least average distance from all points is chosen. Then, by choosing the most suitable line among those perpendicular to this line, these steps are repeated until the variance of a new dimension falls below a certain threshold. The lines obtained at the end of this process form the bases of a linear space. These basis vectors are called principal components.

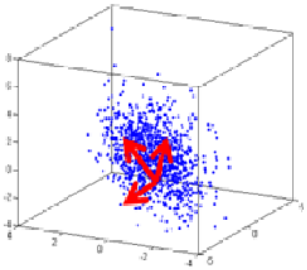

Figure 1

Figure 1.The data is multidimensional and relationships are not explicit.

Figure 2. When viewed from the right angle, the relationship in a complex multidimensional data set is linear.

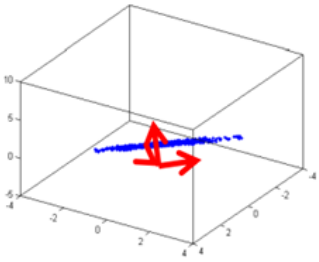

Figure 3

Figure 3. The complex data set is made linear.

Figure 4. The image of the data was obtained in the new coordinate system.

Looking at the data from the appropriate "perspective" means examining the data using this coordinate system.

In PCA, the appropriate coordinate system is sought as follows:

As the 1st axis, the direction with the largest change of data is selected.

As the 2nd axis, the direction perpendicular to the previous 1st axis and at the largest change of data is selected.

As the 3rd axis, the direction perpendicular to the previous 1st and 2nd axes and which is at the largest change of the remaining data is selected.

The fundamental components of the data become independent of each other.

When PCA is applied, the actual size of the p-dimensional space is determined. Basic components have three properties:

They are non-correlated.

The first principal component is the variable that most explains the total variability.

The next principal component is the variable that most explains the remaining variability.

Principal Component Analysis approaches this problem as follows:

By looking at multidimensional data from the right angle, relationships in the data can often be explained.

The purpose of PCA is to find this "right angle".

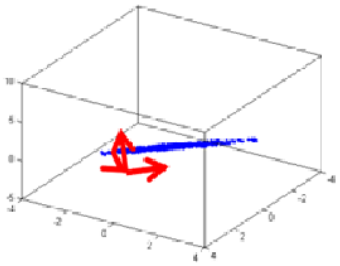

Figure 2

The PCA key point is to choose an appropriate "angle" for visual inspection, i.e. an appropriate coordinate system, to solve the problem.

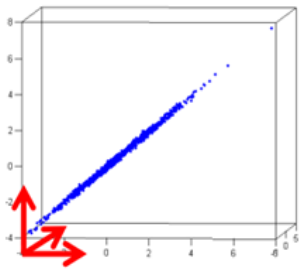

Figure 4

Thus – the direction with the largest remaining change in its data is always chosen as the new axis.

The vertical "largest change" directions chosen like this are called "principal components". The PCA aspects first indicate the aspect that contributes most to the change of data, and then describe the aspects that contribute less. In real applications, one might think that “there is little fundamental impact on multidimensional and at first glance very complex data”. These effects are found as several initial PCA aspects. In this sense, aspects of PCA can often explain and illustrate complex multidimensional data with 2-3 new "features". The concept of "retained variance 2" is used to indicate the sufficient number of

principal components. The total variance of the first principal components to be used should be 90%-95% of the total variance of the original data. In general applications, usually 10-20 first principal components give 90%-95% variation of the data for 1000 dimensional data. In other words, 10-20 PCA components may be sufficient to represent the original data with 95% accuracy. For example, instead of saving all 1000 features to save 1000 feature data, 10-20 first principal components can be recorded and averaged for other components' values. Original data can be saved with 1-2% memory.

As a result:

PCA is a very useful method for size reduction.

PCA represents multidimensional data approximately and with less dimensional data.

PCA finds the largest directions of variance perpendicular to the original data and displays the original data in this coordinate system.

PCA can be used for visual display and review of multidimensional data.

In PCA, machine learning can reduce the size of data, slightly changing PCA features may be unimportant for modeling, thus speeding up the computation related to modeling.

PCA can also be used for data compression.

In summary, PCA is a statistical method. It can be used to describe the pattern in a data set, to describe the data set, to identify similar and different patterns in the data. PCA allows data compression by reducing size. Moreover, while the size is reduced, there is no data loss. This technique is frequently used in the field of image processing in computer science. In the next part of the study, PCA will be applied on a data set and step by step what is done at each stage will be mentioned.

Bayesian Principal Component Analysis–BPCA

Principal component analysis (PCA) is a dimensionality reduction modeling technique that transforms a set of process variables by rotating the representation axes. Maximum likelihood PCA (MLPCA) is an extension that accounts for different noise contributions in each variable. Neither PCA nor any of its extensions use external information about the model or data, such as the range or distribution of the underlying measurements. Such prior information can be extracted from the measured data and used to greatly improve model accuracy. A Bayesian PCA (BPCA) modeling algorithm has been developed that improves the accuracy of estimating parameters and measurements by gaining prior knowledge of the data and the model. The proposed approach integrates modeling and feature extraction, simultaneously solving parameter estimation and data reconciliation optimization problems. Methods for estimating previous parameters from available data are discussed. Also, BPCA is reduced to PCA or MLPCA when used before a uniform. A few examples demonstrate the benefits of BPCA over existing methods even when the measurements violate assumptions about their distribution.

Reducing the size is important because for multivariate statistics, such as clustering unimportant variables, the reliability of the model decreases. Variable selection or size reduction should be done to avoid erroneous results.

Bayesian principal component analysis. In one embodiment, a computer-implemented method for performing Bayesian PCA including inputting a data model; receiving a prior distribution of the data model; determining a posterior distribution; generating output data based on the posterior distribution (Such as, a data model, a plurality of principal components, and/or a distribution); and, outputting the out put data. In another embodiment, a computer-implemented method including inputting a mixture of a plurality of data Spaces, determining a maximum number of principal com ponents for each of the data Spaces within the mixture, and, outputting the maximum number of principal components for each of the data Spaces within the mixture.

Data modeling has become an important tool in solving complex and large real-world computerizable problems. It is a data modeling technique used for data compression, density estimation and data visualization and other applications among data modeling applications. It has proven to be a popular technique for data modeling applications such as data compression, image analysis, visualization, pattern recognition, regression, and time series estimation. Other data modeling applications where PCA can be applied are density modeling for emission intensities in speech recognition, clustering of data for data mining applications, and generating class conditional density models for handwriting recognition.

In one embodiment, a computer-implemented method for performing Bayesian PCA includes inputting a data model; receiving a prior distribution of the data model; determining a posterior distribution; generating output data based on the posterior distribution (Such as, a data model, aplurality of principal components,

and/or a distribution); and, outputting the output data. In another embodiment, a computer-implemented method includes inputting a mixture of a plurality of data Spaces, determining a maximum number of principal components for each of the data Spaces within the mixture, and, outputting the maximum number of principal components for each of the data Spaces within the mixture. A prior distribution such as P(u, W, O') is taken over the parameters of the entered data model. The posterior distribution of the kernel P(u, W. O'D) is then obtained, for example, by multiplying the previous distribution. In one embodiment, the likelihood function and normalization are produced by marginalizing the output data over the parameters to obtain an estimate density.

To implement this framework, it addresses two issues: prior distribution selection and formulation of a traceable algorithm. Thus, the regulations control the effective dimensionality. The hidden field corresponds to the number of key components retained. Further, embodiments of the invention avoid discrete model selection and instead use continuous hyperparameters to automatically determine an appropriate effective dimensionality for the hidden space as part of the Bayesian inference process. The invention includes computer-implemented methods, machine-readable media, computerized systems, and users of various scopes. Other aspects, embodiments, and advantages of the invention beyond those described herein will become apparent from reading the detailed description and referring to the drawings.

Analysis were done by using R package with Rdimtools library.

RESULTS AND DISCUSSION

Component plots of PCA and BPCA were given in Figure 5. Explanation rates of first 5 components were given in Table 1.

Table 1. Explanation rates of first 5 components

	PCA	BPCA
Dim 1	35.78	39.87
Dim 2	56.84	65.98
Dim 3	71.02	76.36
Dim 4	81.04	87.04
Dim 5	87.40	90.03

Results showed that Bayesian Principal Component Analysis was superior to classic Principal Component Analysis.

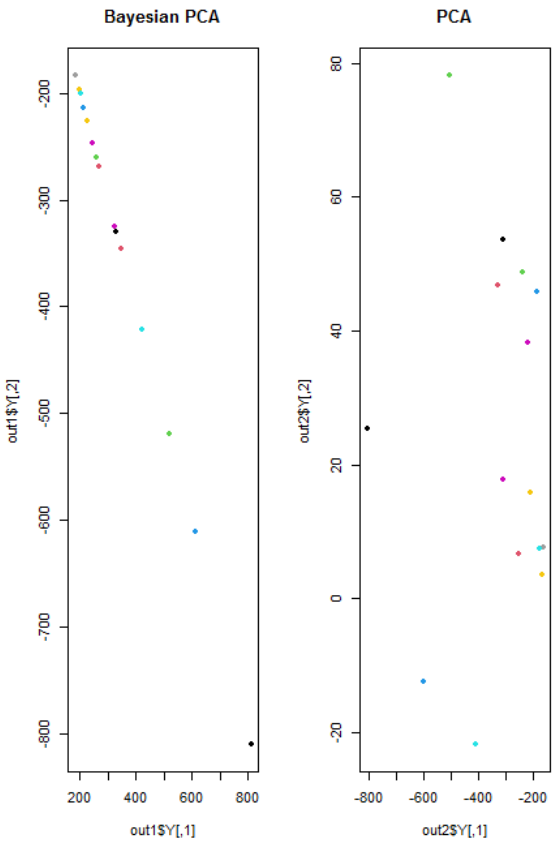

Figure 5. Component plots of PCA and BPCA

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ESTIMATING THE SIGNIFICANCE OF PEARSON CORRELATION COEFFICIENT BY PERMUTATION TEST

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Abstract

The significance of the Pearson correlation coefficient is a controversial topic that the significance level of the correlation coefficient is known as a decreasing function of the sample size. To develop a new perspective on this issue, permutation test which is a nonparametric approach and known as exact statistic was used for this aim. Permutation test and Pearson correlation analysis were used for four different sample sizes (5, 10, 50 and 100) for 10 replication. Results showed that there is no statistical difference between the results of permutation test and parametric t test for Pearson correlation. Also Wilcoxon sign test results showed no difference on the taken decision about the acceptance of the null hypothesis. But 10% of the taken decision were different about the acceptance of the null hypothesis for sample size five. As a result, it can be recommended to use permutation test especially for small sample sizes.

Key words: *Significance, Type I error rate, permutation test, Pearson correlation*

INTRODUCTION

Statisticians have developed techniques that require few or no assumptions when testing hypotheses, especially when the normality assumption is violated. These tests are called non-parametric tests. These include distribution-independent tests where we make almost no assumptions except that the populations are continuous, as well as non-parametric methods in the sense that we are not concerned with the parameters of the populations (Miller and Miller, 2006).

With the permutation test, which is a special type of Monte Carlo test, it is possible to examine whether the difference between the means of the two groups is statistically significant without depending on the assumptions of the classical t test (Templ, 2016). Permutation tests gain popularity in genomic research for many years because straightforward way to obtain reliable statistical inference without making strong distributional assumptions (Buzkova et al, 2011). In permutation tests, correlation coefficients are calculated for all possible combinations and then these obtained values are compared with the correlation coefficients obtained from the original data to determine the significance level. Permutation tests can be applied directly to regression problems (Onder, 2007). Because of its independency from the distribution, permutation tests are successful in many cases where parametric tests are not. The assumptions of permutation tests are exchangeability and relabelability of data. If the null hypothesis is established correctly, exchangeability and relabelability are obtained. If the null hypothesis is correctly established, there will be no effect on the result even when the observations between two groups are exchanged (Onder and Cebeci, 2009). Permutation tests can produce more reliable results in non-normal distributions and when the sample size is less than 15 (Onder, 2007).

In this study, it is aimed to examine the Pearson correlation coefficient of the data set of four different sample sizes by means of the permutation test.

Material and Method

In this study, four different data sets with sample size of 5, 10, 50 and 100 were used with 10 replicates. To analyze the data; NPMANOVA software written by Anderson was utilized. Paired sample *t* test and Wilcoxon sign test were analyzed with SPSS version 20.0 under OMU license.

Permutation Test

When X_1 is constant and n combinations do exists (missing values and duplications are unimportant), the possible combinations can be shown as (X_{1j}, Y_j), $j = 1, 2, ..., n$. Similarly if X_2 is constant, possible combinations can be shown as (X_{2j}, Y_j), $j = 2, 3, ..., n$. Hence there are $n - 1$ combinations in this case, in turn, there are $n-2$ and $n-3$ combinations for X_3 and X_4

respectively. Finally the number of all possible combinations between *X* and Y is *n!*. In a multiple linear regression *t* value for statistical significance is calculated (Kleinbaum et al, 1998).

The number of *t* values is *n!* which will be handled by changing the order of Y. Let t^*, * = *1,2,...,n!* and t_j^* is j^{th} element of t^*. Then $t^* = (t_1, t_2^*, ..., t_k^*)$. When it is assumed that t_j^* is j^{th} element of set of *t* values under the null hypothesis, the experimental distribution of t_j which is j^{th} element of *t* value estimated by OLS (Ordinary Least Squares) can be given as follows:

$$P = \frac{number\ of\ t^* \geq t}{total\ number\ of\ t^*}$$

For all *j = 1, 2, ..., k*, the computing method given above can be applied and calculated for each probability. The significance test of regression equation can be applied as determining the position of t_j^1 . If either $P(t_j < t_j^1)$ or $P(tj > t_j^1)$ is small enough null hypothesis, $H_0: \beta_j = 0$ is rejected by two-tailed test (Onder and Cebeci, 2009).

RESULTS AND DISCUSSION

The results obtained according to the Pearson correlation analysis for each data set are given in Table 1.

Table1. Statistical significance levels of the Pearson correlation method

Groups	Sample Size			
	5	10	50	100
A-B	0,243000	0,483000	0,106000	0,020000
C-D	0,420000	0,000112	0,000010	0,337000
E-F	0,269000	0,000010	0,923000	0,000229
G-H	0,005000	0,692000	0,000010	0,249000
İ-J	0,657000	0,000006	0,000010	0,000010
K-L	0,005000	0,040000	0,000010	0,612000
M-N	0,090000	0,621000	0,000010	0,705000
O-P	0,000192	0,002000	0,368000	0,810000
R-S	0,006000	0,616000	0,395000	0,137000
T-U	0,201000	0,000217	0,869000	0,000010

Permutation test analysis results obtained for all data sets are given in Table 2.

Table 2. Statistical significance levels of Permutation test

Groups	Sample Size			
	5	10	50	100
A-B	0,2445	0,4820	0,10614	0,01980
C-D	0,0925	0,0003	0,00001	0,33865
E-F	0,2624	0,0001	0,92155	0,00026
G-H	0,0158	0,6964	0,00001	0,24940
İ-J	0,6440	0,0002	0,00001	0,00001
K-L	0,1007	0,0021	0,00001	0,61385
M-N	0,1063	0,6236	0,00001	0,70748
O-P	0,0154	0,0015	0,36700	0,81218
R-S	0,0079	0,6172	0,39494	0,13729
T-U	0,1848	0,0005	0,87018	0,00001

Correlation coefficient values of all data sets are given in Table 3.

Table 3. Correlation coefficient values

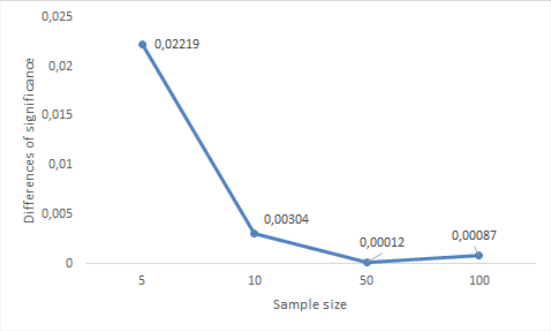
Groups	Sample Size			
	5	10	50	100
A-B	0.643	-0.252	0.232	-0.232
C-D	0.893	0.927	0.975	0.097
E-F	-0.615	0.999	-0.014	0.361
G-H	-0.974	0.144	0.882	0.116
İ-J	0.273	0.965	0.974	0.728
K-L	0.974	0.822	0.996	0.051
M-N	-0.819	0.179	0.910	0.038
O-P	0.997	0.842	-0.030	-0.024
R-S	-0.972	-0.182	0.123	-0.150
T-U	0.686	0.914	0.024	0.678

Table 4. Comparison statistics

n	Sig t test	Pearson	Permutation	Difference	Wilcoxon Sign test
5	0,546	0,1896 ± 0,2182	0,1674 ± 0,1908	0,02219 ± 0,11185	0,317
10	0,456	0,2454 ± 0,3121	0,2424 ± 0,3162	0,00304 ± 0,01235	1,000
50	0,603	0,2661 ± 0,3654	0,2660 ± 0,3653	0,00012 ± 0,00070	1,000
100	0,027	0,2870 ± 0,3160	0,2879 ± 0,3170	0,00087 ± 0,00033	1,000

Wilcoxon sign test was used to analyze whether the coefficient' significance differs on acceptance of the null hypothesis.

Figure 1. Differences by sample sizes for the Pearson correlation coefficient



According to the information obtained as a result of the analyzes, it was understood that there was no statistical difference between the results of the permutation test and parametric t test for Pearson correlation. In addition, Wilcoxon sign test results did not show any difference in the decision to accept the null hypothesis. However, 10% of the decision made differed in accepting the null hypothesis for sample size five. In line with the results obtained, the permutation test can be recommended in estimating the Pearson correlation coefficient of the data sets with a small sample size.

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COMPARATIVE EXAMINATION OF FlOCK AVERAGE AND INDIVIDUAL VALUES MODELING IN LACTATION CURVES

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Abstract

In this study, linear spline, quadratic spline, cubic spline, logarithmic linear, logarithmic quadratic, linear hyperbolic, inverse polynomial, Wilmink, Wood, Cobby and Le Du, Brody and Dhanoa models were considered in modeling dairy cattle lactation curves. Corrected coefficient of determination, mean squared error, Durbin-Watson and Akaike Information Criteria were considered as model comparison criteria. In this study, lactation records of 50 Holstein Dairy cattle were used as material. Modeling was done on both herd average and individual basis. In the study, it was tried to determine which model stood out in herd average and individual modeling and which model comparison criteria were effective. According to the results obtained, it was concluded that the degree of accuracy was higher in individual modeling, and individuals with very good or very bad values were overlooked in the modeling made over the population average.

Key words: *Lactation Curve, Individual Comparison, Herd Average, Modeling*

INTRODUCTION

Milk production in Turkey and in the world is made from ovine and bovine animals. However, a large part of this production is obtained from cattle in the world and in Turkey. The production process, which starts from calving and continues until it is dried, is called "lactation" and it is generally accepted that the lactation period is 305 days in dairy cattle. In the second month following calving, daily milk yield reaches its maximum level (peak period;). After the peak period, the yield tends to decrease gradually (persistence). It is possible to list the factors affecting milk yield under sub-headings as race, lactation period, live weight, age of the animal, milking number, interval and duration, heat, nutrition, environmental temperature, calving season, dryness period and diseases (Özyurt and Özkan, 2009; Atashi et al. 2009). Lactation curve is a graphical representation of milk yields according to certain time intervals (Orman and Ertuğrul, 1999; Orhan and Kaygısız, 2002; Kaygısız et al., 2003). Knowing the functional structure of the lactation curve of the flock at hand provides benefits to the breeder in many ways. Since the lactation curves to be made on the basis of herd use the values of the herd average, the curve to be obtained will represent the general structure of the herd. In this case, the selection of individuals higher or lower than the herd average becomes difficult. On the other hand, obtaining individual lactation curves will provide great convenience in the selection of individuals to be separated for breeding and weeding in the herd. This will lead to an increase in the degree of accuracy in selection. On the other hand, it will prevent erroneous selections in selection and sorting. In this study, a comparison of the lactation curves and calculated values obtained from the herd average and individual values will be made and the results will be evaluated in terms of breeding and breeding.

MATERIALS AND METHODS

In this study, 305-day lactation milk yields of 100 randomly selected Holstein dairy cattle were used. The Wood model (Orhan and Kaygısız, 2002; Keskin and Tozluca, 2004), which is widely used and gives reliable results, was taken into account in modeling the lactation curves. Wood's model was first applied to an individual with both a very good lactation tendency and a bad lactation tendency, taking into account the population mean values. Afterwards, the mean values of the population and the mean squared error of individual values (very good and bad), corrected determination coefficients, Durbin-Watson autocorrelation values and Akaike Information Criteria values were calculated.

Wood's Model Wood's equation (Wood, 1967), which is widely used in modeling lactation curves and gives reliable results, is $Y(t)= at^{b}e^{-}$

$^{(ct)}$. Here, Y(t): t of lactation. day milk yield (kg), a: initial milk yield, b: slope until reaching the highest yield, c: slope after the highest yield, t: time (day), e: natural logarithm base.

The equations for the mean squared error (MSE), adjusted coefficient of determination ($\bar{R}^2$), Durbin-Watson autocorrelation test (DW) and Akaike information criterion (AIC) used in the evaluation of the models are as follows.

MSE=ESS/(p-2)

in equality , HKT: Error sum of squares, n: number of observations, p: number of parameters in the model. The model with the smallest value is considered to be the most suitable model.

$\bar{R}^2=1-[(n-1)(n-P)](1-R^2)$

In the equation, n: number of observations, p: parameter in the model number, R^2: coefficient of determination. The value of the coefficient of determination is a measure of how much of the curve model created according to the data set can represent the total change in the data set. A high coefficient of determination means that the fit of the model to the point distribution is high.

$$DW = \frac{\sum_{t=2}^{n}(e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^2}$$

In the equation, e_t : error at time , e_{t-1} : error at time t-1 is an error. The DW value always lies between 0 and 4, and if the value is 2, it is assumed that there is no autocorrelation.

AIC=nln(HKT/n)+2k

In the equation, HKT: Error sum of squares, n: Number of Observation Pairs, k: indicates the number of parameters in the model. The model with the smallest value is considered to be the most suitable model. Prameter estimates and lactation curves were made in the SAS package program. (SAS, 1999).

RESULTS AND DISCUSSION

In dairy cattle, expressing their production with appropriate models allows estimating their production in a lactation period and throughout their lifetime. Prediction of milk production is extremely important for accurate breeding. For this reason, the selection of suitable models is extremely important in terms of time and cost. Selection of the most suitable model will allow selection and selection over individual lactation curves at the start of selection. This will lead to an increase in the degree of accuracy in selection studies. In lactation curve modeling based on herd averages, selection of individuals with superior genetic structure or selection of individuals with low milk yield is relatively difficult compared to individual modelling.

When the results in Table 1 are examined, the mean square error of three different lactation curves, the corrected coefficient of determination. It is seen that the best model in terms of Durbin-Watson autocorrelation values and Akaike Information Criteria values is model number 2. The model number 3 has the worst results statistically ppears to have better results.

Lactation curves obtained from individuals with good and bad yields on the average of the population are given in Figure 1, Figure 2 and Figure 3, respectively. When examined in all three ways, the positions of the points according to the curve and their general distributions support the results in Table 1.

Table 1. Values from three different data sets.

1. Modeling Style	EMS	$\bar{R}^2$	DW	AIC
Population	2.940	0.965	2.334	-6.015
2. individual good	1.070	0.981	2.012	-23.6
3. individual bad	4.158	0.924	1.125	2.457

EMS: Error mean squares, **$\bar{R}^2$:** Adjusted coefficient of determination, **DW:** Durbin-Watson autocorrelation test, **AIC:**:Akaike Knowledge Criteria

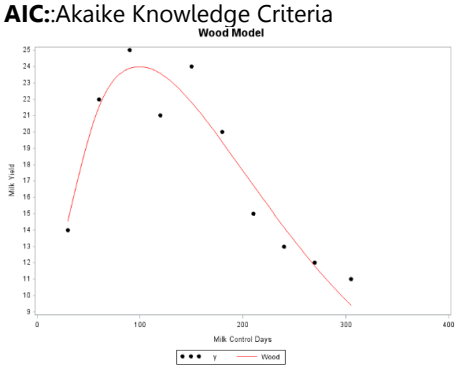

Figure 1 . The lactation curve of the population mean.

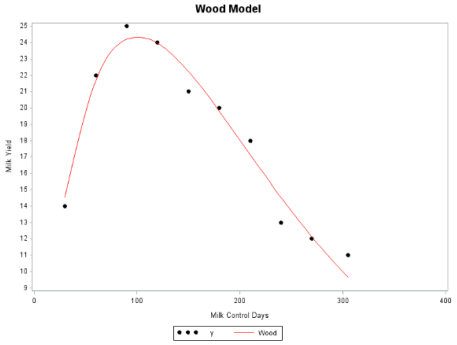

Figure 2 . Lactation curve of the individual with good lactation efficiency.

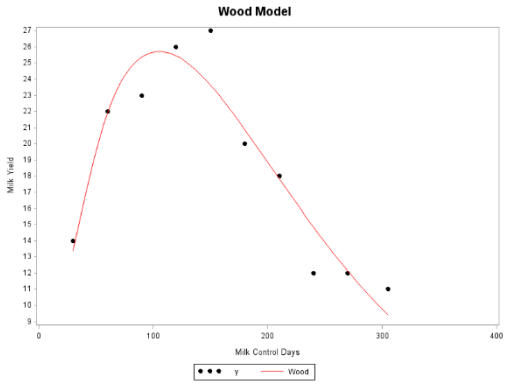

Figure 3 . Lactation curve of individuals with poor lactation efficiency.

CONCLUSIONS

As a result, the values to be obtained by a modeling based on the population mean are the values of the population. The interpretations to be made here belong only to the population and are not suitable for interpretation on an individual basis. When evaluated according to model evaluation criteria, it can give an idea about whether the herd is managed properly in terms of breeding. Or it can give a preliminary idea about the presence of low or high yielding animals in the herd. However, during the selection or weeding phase, the breeder will feel the need to identify low and high yielding individuals. At this point, it is seen that these results obtained over the population average are not very healthy and suitable for use. Although it is time consuming, it is imperative to obtain model evaluation criteria and lactation curves by individual modelling. In this case, the researcher will easily be able to select high productive individuals by selection, and will be able to weed out low productive individuals without hesitation. Considering that it will take a long time for the selection and sorting results to be reflected in the herd, it is obvious how accurate individual modeling is.

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MULTIPLE-RESPONSES MARS DATA MINING ALGORITHM MODELING THROUGH ehaGof PACKAGE IN REGRESSION TYPE PROBLEMS

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Abstract

In the statistical modeling studies carried out in the last ten years; The prediction performances of decision trees, artificial neural networks and MARS (Multivariate Adaptive Regression Splines) algorithms are comparatively examined.The MARS algorithm, which is an improved version of the CART algorithm, has been the focus of researchers in determining the high-level relationships between the studied variables. There is still a lack of information in the literature about multi-response MARS models for regression-type problems in livestock. Therefore, this study was conducted to develop an R script of a multi-response MARS model for regression-like problems using the "cbind function" in R software. The R script was developed to calculate eligibility criteria for each continuous response. When the Earth package has more than one (k) continuous dependent variable (multiple responses), it creates k concurrent prediction models. This package attempts to minimize the sum of the GCV values of the k dependent variables. In the study; To select the best predictive models, the ideal tuning parameters, namely the number of selected terms (k) and the degree of interaction (degrees), were assigned for the lowest GCV value.

Our work will be a respected reference for researchers in different scientific fields to construct a prediction equation for continuous response of the R script file developed on the MARS multi-response model for regression type problems.

Key words: *MARS, multiple-responses, earth package, data mining, CART*

DETERMINATION OF CARCASS WEIGHT AND SLAUGHTER WEIGHT WITH THE MULTIRESPONSE MULTIVARIATE ADAPTIVE REGRESSION SPLINES FOR JAPANESE QUAILS (COTURNIX COTURNIX JAPONICA)

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Abstract

The main aim of the study was to determine the carcass weight and slaughter weight with some carcass characteristics, early and late thermal manipulation in incubation period and body weight for different age of Japanese quails. For this aim, Multiresponse Multivariate Adaptive Regression Splines (MMARS) was utilized to determine the carcass weight and slaughter weight of Japanese quails. The MMARS model with 21 terms with the interaction effect for determining the best model for carcass weight and slaughter weight prediction within the scope of the lowest RMSE value. According to the MMARS model reflected that sex (for male), age (for 6, 7 and 8 week), rump weight, breast weight, back weight, leg weight, egg weight, different thermal manipulation incubation (for control and late embryogenesis) were affected to determine the carcass weight and slaughter weight for Japanese quails. In conclusion, it could be recommended that MMARS method is useful for determining the carcass and slaughter weight for Japanese quails.

Key words: *Japanese quail, carcass weight, slaughter weight, MMARS, carcass characteristics*

INVESTIGATION OF GROWTH CURVE PARAMETERS WITH RICHARD MODEL

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Abstract

This study was carried out to determine the fit of individual growth curves in Richard modeling of Japanese quails with both female and male quail data. A total of 810 quail data were used as material in the study. For the Richard model in Japanese quails MSE, adjusted R2, AIC and DW values were compared in both female and male quails. In addition, the growth curve parameter estimates of the model are also shown. This study was carried out to determine the fit of individual growth curves in Richard modeling of Japanese quails with both female and male quail data. A total of 810 quail data were used as material in the study. For the Richard model in Japanese quails MSE, adjusted R2, AIC and DW values were compared in both female and male quails. In addition, the growth curve parameter estimates of the model are also shown.

Key words: *Richard, Modeling, Parameters*

DETERMINATION OF MASTITIS LEVEL USING ARTIFICIAL NEURAL NETWORKS FROM THERMAL IMAGES

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Abstract

In this study, it was aimed to determine the severity of mastitis from milk cow udders using image processing methods and artificial neural networks. The udders of 200 milking cows in a commercial enterprise were photographed with the help of a thermal camera before milking. With the help of the gimbal, the camera-induced shaking of the photos is prevented. Somatic cell count was determined by taking milk samples from photographed udders, and udders photographs and somatic cell counts were transferred to the computer. The California Mastitis Test scores of the udders were tried to be estimated by processing the obtained images. As a result of the study, the estimation value obtained from the artificial neural network analysis was found to be 0.55. The results showed that artificial neural networks cannot be used to determine California Mastitis Test scores.

Key words: *Mastitis, Artificial neural networks, Milk, Cow udders, Thermal camera, Thermal image*

USE OF DEEP LEARNING TO DETERMINE THE FRESHNESS OF EGG

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Abstract

The freshness of the egg is important for both hatching and human consumption. It is quite difficult to determine the freshness of the egg without damaging it with classical methods. Deep learning is a powerful method used to classify data without processing or with much less processing. In this study, 50 eggs were photographed as experimental material for 29 days and the images obtained were used as data. It is aimed to determine how many days old the eggs are, which are foldered according to the days of the photos obtained. As a result of the study, 91.78% valuation accuracy value was obtained. Obtaining inputs without preprocessing shows that Deep learning method can be used when fast decision is required and the machine needs to make its own decision.

Key words: *Egg, Freshness, Deep learning, Non destructive*

ANALYSIS OF COVARIANCE IN COMPLETELY RANDOMIZED DESIGNS USING R AND AN APPLICATION

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Abstract

In completely randomize design, the trial units in the groups must be homogeneous. This homogeneous material is randomly divided into as many groups as the number of applications. After that applications are randomly distributed into these groups. In many studies, homogeneous material may not be provided. In this case, initial measurements of the material are made and considered as covariate in the analysis. This heterogeneity in the material is tried to be eliminated by analysis making covariance analysis. In this study, the construction and interpretation of covariance analysis in completely random trials is introduced using R software.

Key words: *Covariance, Multiple comparison, Randomize design*

THE EFFECTIVENESS OF USING BULLS WITH A HIGH IMMUNE RESPONSE IN THE IMPROVEMENT OF MILK PRODUCTION TRAITS OF HOLSTEIN-FRIESIAN COWS

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Abstract

Currently, there is a dominant trend in livestock farming to reduce the use of antibiotics. Therefore, research that achieves this goal by genetically improving the immunity of animals may be of interest. The aim of the research was to evaluate the effectiveness of the use of bulls with an increased immune response in improving the milk yield and composition of Polish Holstein-Friesian cows. The research covered 5,093 cows calved in 2013-2020, kept in 7 Polish herds. Among them, 1,014 cows were the offspring of bulls with an increased immune response (named HIR), including 642 animals related to them in the first generation (group HIR50) and 373 in the second or third generation (HIR25). 4,078 animals were not related to HIR bulls (HIR0). The cows included in the study were controlled in terms of lactation (1 and 2), lactation milk yield (kg) and content of fat and protein, lactose and dry weight (%) in milk. The multivariate analysis of variance model took into account the influence of the following main factors: herd, calving year and calving season, age at first calving, genotype participation of HIR bull (HIR) in the cow genotype and interactions: HIR × herd, year × calving season. The significance of differences between the compared groups was tested using the Scheffé test. The analysis of variance showed a statistical effect of the share of HIR bulls on the milk yield and its protein content in the first and second lactation, and on lactose in the first lactation. It was found that daughters of HIR bulls (HIR50) by about 425 kg in the first (12,072.31 kg vs 11,646.88) and 819.48 kg in the second (13,294.32 kg vs 12,474.84 kg) statistically outperformed their peers unrelated to HIR bulls (HIR0). Daughters of HIR bulls (HIR50) significantly exceeded their HIR0 and HIR25 peers in terms of lactose content in milk in the first lactation, by 0.04 percentage point, respectively. At the same time, the HIR50 group was inferior to the other groups in terms of milk protein content in the first lactation (3.32 vs 3.41 for HIR0, 3.39 for HIR25) and in the second lactation (3.31 vs 3.39 for HIR0, 3.35 for HIR25). The conducted research leads to the conclusion that the use of bulls with increased immunological resistance in insemination of cows of the Holstein-Friesian breed may promote higher milk yield, accompanied by a decrease in its protein content.

Key words: *dairy cattle; milk yield; bulls; high immune response*

LONGEVITY OF POLISH HOLSTEIN-FRIESIAN COWS MILKED AUTOMATICALLY

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Abstract

Research findings show that longevity is playing an increasing role in breeding programmes because it has a significant effect on the profitability of dairy cattle breeding. Longer use of cows has a positive effect on the minimization of milk production costs, as well as the reduction of outlays on heifers rearing. Therefore, this study was undertaken to evaluate the effect of changing the milking system from conventional to automatic on the longevity of Polish Holstein-Friesian dairy cows. The study was conducted on a group of 1480 Polish Holstein-Friesian (PHF) which were used in two herds located in Poland equipped with conventional (CMS) and then automatic milking systems (AMS) made by Lely - milking robots "Astronaut A4". The cows included in the study were controlled for the following traits: number of parturitions, life span, and length of productive life, lifetime yield of milk, fat and protein, and cause of culling. Multivariate analysis of variance was applied to determine the factors responsible for the controlled traits of cow longevity, milk yield and composition. In turn, causes of culling were analysed using the χ2 test of independency The conducted multivariate analysis of variance showed a highly significant effect of changing milking system from CMS to AMS on all the controlled traits of longevity as well as life yield of milk, protein and fat. On the basis of the conducted research, it was shown that cows used in cowsheds during the period of automatic milking were characterised by a highly significant 0.26 higher number of calving intervals, life expectancy was prolonged by 6 days, while use by 8 days, improved milk yield than during the period of application of conventional milking. This fact allows us to conclude that further implementation of the automatic milking system in dairy farms should lengthen the lifespan, use of cows, increase the lifetime yield of milk, and thus affect the profitability of production. The study found a highly significant effect of herd × milking system interaction on the number of births. In herd A (new building), cows were milked about 276 days longer than in herd B (adopted building), while their total life span was 283.94 days longer. This allows us to conclude that a significantly better effect of the robotic milking system in terms of improved cow longevity and milk yield can be expected when the automatic milking system is installed in a new barn than in a modernized ones. The χ2 test did not show a statistical relationship between the milking system and reasons for cows not milking. Regardless of the milking system, cows were most often culled due to, in decreasing order: sterility and diseases of the reproductive system, random and other accidents, udder diseases, diseases of the locomotor system and metabolic disorder, digestive and respiratory system diseases.

Key words: *dairy cattle, automatic milking system, longevity*

EFFECTS OF MELATONIN ON THE MATURATION OF BOVINE OOCYTES

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Abstract

A total of 438 cumulus-oocyte complexes in 5 replications were used in this study to determine the effects of melatonin on the maturation and subsequent in vitro development of bovine oocytes. Melatonin, which is synthesized rhythmically by the pineal gland, affects the reproductive system and acts as a free radical scavenger. To determine the effects of melatonin at a concentration of 10-11 M in the follicular fluid, maturation media containing melatonin at a concentration of 0, 10-5, 10-7, 10-9 M were prepared. Cumulus oocyte complexes were taken from the ovaries obtained from the cattle slaughtered in the slaughterhouse; the ones of suitable quality were randomly distributed to the experimental groups and incubated for 20-24 hours in air at 5% CO2 and maximum humidity conditions. As a result of incubation, the proportion of embryos reaching the cumulus enlargement and cleavage stage was determined. As a result of the research, it was determined that cleavage rates were the highest (P<0.05) in incubation with media containing 10-9 M melatonin, while increasing melatonin concentration decreased the cleavage rate significantly (P<0.05). However, it was determined that the effect of melatonin on cumulus enlargement was insignificant. In conclusion, our present study shows that melatonin can significantly increase the efficiency of bovine oocyte maturation in vitro. This beneficial effect of melatonin is mediated by melatonin receptors.

Key words: *Bovine, IVF, Melatonin, Maturation*

GENETIC AND NON-GENETIC RISK FACTORS FOR MAJOR DISEASES IN HOLSTEIN DAIRY CATTLE OF AN INDUSTRIAL HERD

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Abstract

Nowadays, breeding is mostly aimed to increase livestock resistance against diseases while improving level of yield and consequently increasing the economic lifespan (longevity) of the animal. Health traits in the profitability of dairy cattle farms are related to yield traits, but due to the lack of reliable information about health disorders, the inclusion of these traits in selection programs faces limitations. The possibility of taking diseases into account in the selection of dairy cows depends on the economic importance of the diseases. The aim of this study is to estimate the genetic parameters of yield traits and some disease traits. In this study, the health and yield information of 1600-Azar Negin herd during 2018-2020 as well as their pedigree information were examined. Today, breeding mostly concentrated on breeding bull to resist against diseases while increasing yield and subsequently increasing the economic life of the animal are taken into consideration. It is while, in the past, these breeding goals focused on the productive traits of bull. The traits studied in this work include mastitis, infertility, lameness, 305-day milk production, fat percentage and milk protein percentage. The aim of this study was to estimate the genetic parameters of yield traits and some disease traits. In this study, we aimed to estimate the contribution of environmental and genetic factors affecting reproductive and productive abnormalities and to find the relationship between the two in animals and yield and reproductive traits among Azar Negin commercial herd. For this purpose, disease and yield information of 1600-Azar Negin industrial herd were studied and analyzed by ASReml software and predictable results were expressed in two parts. The first part of the results was the effect of environmental factors on the incidence of diseases. In the second part, the results of the genetic value of animals for the studied diseases (infertility, lameness and mastitis) were expressed. The results of descriptive statistics showed that infertility with an incidence rate of 2.27% has the highest incidence rate among other diseases. Lameness was the second most common disease with 1.38% and mastitis had the lowest incidence with 1.18%. Logistic regression analysis was used to investigate the effect of milk yield (as a continuous variable) and other factors on the incidence of a disease (as a two-sentence response variable). Other constant effects include birth order, calving year, calving season and birth status. The results of logistic regression analysis showed that the effect of calf birth is significant and positively related to mastitis, infertility and lameness ($p<0.05$). The calving year and calving season have a significant relationship with infertility. Lameness is significantly associated with 305-day milk production. Examination of Spearman correlation between disease traits and production traits shows that mastitis has a positive and significant relationship only with birth order (0.17). The relationship between infertility and calving year (-0.08), calving status (0.06) and birth order (0.08) was significant. Lameness was also significantly associated with calf birth status (0.08) and birth order (0.1). Disease traits (infertility, lameness and mastitis) had a positive genetic correlation with calving frequency, indicating that cows are genetically more susceptible to disease by increasing calving frequency. The results of the breeding value of male bull related to yield traits and disease traits showed that the more increased the protein and fat of milk, the more susceptible the bull to disease. Male animals with high breeding value for fat and milk protein had undesirable breeding value for mastitis. Also, no relationship was found between desirable male bull in terms of breeding value of milk yield and male breeding with undesirable breeding value for mastitis. In general, most disease traits have a positive genetic correlation with calving, which indicates that cows are genetically more susceptible to disease by increasing calving. In this study, the genetic correlation of 305-days milk yield with lameness was positive and with mastitis and infertility was negative.

Key words: *Dairy cattle, Holstein, milk, Disease, Genetic resistance*

ANTIBIOTIC RESISTANCE PROFILES OF *HALOARCULA SALARIA*, *HALOBACTERIUM SALINARUM* AND *HALOARCULA TRADENSIS*

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Abstract

*Salt-loving halophilic microorganisms such as extremely halophilic archaea inhabit saline environments. In the leather industry, the salt-containing extremely halophilic archaea producing protease and lipase may contaminate hides and skins during the preservation period and their growth causes red heat on salted hides and skins. Protease and lipase enzymes produced by extremely halophilic archaea may cause malodor and the loose grain, and as a result of these enzymatic activities, low-quality leather products are obtained. The antibiotic-resistant extremely halophilic archaea may be found on salted hides and skins. In the present study, the antibiotic resistance profiles of proteolytic and lipolytic extremely halophilic archaeal isolates (*Haloarcula salaria *AT1,* Halobacterium salinarum *22T6,* Haloarcula tradensis *7T3), which were obtained from deteriorated salted sheepskin samples, against penicillin G (10 units), bacitracin (0.04 units), ampicillin (10 µg), novobiocin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), rifampicin (5 µg), streptomycin (25 µg), and erythromycin (15 µg) were examined by Kirby-Bauer disc diffusion method.* Haloarcula salaria *AT1,* Halobacterium salinarum *22T6,* Haloarcula tradensis *7T3 were resistant to penicillin G (10 units), bacitracin (0.04 units), ampicillin (10 µg), gentamicin (10 µg), streptomycin (25 µg), and tetracycline (30 µg). The inhibition zone diameters for Haloarcula salaria against chloramphenicol (30 µg), novobiocin (5 µg), rifampicin (5 µg), and erythromycin (15 µg) were respectively measured as 15 mm, 40 mm, 13 mm, and 12 mm. While the inhibition zone of 30 mm for Halobacterium salinarum was observed against novobiocin (5 µg), inhibition zones for Haloarcula tradensis against novobiocin (5 µg) and rifampicin (5 µg) were respectively measured as 40 mm and 10 mm. In conclusion, multi-drug resistant extremely halophilic archaeal strains were isolated from salted sheepskins obtained from the leather industry.*

Key words: *leather industry, Haloarcula salaria AT1, Halobacterium salinarum 22T6, Haloarcula tradensis 7T3, protease enzyme, lipase enzyme, antibiotics*

INTRODUCTION

In the twentieth century, one of the most important achievements in medicine is the discovery of antibiotics (Boyd et al., 2021). However, antibiotic resistance genes and antibiotic-resistant microorganisms are found in nature (Hall and Barlow, 2004; Wright, 2007). The main reasons for antibiotic resistance are misuse and overuse of antibiotics that cause the development of new antibiotic-resistant microorganisms. Moreover, poor infection prevention and their control contribute to the development of new antibiotic-resistant microorganisms. These microorganisms distribute in humans, farm animals, aquatic animals, and the environment (Ji et al., 2012; Watts et al., 2017; Xiong et al., 2018; Zuhang et al., 2021). Antibiotic resistance may occur by genetic changes among microorganisms and mutation (Xiong et al., 2018; Zuhang et al., 2021). Aquatic environments such as drinking water, lake, hypersaline lakes, salterns, wastewater, and sea are the reservoirs for antibiotic resistance genes (Arahal et al., 1996; Karkman et al., 2018; Amarasiri et al., 2020).

Researchers stated that antibiotic resistant extremely halophilic archaea was found in Tuz Lake (Birbir et al., 2007). Tuz Lake, which is a hypersaline lake found in Central Türkiye, harbors extremely halophilic archaeal genera such as *Haloquadratum*, *Haloarcula, Halorhabdus*, *Halorubrum, Halonotius, Natronomonas, Halolamina, Halobacterium, Halosimplex, Halomicrobium,* and *Haloplanus* (Birbir et al., 2007; Akpolat et al., 2021).

The salt produced in Tuz Lake is used in the leather industry for preserving hides and skins (Birbir and Sesal, 2003). In a previous study, the researchers tested the susceptibilities of extremely halophilic archaea against amikacin (30 µg), ampicillin (10 µg), bacitracin (10 units), cefadroxil (30 µg), chloramphenicol (30 µg),

ciprofloxacin (5 µg), erythromycin (15 µg), neomycin (30 µg), novobiocin (5 µg), penicillin G (10 units), spiramycin (100 µg), streptomycin (25 µg), and sulfamethoxazole-trimethoprim (25 µg) (Birbir et al., 2007). The researchers reported that while extremely halophilic archaeal isolates were resistant to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), neomycin (30 µg), penicillin G (10 units), spiramycin (100 µg), and streptomycin (25 µg), but all were sensitive to bacitracin (10 units), novobiocin (5 µg). In addition, some of the extremely halophilic archaeal isolates were found to be susceptible to amikacin (30 µg), cefadroxil (30 µg), and sulfamethoxazole-trimethoprim (25 µg) in that study (Birbir et al., 2007). That study showed that the antibiotic-resistant extremely halophilic archaea found in preservation salt may contaminate hides and skins during the curing process in the leather industry.

The test isolates (*Haloarcula salaria* strain AT1, *Halobacterium salinarum* strain 22T6), *Haloarcula tradensis* strain 7T3) used in the present study were previously isolated from different saline environments by the researchers. *Haloarcula salaria* strain HST01-2R^T and *Haloarcula tradensis* strain HST03^T were isolated from salt in a fish sauce sample in Thailand (Namwong et al., 2011). The researchers applied different antibiotics against those two extremely halophilic archaeal species. They reported that *Haloarcula salaria* strain HST01-2R^T) was resistant to ampicillin (30 µg), chloramphenicol (30 µg), gentamicin (30 µg), rifampicin (30 µg), streptomycin (30 µg), tetracycline (30 µg), and novobiocin (5 µg), but susceptible to bacitracin (10 µg) (Namwong et al., 2011). *Haloarcula tradensis* strain HST03^T was reported as resistant to ampicillin (30 µg), chloramphenicol (30 µg), gentamicin (30 µg), rifampicin (30 µg), streptomycin (30 µg), and tetracycline (30 µg), but *Haloarcula tradensis* strain HST03^T was susceptible to bacitracin (10 µg) and novobiocin (5 µg) (Namwong et al., 2011). *Halobacterium salinarum* was isolated from brine samples obtained from saltern crystallizer ponds in the Israel Salt Company (Oren and Litchfield, 1999) and halite crystals obtained from Badwater salt pan in California (Mormile et al., 2003). *Halobacterium salinarum* was found to be sensitive to anisomycin and novobiocin, but resistant to ampicillin, chloramphenicol, gentamicin, streptomycin, tetracycline, kanamycin, vancomycin (Grant, 2001; Oren, 2014).

Although antibiotic resistance profiles of extremely halophilic archaea found in hypersaline environments were examined in the previous studies, antibiotic resistance profiles of extremely halophilic archaea isolated from deteriorated salted sheepskins have not been investigated yet. Hence, the goal of the study was to examine the resistance of *Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6, *Haloarcula tradensis* 7T3 isolated from deteriorated sheepskins with red heat against penicillin G (10 units), bacitracin (0.04 units), ampicillin (10 µg), novobiocin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), rifampicin (5 µg), streptomycin (25 µg), and erythromycin (15 µg) by Kirby-Bauer disc diffusion method.

MATERIALS AND METHODS

Antibiotic susceptibility tests

Susceptibility or resistance of extremely halophilic archaea (*Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6, and *Haloarcula tradensis* 7T3) to novobiocin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), rifampicin (5 µg), streptomycin (25 µg), and erythromycin (15 µg) was determined by Kirby-Bauer disc diffusion method (Bauer et al., 1966). Novobiocin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), rifampicin (5 µg), streptomycin (25 µg), and erythromycin (15 µg) were purchased from Oxoid (Basingstoke, Hants, UK).

Pure culture of *Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6, and *Haloarcula tradensis* 7T3 were grown into Brown liquid medium containing 2 g KCl, 20 g $MgSO_4.7H_2O$, 1 g $CaCl_2.H_2O$, 250 g NaCl, 3 g tri-Na-citrate, 5 g yeast extract, 1000 ml sterile distilled water at 39°C for 10 days. After the incubation, steril saline solution (25% NaCl) containing each isolate was adjusted to 10^8 CFU/ml. Later, each isolate was evenly spread on a Brown agar medium. The antibiotic discs were placed on the surface of the inoculated Brown agar medium and then the plates were incubated at 39°C for 10 days. At the end of the incubation, the inhibition zones around the antibiotic discs were measured.

RESULTS AND DISCUSSION

Due to the absence of peptidoglycan in the cell wall of extremely halophilic archaea, *Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6, *Haloarcula tradensis* 7T3 were resistant to penicillin G (10 units), bacitracin (0.04 units), and ampicillin (10 µg) (Table 1). Inhibition zone diameters for *Haloarcula salaria*, *Halobacterium salinarum*, and *Haloarcula tradensis* against novobiocin were respectively measured as 40 mm, 30 mm, and 40 mm. Inhibition zones of *Haloarcula salaria* against chloramphenicol, rifampicin, and erythromycin

were respectively detected as 15 mm, 13 mm, and 12 mm. The inhibition zone of *Haloarcula tradensis* against rifampicin was 10 mm (Table 1).

Birbir and her colleagues (2007) stated that while extremely halophilic archaeal isolates were resistant to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), neomycin (30 µg), penicillin G (10 units), spiramycin (100 µg), and streptomycin (25 µg), but they were sensitive to bacitracin (10 units), novobiocin (5 µg). Our test isolates were resistant to ampicillin (10 µg), bacitracin (0.04 units), penicillin (10 units), streptomycin (25 µg), *Halobacterium salinarum* and *Haloarcula tradensis* were resistant to chloramphenicol (30 µg) and erythromycin (15 µg) (Table 1).

The researchers previously isolated our test isolates (*Haloarcula salaria*, *Halobacterium salinarum,* and *Haloarcula tradensis*) from a food salt sample, brine sample, and halite crystal (Oren and Litchfield, 1999; Mormile et al., 2003; Namwong et al., 2011). *Haloarcula salaria* strain HST01-2R^T was found to be susceptible to bacitracin (10 µg), but resistant to chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (30 mg), gentamicin (30 µg), rifampicin (30 µg), streptomycin (30 µg), and novobiocin (5 µg) (Namwong et al., 2011). In the present study, while *Haloarcula salaria* was resistant to bacitracin (0.04 units), tetracycline (30 µg), ampicillin (30 µg), gentamicin (30 µg), and streptomycin (30 µg), it was susceptible to chloramphenicol (30 µg), rifampicin (30 µg), erythromycin (15 µg), and novobiocin (5 µg) (Table I).

Namwong et al. (2011) reported that *Haloarcula tradensis* strain HST03^T was susceptible to bacitracin (10 µg) and novobiocin (5 µg), but resistant to chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (30 µg), gentamicin (30 µg), rifampicin (30 µg), and streptomycin (30 µg). In this study, while *Haloarcula tradensis* was resistant to erythromycin (15 µg), chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (30 µg), gentamicin (30 µg), penicillin (10 U), bacitracin (0.04 U) and streptomycin (30 µg), it was susceptible to rifampicin (30 µg), and novobiocin (5 µg) (Table 1).

Table 1. Antibiotic susceptibility of *Haloarcula salaria*, *Halobacterium salinarum*, *Haloarcula tradensis*

Antibiotics	***H. salaria***	***H. salinarum***	***H. tradensis***
Gentamicin (10 µg)	-	-	-
Streptomycin (25 µg)	-	-	-
Chloramphenicol (30 µg)	15 mm	-	-
Tetracycline (30 µg)	-	-	-
Ampicillin (10 µg)	-	-	-
Penicillin (10 units)	-	-	-
Bacitracin (0.04 units)	-	-	-
Novobiocin (5 µg)	40 mm	30 mm	40 mm
Rifampicin (5 µg)	13 mm	-	10 mm
Erythromycin (15 µg)	12 mm	-	-

CONCLUSIONS

In the present study, *Halobacterium salinarum, Haloarcula tradensis,* and *Haloarcula salaria* showed resistance to nine (gentamicin, streptomycin, chloramphenicol, tetracycline, ampicillin, bacitracin, penicillin, rifampicin, erythromycin), eight (gentamicin, streptomycin, chloramphenicol, tetracycline, ampicillin, bacitracin, penicillin, erythromycin), and six (gentamicin, streptomycin, tetracycline, ampicillin, bacitracin, penicillin) antibiotics, respectively. This study proved that multidrug-resistant extremely halophilic archaea were found on the deteriorated salted sheepskins with red heat. Due to the detection of the multidrug-resistant extremely halophilic archaea producing proteolytic and lipolytic enzymes on the salted sheepskins, we recommend annihilation of these destructive archaeal isolates found in the preservation salt via direct or alternating electric current treatment or effective antimicrobial agents to prevent spoilage of salted hides and skins.

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IMPROVING SUSTAINABLE HUMAN-ANIMAL-ENVIRONMENT INTERRELATIONSHIP IN AFRICA: A DRIVE TO TACKLING ZOONOSIS IN NIGERIA WITH ONE HEALTH APPROACH

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Abstract

The health of humans and animals are interlinked with the environment. Majority of infectious diseases that affect human health are zoonotic i.e. originate from animals, and occur at the human-animal-environment interface. With the emergence of antimicrobial resistances and environmental pollution, addressing these diseases needs an interdisciplinary and intersectoral expertise. Nigeria is the first country in Africa to launch a One Health plan signed by the Ministers of Health Agriculture and Environment. Nigeria has developed a One Health strategic plan to meet its human, animal and environmental health challenges. This approach drives innovations that are important to manage the outbreaks we experience and offers synergy across our various Ministries, but there are several challenges at the level of implementation. The major bottlenecks in implementing One Health include absence of a legal framework to implement One Health, poor coordination among different governmental and private agencies, lack of proper surveillance of animal diseases, poor data-sharing mechanism across sectors, and limited budget. Implementing systematic zoonotic surveillance; regulated antibiotic use among humans and animals; development of a zoonotic registry in the country; constitution of a wide network of academic, research, pharmaceutical, and various implementation stakeholders from different sectors is the need of the hour to effectively use One Health in order to combat increasing zoonotic diseases.

Key words: *One health, Infectious diseases, Antimicrobial resistances, Zoonotic diseases*

THE EFFECT OF *Lactobacillus brevis* STRAIN ON SILAGE QUALITY IN TRITICALE SILAGES

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Abstract

The present study was carried out to investigate the effects of Lactobacillus brevis (LB) MF098783 lactic acid bacteria strain isolated from homemade pickles on triticale (Triticosecale Wittmack) silage as an inoculant, silage quality, aerobic stability, and microorganism growth. Working groups; control (T), Lactobacillus brevis lactic acid bacterial strain groups LB 10^6 ($TLAB^6$), LB 10^8 ($TLAB^8$) and LB 10^9 ($TLAB^9$) were created. Triticale was harvested during the dough maturity period, vacuumed into 1 kg bags with 5 repetitions and left for fermentation for 90 days. After fermentation, chemical, physical and microbiological analyzes were performed on silage samples. From the results of chemical analysis; nutrient contents, aerobic stability, water-soluble carbohydrates and physical analysis; Among the pH, temperature, L, a, b, chroma, h values, only the pH and temperature values from physical analyzes were found to be statistically significant (P<0.05). The pH values were found to be 5.60, 4.95, 4.99 and 4.88, respectively. In microbiological analysis: lactic acid, yeast-mold and after aerobic stability yeast-mold analyzes were found to be statistically insignificant (P>0.05). Lactic acid bacteria counts were determined as 2.88, N/A, 1.30 and 2.11 log10 cfu/g KM, respectively, and no mold was found in silages after staring and after aerobic stability analyses. As a result, the addition of LB to triticale silage contributed to the prevention of unwanted microorganism and yeast growth.

Key words: *aerobic stability, silage microbiology, Lactobacillus brevis, triticale silage*

INTRODUCTION

Ensiling is a microbial process and ensures that roughage with high water content is preserved for a long time without deterioration by improving the quality of roughage that will positively affect the yield performance of ruminants with minimum nutrient loss(Bai et al., 2022; Wang et al., 2022).

The most preferred maize is because of its high energy content for silage, being easy to ensilage and storing, as well as being delicious for animals. However, due to the fact that maize is not resistant to drought, it needs a lot of irrigation or the amount of precipitation in the region to be cultivated is low, farmers resort to different forage plant alternatives.

These alternative forage crops include sudan grass, oats, wheat, or triticale, etc. It can be used as an alternative forage crop to triticale, whose dry matter yield varies between 9-11 tons/ha (Özdüven et al., 2010).

Various additives are used to increase silage quality. Among these additives, lactic acid bacteria inoculants are mostly preferred to improve silage fermentation and aerobic stability (Han et al., 2022). Bacterial inoculants generally contain Lactobacillus, Pediococcus and Enterococcus bacteria (Erbil, 2012). Lactic acid bacteria generally contribute to the prevention of unwanted microorganism growth by increasing the lactic acid density in the silage microflora (Filya, 2001). Lactic acid bacteria (LAB) are divided into two as homofermentative and heterofermentative lactic acid bacteria according to the fermentation form of sugar in the environment to lactic acid (Basmacıoğlu and Ergül, 2002). Homofermentative LAB ferment the sugar in the environment to lactic acid via the glycolic pathway. In addition, it is known that they cannot use the phosphogluconate /phosphoketolase pathway in this process. On the other hand, $NADH+H^+$, which is formed from the dehydrogenation of glyceraldehyde-3-phosphate, is used to reduce pyruvic acid to lactic acid in the last step of the fermentation process (Çon ve Gökalp, 2000; Yılmaz, 2015).

Heterofermentative LAB can ferment sugars (hexose) to acetic acid in the presence of ethanol, carbon dioxide, or suitable electron acceptor other than lactic acid. On the other hand, it is known that they can ferment pentose sugars only to lactic acid. *Lactobacillus brevis* and *Lactobacillus buchneri* are the most used lactic acid bacteria in this group. LAB, in this group,

generally increase the aerobic stability of silage. On the other hand, it has been reported that feed consumption may be adversely affected due to the increase in the amount of acetic acid in the silage (Basmacıoğlu ve Ergül, 2002; Demirci, 2009; Yılmaz, 2015). Many studies have reported that the use of homofermentative LAB can be effective in rapidly decreasing the pH level, increasing the lactic acid density and consequently reducing dry matter loss by improving silage fermentation, while heterofermentative lactic acid bacteria can be effective in preventing yeast and mold formation, improving aerobic stability and increasing the amount of acetic acid (Han et al., 2022). In this study, the objective of the determine the effects of triticale silage of Lactobacillus brevis, a heterofermentative lactic acid bacteria, on fermentation, aerobic stability, and microorganism growth.

MATERIALS AND METHODS

In the study, triticale plant was obtained from the research field of Kırşehir Ahi Evran University Field Crops Department (Latitude: 39.1286"N, Longitude: 34.1078"E). Triticale harvested during the dough formation period was chopped to be 1.5-2 cm long. After the chopping process was completed, 1000 g of plant material was placed in 2 kg plastic bags and each group was sprayed with the heterofermentative *Lactobacillus brevis* MF098783 strain isolated from the pickle at a concentration of 1×10^6 cfu/g, 1×10^8 cfu/g and 1×10^9 cfu/g, respectively. After the incubation process, the air in the packages was vacuumed with the help of a vacuum device (Packtech PT-VKM-CPRO). In the study, a total of 20 silages were prepared with 5 replications in each group and left to fermentation in a dark environment at 20-22 °C under laboratory conditions for 90 days. Study groups were formed as control (T), triticale + L. brevis 10^6 ($TLAB^6$), triticale + L. brevis 10^8 ($TLAB^8$) and triticale + L. brevis 10^9 ($TLAB^9$), respectively. Four groups of three parallel samples were taken from the silages whose fermentation process was completed; (1) chemical [dry matter (DM), ash, ether extract (EE), crude protein (CP), crude fiber (CF), ADF, NDF, total soluble matter (TSM)], (2) data determined by calculations and energy values [DCP: Digestible crude protein,
TDN: Total digestible nutrient, DE: Digestible energy, ME: Metabolic energy, NE_L: Net energy-lactation, NE_M: Net energy-maintenance, NE_G: Net energy-gain] (3) physical (temperature, color, pH), (4) microbiological (lactic acid bacteria, yeast and mold counts) and (5) statistical analyzes were performed. DM, CP, EE, ash analysis according to AOAC (1998) standard procedure; CF, ADF and NDF analysis according to Van Soest et al. (1991) (ANKOM 200 Fiber Analyzer); pH values Chen et al. (1994); total soluble matter (TSM) contents were determined as explained by Singh et al. (2020). After the silage samples were opened, the L*, a*, and b* color values were measured from three different parts of the silage with the Konica-Minolta CR-410 color meter. Using the a* and b* values, the Chroma (C*, saturation index) and hue angle (h°) values were calculated according to describe of AMSA, (2012). In the study, the lactic acid bacteria, yeast, and mold counts contained in the silages were determined by the method reported by Seale et al. (1990). On the fifth day after open, the CO_2 value and pH values were determined as described by Ashbell et al. (1991). Metabolizable energy and protein values of silages with total carbohydrate (TC), hemicellulose, nitrogen free extract (NFE) and nitrogen free carbohydrate (NFC) were calculated as reported by Filik (2020). The relative feed value (RFV) and relative forage quality (RFQ) calculations of the silages were calculated according to the reported by Kılıç and Abdiwali (2016) and Filik (2020). SAS (2001) package program was used in statistical analysis, and the linear relations between the experimental groups were determined by applying orthogonal polynomial contrast with the General Linear Model (PROC GLM) procedure in accordance with the trial model of the study (random plots trial plan). The difference between the groups was made using the Duncan Multiple Comparison Method (Genç & Soysal, 2018).

RESULTS AND DISCUSSION

The chemical analysis results of the study, which aimed to determine the effects of different dilution ratios of the heterofermentative LAB Lactobacillus brevis on fermentation, aerobic stability, and microorganism growth in triticale silages as inoculants, are given in Table 1.
Dry matter contents were determined as 935.50, 939.55, 940.60 and 938.80 (g/kg) in T, TLAB6, TLAB8 and TLAB9 groups, respectively. DM increased in the treatment groups compared to the control group, and the differences between the groups were found to be very significant ($P<0.01$). In support of our study, Lee et al. (2016) reported that the dry matter content of triticale silages with Lactobacillus plantarum, Lactobacillus plantarum + enzyme, Lactobacillus plantarum + Lactobacillus buchneri and Lactobacillus plantarum + Lactobacillus buchneri + enzyme added increased compared to the control group. Total soluble matter (TSM) contents of silages were determined as 28.90, 26.98, 27.08 and 27.65 (% Bx), respectively, and the difference between the groups was found to

be statistically insignificant (P>0.05). Doğan (2019) reported that TSM values were found as 15.40, 16.20 and 14.43 in triticale silages [control, HMLAB+E (SILAID: Homofermentative LAB + enzyme, Lactobacillus plantarum, Enterecoccus faecium, cellulase, amylase, hemicellulase, and pentosanase) and HM+HTLAB+E (MICROBIOS: Homofermentative + heterofermentative LAB+ enzyme, Lactobacillus buchneri, Propionibacterium shermanii, Enterococcus faecium, Lactobacillus plantarum, cellulase, hemicellulose, and amylase)] harvested in the milk setting period, and 12.32, 10.63 and 13.26 in the silages harvested in the pulp setting period (P>0.05). According to Jia et al. (2021) determined TSM values as 15.3, 8.5, 12.6 and 13.1 in oat silages prepared [control, Lactobacillus buchneri, Lactobacillus rhamnosus, and Lactobacillus plantarum] by harvesting in the period before earing, and as 12.3, 10.3, 15.7 and 21.6 in the grain setting period (P<0.001). Chen et al. (2020) determined the TSM content in oat bale silages [control and Lactobacillus buchneri, Lactobacillus plantarum, and Pediococcus pentosaceus] as 3.0 and 14.6, respectively (P<0.001).

Table 3. Chemical analysis results of triticale silages

Groups	T	TLAB[6]	TLAB[8]	TLAB[9]	P
DM	935.50±0.00^{c}	939.55±0.05^{ab}	940.60±0.60^{a}	938.80±0.50^{b}	0.0030
OM	93.56±0.15	93.52±0.07	93.80±0.03	93.67±0.04	0.2634
Ash	6.44±0.15	6.48±0.07	6.20±0.04	6.33±0.04	0.2634
CP	10.76±0.06^{b}	11.00±0.06^{a}	10.31±0.01^{c}	10.41±0.07^{c}	0.0021
EE	4.49±0.04^{a}	4.30±0.03^{b}	4.30±0.04^{b}	4.33±0.02^{b}	0.0323
CF	25.70±0.14^{a}	24.91±0.04^{b}	24.80±0.09^{b}	24.71±.06^{b}	0.0052
ADF	31.35±0.06^{a}	30.00±0.07^{b}	29.73±0.01^{c}	29.58±0.04^{c}	<.0001
NDF	55.42±0.70	54.00±0.14	56.23±0.37	55.61±0.13	0.0690
Hcel	24.07±0.76^{b}	24.00±0.07^{b}	26.50±0.36^{a}	26.03±0.17^{a}	0.0290
TC	78.32±0.25^{b}	78.23±0.15^{b}	79.19±0.08^{a}	78.95±0.01^{a}	0.0265
NFC	22.90±0.45	24.24±0.00	22.97±0.45	23.34±0.12	0.1219
NFE	52.63±0.40^{b}	53.33±0.11^{b}	54.39±0.00^{a}	54.24±0.06^{a}	0.0112
ADFom	24.91±0.09^{a}	23.52±0.14^{b}	23.52±0.04^{b}	23.25±0.00^{b}	0.0006
NDFom	48.98±0.86	47.52±0.21	50.02±0.33	49.28±0.16	0.0813
TSM	28.90±0.79	26.98±0.15	27.08±0.71	27.65±0.28	0.1057

DM: Dry matter (g/kg), OM: Organic matter (%), CP: Crude protein (%), EE: Ether Extract (%), CF: Crude FiBER (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%), Hcel: Hemicellulose (%), TC: Total carbohydrates (g/kg), NFC: Nitrogen free carbohydrates (g/kg), NFE: Nitrogen free extract (g/kg), TSM: Total soluble matter, T: Triticale, TLAB6: Triticale + Lactobacillus brevis 1 x 10^{6}, TLAB8: Triticale + Lactobacillus brevis 1 x 10^{8}, TLAB9: Triticale + Lactobacillus brevis 1 x 10^{9}. *[1]ADFom = ADF – ash, NDFom = NDF – ash, [2] a, b, c Mean values within the same column with no common letters differ significantly (P<0.05)*

In the present study, CP contents of silages were determined as 10.76, 11.00, 10.31, and 10.41%, respectively. CP content showed a statistically significant increase in TLAB6 group compared to control and other treatment groups (P<0.001). Özdüven et al. (2010) determined the CP values of triticale silages to which they added LAB, enzyme, or their mixtures [control, +LAB (*Lactobacillus plantarum + Enterococcus faecium*), +Enzyme (amylase, cellulase, hemicellulase, pentosanase), +LAB + enzyme (*Lactobacillus plantarum, Streptococcus faecium, Pediococcus acidilactici* + amylase, cellulase, hemicellulase), pentosanase)] as 8.5, 8.6, 8.7 and 8.6%, respectively (P>0.05). Doğan (2019) reported that CP values were found as 7.95, 7.76, and 7.76 in triticale silages harvested in the milk setting period, and 8.19, 8.09 and 7.74 in the silages harvested in the pulp setting period (P>0.05). Romero et al. (2017) determined the CP values of oat silages prepared by using two different ensiling methods (polyethylene bags and plastic drums; control and *Lactobacillus buchneri + Pediococcus pentosaceus*) as 6.82-7.07 and 7.03-6.84, respectively (P>0.05). Demirci (2009) investigated the effects of homofermentative (HM) and heterofermentative (HT) lactic acid bacteria on Hungarian vetch and oat bale silages [control, HM+HT (*Lactobacillus plantarum + Enterococcus faeicum*) and HT (*Lactobacillus buchneri*)] that determined the CP values as 13.59, 13.61 and 13.28%, respectively (P<0.05).

The ADF contents of triticale silages were 31.35, 30.00, 29.73 and 29.58%, respectively; NDF was determined as 55.42, 54.00, 56.23 and 55.61%. While differences between groups in ADF values were very significant (P<0.01), differences between groups in NDF values were insignificant (P>0.05). Demirci (2009) found ADF contents 39.15, 37.98 and 34.77%; NDF values were determined as 55.48, 62.92 and 52.70%, respectively. Romero et al. (2017) determined ADF values as 35.0-35.4 and 34.6-35.0, and NDF values as 65.5-67.1 and 65.6-65.5, respectively (P>0.05). Doğan (2019) reported that ADF-NDF

values were found as 40.39, 38.77, 37.99-60.73, 61.02, 56.97% in triticale silages harvested in the milk setting period, and 36.73, 37.16, 38.74-59.91, 59.50, 55.98% in the silages harvested in the pulp setting period, respectively (P>0.05). The total carbohydrate contents of the study groups were 78.32, 78.23, 79.19 and 78.95 (P<0.05); NFC contents 22.90, 24.24, 22.97 and 23.34 (P>0.05); NFE contents were determined as 52.63, 53.33, 54.39 and 54.24 (P<0.05), respectively. When the chemical analysis results of the treatment groups were examined, OM and CP values increased, while ADF and EE values decreased compared to the control group. The present result is an indication that the bacteria are working correctly, and other research results also support our results (Demirci 2009; Özdüven et al. 2010; Doğan 2019).

The DCP and TDN contents of the silages were found as 6.00, 6.21, 5.59, 5.68 (P<0.01) and 59.80, 60.10, 59.39, 59.50 (P<0.01), respectively. When the energy contents of the silages were examined, DE, ME, NEL, NEM and NEG values were respectively 2.64, 2.65, 2.62 and 2.63 (P<0.05); 2.16, 2.17, 2.15 and 2.15 (P<0.001); 1.35, 1.35, 1.34 and 1.34 (P>0.05); 1.30, 1.31, 1.29 and 1.30 (P<0.05); 0.73, 0.74, 0.72 and 0.72 (P<0.001).

Table 2. DCP, TDN and Energy Contents of Silages

Groups	T	TLAB[6]	TLAB[8]	TLAB[9]	*P*
DCP	6.00±0.04[b]	6.21±0.05[a]	5.59±0.01[c]	5.68±0.06[c]	0.0017
TDN	59.80±0.05[b]	60.10±0.06[a]	59.39±0.02[c]	59.50±0.07[c]	0.0022
DE	2.64±0.01[b]	2.65±0.00[a]	2.62±0.00[c]	2.63±0.00[cb]	0.0138
ME	2.16±0.00[b]	2.17±0.00[a]	2.15±0.00[c]	2.15±0.00[c]	<.0001
NE_L	1.35±0.01	1.35±0.00	1.34±0.01	1.34±0.00	0.1376
NE_M	1.30±0.00[b]	1.31±0.00[a]	1.29±0.00[b]	1.30±0.01[b]	0.0190
NE_G	0.73±0.00[b]	0.74±0.00[a]	0.72±0.00[c]	0.72±0.00[c]	<.0001

DCP: Digestible crude protein (%), TDN: Total digestible nutrients (%), DE: Digestible energy (Mcal/kg), ME: Metabolic energy (Mcal/kg), NE_L : Net energy-lactation (Mcal/kg), NE_M : Net energy-maintenance (Mcal/kg), NE_G : Net energy-gain (Mcal/kg), T: Triticale, TLAB6: Triticale + Lactobacillus brevis 1 x 10^6, TLAB[8]: Triticale + Lactobacillus brevis 1 x 10^8, TLAB[9]: Triticale + Lactobacillus brevis 1 x 10^9. [1] a, b, c Mean values within the same column with no common letters differ significantly (P<0.05)

The RFV and RFQ values of the silages were calculated as 108.25, 112.90, 108.78, 110.19 (P<0.05) and 105.28, 108.59, 103.06, 104.40 (P<0.05), respectively. RFV values of all silages in accordance with the calculations II. quality feed (103-124) (Kılıç and Abdiwalli, 2016). The RFQ values of silages were concluded that all silages are feeds (100-120) that can be used in the feeding of heifers and dry cows aged 18-24 months (Filik, 2020).

Table 3. Feed quality characteristics of silages

Groups	T	TLAB[6]	TLAB[8]	TLAB[9]	P
DDM	64,48±0,05c	65,63±0,05b	65,75±0,01a	65,86±0,03a	<.0001
DMI	2,17±0,02b	2,23±0,00a	2,14±0,01b	2,16±0,01b	0,0491
RFV	108,25±1,29b	112,90±0,38a	108,78±0,70b	110,19±0,20ab	0,0413
RFQ	105,28±1,41b	108,59±0,38a	103,06±0,63b	104,40±0,12b	0,0316

DDM: Digestible dry matter (%), DMI: Dry matter intake, RFV: Relative feed value, RFQ: Relative forage quality, T: Triticale, TLAB[6]: Triticale + Lactobacillus brevis 1 x 10^6, TLAB[8]: Triticale + Lactobacillus brevis 1 x 10^8, TLAB[9]: Triticale + Lactobacillus brevis 1 x 10^9. [1] a, b, c Mean values within the same column with no common letters differ significantly (P<0.05)

The pH_1 values of the silages were determined as 5.60, 4.95, 4.99 and 4.88, respectively. The pH values of the LAB supplemented groups decreased significantly compared to the control group and the differences between the groups were found to be very significant (P<0.001). Demirci (2009) found the pH values of Hungarian vetch and oat bale silages in his study investigating the effect of homofermentative (HM) and heterofermentative (HT) lactic acid bacteria [control, in HM+HT (Lactobacillus plantarum + Enterococcus faeicum) and HT (Lactobacillus buchneri) groups] to be 4.63, 4.56 and 4.33, respectively (P<0.05). Özdüven et al. (2010) reported pH values of triticale silages as 4.5, 3.8, 4.1 and 3.7, respectively (P<0.05). Doğan (2019) reported pH 4.48, 4.24, 4.19 for triticale silages harvested with bacterial inoculants during the milk setting period, and 4.83, 4.27 and 4.23 for those harvested during the pulping period (P<0.001). Romero et al. (2017) reported pH values to be 6.10 and 6.04 in the method using polyethylene bags, and 6.13 and 6.16 in the plastic drum method, respectively (P<0.05). Triticale silages seem to have appropriate pH values to prevent unwanted microorganism growth. As a matter of fact, Filya (2001) reported that Clostridial spores and Enterobacteria

generally develop in environments where the pH is around 6-7 but cannot thrive in environments where it is below 5.

Table 4. Physical analysis results of silages

Group	T	TLAB^6	TLAB^8	TLAB^9	P
DM	47.00±0.24^a	45.25±0.73^b	45.78±0.71^{ab}	45.39±0.54^{ab}	0.0409
pH$_1$	5.60±0.01^a	4.95±0.02^b	4.99±0.02^b	4.88±0.03^c	<.0001
Temperature	23.00±0.07^b	23.38±0.05^a	21.13±0.05^d	21.43±0.03^c	<.0001
L*	44.60±0.57	44.27±2.68	46.88±2.68	40.36±0.90	0.1846
a*	2.16±0.18	2.43±0.10	2.72±0.26	2.47±0.18	0.2712
b*	15.47±0.57	15.56±1.31	16.25±0.93	14.00±0.46	0.3647
C*	15.62±0.58	15.74±1.30	16.49±0.92	14.22±0.44	0.3639
h°	82.09±0.45	81.02±0.50	80.45±0.95	79.96±0.91	0.2567

DM: Dry matter, L*: Lightness, a*: redness, b*: yellowness, h°: hue angle, C*: Chroma or saturation, T: Triticale, TLAB6: Triticale + *Lactobacillus brevis* 1 x 10^6, TLAB8: Triticale + Lactobacillus brevis 1 x 10^8, TLAB9: Triticale + Lactobacillus brevis 1 x 10^9. [1] [a, b, c] Mean values within the same column with no common letters differ significantly (P<0.05)

Microorganism count results of silages after opening are given in Table 5. According to the results, lactic acid bacteria were found only in the control (T) group (1.00), and LAB was not found in the other groups. The amount of yeast was found to be 3.67, 1.00 and 1.00 in the T, TLAB^6 and TLAB^8 groups, respectively, and no yeast was found in the TLAB^9 group (P>0.05). However, mold formation did not occur in any group. The present results, it is thought that lactic acid bacteria, which perform anaerobic respiration in the vacuum package, ferment quickly and stop their reproductive activities due to the high internal pressure of the vacuum package necessary for the continuation of fermentation. However, due to the transition of the lactic acid bacteria used to the spore state, reproductive activities could not be detected in the measurements made when they were opened. Contrary to this situation, it is seen that reproductive activities have started after starvation in the control group.

Table 5. Microorganism count results at opening time of silages

Groups	T	TLAB^6	TLAB^8	TLAB^9	P
LAB, log$_{10}$ cfu/g	1.00±0.00	N/A	N/A	N/A	N/A
Yeast, log$_{10}$ cfu/g	3.67±0.33	1.00±0.00	1.00±0.00	NF	0.0725
Mold, log$_{10}$	N/A	N/A	N/A	N/A	N/A

T: Triticale, TLAB^6: Triticale + Lactobacillus brevis *1 x* 10^6, TLAB^8: Triticale + Lactobacillus brevis 1 x 10^8, TLAB^9: Triticale + Lactobacillus brevis 1 x 10^9. NF: Not found. [1] a, b, c Mean values within the same column with no common letters differ significantly (P<0.05)

The results of pH_2, CO_2 and microorganism counts of silages after aerobic stability are given in Table 6. pH_2 values in T, TLAB^6 and TLAB^8 groups were determined as 5.81, 4.87, 4.85 and 4.80, respectively (P<0.001). The current pH results show that, as Filya (2001), it is seen that the development of pathogenic microorganisms is adversely affected and below 5. Mold formation in the groups did not occur after the aerobic stability test. Lactobacillus brevis lactic acid bacteria is thought to have a positive effect on the inhibition of mold growth at all doses in triticale silages.

Table 6. pH_2, CO_2 and microorganism count results of silages after aerobic stability

Group	T	TLAB^6	TLAB^8	TLAB^9	P
pH_2	5.81±0.12^a	4.87±0.03^b	4.85±0.01^b	4.80±0.01^b	<.0001
CO_2	4.86±0.55^a	3.21±0.19^b	2.58±0.32^b	2.51±0.00^b	0.0212
AAS Yeast log$_{10}$ cfu/g	64.00±1.53	N/A	N/A	N/A	N/A
Mold, log$_{10}$	N/A	N/A	N/A	N/A	N/A

T: Triticale, TLAB^6: Triticale + *Lactobacillus brevis* 1 x 10^6, TLAB^8: Triticale + Lactobacillus brevis 1 x 10^8, TLAB^9: Triticale + Lactobacillus brevis 1 x 10^9., ASS: After aerobic stability, N/A: Not available. [1] [a, b, c] Mean values within the same column with no common letters differ significantly (P<0.05).

Özdüven et al. (2010) found lactobacilli, yeast and mold numbers of triticale+LAB silages to be 4.6, 6.0, 5.7, 6.1 (P<0.05), 5.2, 5.1, 5.2, 5.1 (P>0.05) and 3.2, 2.3, 2.8, 2.2 (P<0.05), respectively. In the same study, pH values after aerobic stability test were 5.6, 5.7, 5.7, 5.7; CO2 values were 40.8, 58.6, 48.8, 55.2, yeast values were 5.7, 7.5, 6.9, 7.1; mold values were found as 4.9, 5.3, 5.0 and 5.2, respectively (P<0.05). Doğan (2019) found that lactobacilli values of 5.08, 6.71, 6.57 and 6.22,

6.47, 6.35; reported that the yeast counts were 5.48, 4.83, 6.14 and 5.07, 4.96, 4.93 for the milk and dough stage (P<0.001). When the mold values were examined, it was reported that it was 4.65, 5.12, 0.00 and 4.33, 4.30, 0.00 in milk and dough formation silages, respectively (P<0.001). After the aerobic stability test, pH, CO2, yeast and mold count results were found in the milk and dough formation periods, 5.11, 5.08, 4.84 and 5.30, 5.09, 4.96 (P<0.05); 70.85, 60.18, 0.00 and 53.36, 5.77, 1.99 (P<0.001); 7.29, 7.31, 6.03 and 7.42, 6.91, 6.68 (P<0.05); 8.10, 7.89, 7.14 and 8.17, 7.47, 6.29 (P<0.05), respectively.

CONCLUSIONS

As a result, the use of heterofermentative Lactobacillus brevis MF098783 lactic acid bacteria strain did not provide the expected increase in lactobacilli density in silages; It is possible to say that silages have positive effects on fermentation, microorganism growth and aerobic stability by lowering their pH levels. On the other hand, it did not have a significant effect on the nutrient parameters of the silages.

ACKNOWLEDGEMENTS

This article was prepared out from the MSc thesis of the first author. The second author was the supervisor of the thesis. This work was supported by Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: ZRT.A4.21.019.

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THE EFFECT OF *Lactobacillus plantarum* STRAIN ON SILAGE QUALITY IN RYE SILAGES

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Abstract

In this study, the homofermentative lactic acid bacteria lactobacillus plantarum was regulated to determine aerobic stability, fermentation microorganism growth in rye silage. Rye was harvested during the grain maturity period. After the shredding process was completed, 100 g of plant material was placed in 2 kg plastic bags and applied by spraying lactobacillus plantarum lactic acid bacteria in concentrations of 1×10^{6}, 1×10^{8} and 1×10^{9} cfu/g for each bacteria separately. In the study, a total of 20 silages with 5 replications were prepared and left for fermentation under laboratory conditions (25-30 °C) for 90 days. After the silages were opened, chemical, physical, and microbiological tests were applied. It was then subjected to a 5-day aerobic stability test. Among the chemical analysis results, nutrient content, aerobic stability, water-soluble carbohydrates, physical analysis pH, temperature, L, a, b, chroma, h values, only physical analysis pH and temperature values were found to be statistically significant ($P<0.05$). The pH values were found as 6.26, 5.29, 5.16, 4.88, respectively. In microbiological analysis: lactic acid, yeast-mold and yeast-mold analyzes after aerobic stability were found to be statistically insignificant ($P>0.05$). As a result, it was concluded that the addition of LP in rye silage contributed to the prevention of unwanted microorganism and yeast growth.

Key words: *silage, rye, lactobacillus plantarum, inoculant*

INTRODUCTION

Agricultural production areas are very important in the development of countries and in the livestock sector. It has been reported that the studies carried out to solve the feed and nutrition problems due to the development of the livestock industry contribute significantly to the country's economy (Ergün and Bayram, 2021). Silage, which is frequently used in animal nutrition, can be defined as the cultivation of green fodder plants with high water content in an oxygen-free environment with lactic acid bacteria. (Uğurlu, 2019). Rye is preferred as an alternative forage source to meet the dietary fiber needs of ruminant animals (Moore, 2014). Evaluation of the silo ability potential of forage crops such as rye alone, with other forage crops and with or without inoculant additives is important in terms of the diversity and economics of the prepared silage feeds. Diversity of silage materials is also very important for the use of silage in livestock to become widespread and for businesses to benefit economically (Koç, 2019).

Rye (*Secale cereal* L.) forage crop is more resistant to strong cold and high temperatures than other cereal crops, however, since it is harvested during the spike and dough period, the amount of dry matter (DM) determines the product quality according to the harvest time (Uğurlu, 2019). While silage fermentation increases the amount of DM in the rye plant harvested during the dough formation period, the increase in the amount of lignin in the environment together with the decrease in the amount of water-soluble carbohydrates (WSC) decreases the fermentation and nutrient digestibility (Filya, 2004). Lactic acid bacteria (LAB) are very important to improve the fermentation quality and digestibility of silage (Paradhipta et al., 2020). In this context, lactic acid bacteria (LAB) containing lactic acid-producing *Lactobacillus*, *Streptococcus* or *Pediococcus bacteria* are preferred to ensure that fermentation is effective and fast (Hotun, 2019). It is known that the addition of LAB to rye products improves the fermentation quality. For this reason, the use of cell wall and starch-degrading enzymes together with lactic acid bacteria improves bacterial fermentation in silage, while reducing the contents of acid insoluble fiber (ADF) and neutral detergent insoluble fiber (NDF). However, it increases the digestibility of nutrients (Uğurlu, 2022). In this study, the effects of Lactobacillus plantarum, a homofermentative lactic acid bacteria, on

fermentation, aerobic stability and microorganism growth on rye silage were determined.

MATERIALS AND METHODS

In the study, rye plant was obtained from the research field of Kırşehir Ahi Evran University Field Crops Department (Latitude: 39.1286"N, Longitude: 34.107"E). Rye harvested during the grain maturity period was chopped to be 1.5-2 cm long. After the chopping process was completed, 1000 g of plant material was placed in 2 kg plastic bags and each group was sprayed with the heterofermentative Lactobacillus plantarum MF098786 strain isolated from the pickle at a concentration of 1×10^6 cfu/g, 1×10^8 cfu/g and 1×10^9 cfu/g, respectively. After the incubation process, the air in the packages was vacuumed with the help of a vacuum device (Packtech PT-VKM-CPRO). In the study, a total of 20 silages were prepared with 5 replications in each group and left to fermentation in a dark environment at 20-22 °C under laboratory conditions for 90 days. Study groups were formed as control (C), rye + L. plantarum 10^6 (LAB^6), rye + L. plantarum 10^8 (LAB^8) and rye + L. plantarum 10^9 (LAB^9), respectively. Four groups of three parallel samples were taken from the silages whose fermentation process was completed; (1) chemical [dry matter (DM), ash, ether extract (EE), crude protein (CP), crude fiber (CF), ADF, NDF, total soluble matter (TSM)], (2) data determined by calculations and energy values [DCP: Digestible crude protein,
TDN: Total digestible nutrient, DE: Digestible energy, ME: Metabolic energy, NEL: Net energy-lactation, NEM: Net energy-maintenance, NEG: Net energy-gain] (3) physical (temperature, color, pH), (4) microbiological (lactic acid bacteria, yeast and mold counts) and (5) statistical analyzes were performed. DM, CP, EE, ash analysis according to AOAC (1998) standard procedure; CF, ADF and NDF analysis according to Van Soest et al. (1991) (ANKOM 200 Fiber Analyzer); pH values Chen et al. (1994); total soluble matter (TSM) contents were determined as explained by Singh et al. (2020). After the silage samples were opened, the L*, a*, and b* color values were measured from three different parts of the silage with the Konica-Minolta CR-410 color meter. Using the a* and b* values, the Chroma (C*, saturation index) and hue angle (h°) values were calculated according to describe of AMSA, (2012). In the study, the lactic acid bacteria, yeast, and mold counts contained in the silages were determined by the method reported by Seale et al. (1990). On the fifth day after open, the CO2 value and pH values were determined as described by Ashbell et al. (1991). Metabolizable energy and protein values of silages with total carbohydrate (TC), hemicellulose, nitrogen free extract (NFE) and nitrogen free carbohydrate (NFC) were calculated as reported by Filik (2020). The relative feed value (RFV) and relative forage quality (RFQ) calculations of the silages were calculated according to the reported by Kılıç and Abdiwali (2016) and Filik (2020). SAS (2001) package program was used in statistical analysis, and the linear relations between the experimental groups were determined by applying orthogonal polynomial contrast with the General Linear Model (PROC GLM) procedure in accordance with the trial model of the study (random plots trial plan). The difference between the groups was made using the Duncan Multiple Comparison Method (Genç & Soysal, 2018).

RESULTS AND DISCUSSION

In this study, silages prepared from rye forage plant were treated with Lactobacillus plantarum, a homofermentative lactic acid bacteria. In the study, it was aimed to determine the effects of the lactic acid bacteria used on fermentation properties, aerobic stability, and microorganism growth when the fermentation period is completed.

Chemical analysis results of silages were shown in Table 1. When the DM contents of the silages were examined, it was determined as 940.75, 936.20, 937.10, 936.15 g/kg in the C, LAB^6, LAB^8, LAB^9 groups, respectively ($P<0.05$). Uğurlu (2019) added three different commercial inoculants to rye silage Biosil (*Lactobacillus plantarum* DSM 8862, *Lactobacillus plantarum* DSM 8866, LABI), Silaprilis Pro (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Propionibacteria acidipropionici*, xylenase, β-glucanase, LABII), Sil-All (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* ve *Propionibacteria acidipropionici*, amylase, cellulase, xylenase, β-glucanase, LABIII) in the control, LABI, LABII and LABIII groups, DM contents were found as 381.74, 391.99, 394.10 and 402.57, respectively ($P<0.05$). In our study, the organic matter content (OM%) was determined as 93.00, 92.37, 92.52 and 92.56 ($P<0.05$) in the C, LAB^6, LAB^8 and LAB^9 groups, respectively. Ash and crude protein (CP) values of control, LAB^6, LAB^8, LAB^9 were 7.01, 7.63, 7.48, 7.44 ($P<0.05$) and 9.76, 11.23, 10.98, 10.5 ($P<0.001$) respectively. Joo et al. (2015) investigated the effect of *Lactobacillus plantarum* (LP), *Lactobacillus buchneri* (LB) and their mixtures in rye silage, in the control, LP, LB, LP+LB groups, the ash and CP values were 7.06, 6.96, 6.89, 7.07 and 9.51, 9.81, 9.41, 9.71. When Table 2 is examined, the EE contents of the C,

LAB^6, LAB^8, and LAB^9 groups were found to be 4.49, 4.66, 4.77, and 4.76 (P<0.05), respectively. As shown in our results, the ADF contents of silages C, LAB^6, LAB^8, LAB^9 were determined as 36.92, 35.94, 36.17 and 34.89 (P<0.05), respectively. Similarly, Ike (2019) found homofermentative *Lactobacillus plantarum* and *Enterecoccus faecium* (SILAID LAB, HMLAB), heterofermentative *Lactobacillus buchneri* (Pioneer, HTLAB) lactic acid bacteria and enzyme (cellulase, pentosanase and SILAID, E) in common vetch, wheat, oat mixed silages mixtures were added in the control, HMLAB, HMLAB+E, HTLAB, HTLAB+E and HM+HTLAB+E groups, the ADF contents were determined as 34.69, 35.44, 32.77, 36.24, 33.94 and 32.41 (P<0.05), respectively. Digestible crude protein (DCP) and energy contents of silages were given in Table 2. As a result of the study results, digestible crude protein (DCP), total digestible nutrients (TDN) and digestible energy (DE) values were 5.09, 6.43, 6.20, 5.78 (P<0.001), 58.46, 60.08, 59.81, 59.31 (P<0.01) and 2.58, 2.65, 2.64, 2.62 (P<0.01), respectively. Metabolic energy (ME), net energy-lactation (NE_L), net energy-maintenance (NE_M) and net energy-gain (NE_G) values were 2.12, 2.17, 2.17, 2.15 (P<0.01), 1.32, 1.35, 1.35, 1.34 (P<0.05), 1.36, 1.31, 1.31, 1.29 (P<0.01) and 0.69, 0.74, 0.73, 0.72 (P<0.001) respectively. The feed quality characteristics of silages are given in Table 3. When the digestible dry matter (DDM) values were 60.14, 60.90, 60.73, 61.72 (P<0.05), respectively. On the other hand, dry matter intake (DMI), relative feed value (RFV) and relative forage quality (RFQ) values were 1.92, 1.90, 1.89, 2.02, 89.22, 89.52, 88.71, 96.64 and 90.96, 92.62, 91.63, 97.38 (P>0.05), respectively.

After opening dry matter (AODM), pH_1 and physical properties of silages are given in Table 4. In our study, AODM was 47.79%, 45.26, 46.68, 46.97, respectively, (P<0.01). Filya (2002), control in maize and sorghum silages, IA (*Lactobacillus plantarum*, *Enterococcus faecium*, Inoculant 1199, Pioneer), IB (*Pediococcus acidilactici*, *Lactobacillus plantarum*, *Enterococcus faecium*, H/M F Inoculant No. 9927) and IC (*Enterococcus faecium*, Lacticil M74) groups, DM contents for corn and sorghum silage were 35.2%, 33.6, 33.5 and 35.0, and 28.1, 27.4, 27.9 and 27.0, respectively. In the study, pH values of C, LAB^6, LAB^8, LAB^9 groups of LAB inoculants added to rye silages at different rates were determined as 6.26, 5.29, 5.16, 4.88, (P<0.001) respectively. A similar study, İke (2019) reported that the pH values of common vetch, wheat, and oat mixed silages in the control and HMLAB groups were 4.16 and 4.09, respectively (P<0.05). Ozduven et al. (2010) investigated the effects of lactic acid bacteria in triticale silages, LAB (*Lactobacillus plantarum* + *Enterococcus faecium*), Enzyme (amylase, cellulase, hemicellulase, pentozanase), LAB + enzyme (*Lactobacillus plantarum*, *Streptococcus faecium*, *Pediococcus acidilactici*, amylase, cellulase, hemicellulose, pentosanase) groups, the *lactobacilli* contents were 4.6, 6.0, 5.7 and 6.1 (P<0.05); yeast amounts of 5.2, 5.1, 5.2 and 5.1 (P>0.05) and mold amounts were determined as 3.2, 2.3, 2.8 and 2.2 (P<0.05), respectively. Uğurlu (2022) reported that the addition of LAB to rye silage significantly affects aerobic stability.

Table 4. Chemical analysis results of rye silages

Group	C	LAB^6	LAB^8	LAB^9	P
DM	940.75±0.65^a	936.20±0.20^b	937.10±0.80^b	936.15±0.25^b	0.0105
OM	93.00±0.16^a	92.37±0.03^b	92.52±0.07^b	92.56±0.11^b	0.0466
Ash	7.01±0.16^b	7.63±0.03^a	7.48±0.07^a	7.44±0.11^a	0.0466
CP	9.76±0.09^c	11.23±0.07^a	10.98±0.08^a	10.51±0.09^b	0.0008
EE	4.49±0.00^b	4.66±0.06^a	4.77±0.03^a	4.76±0.03^a	0.0130
CF	29.98±0.37	28.74±0.02	28.79±0.02	29.05±0.40	0.0920
ADF	36.92±0.02^a	35.94±0.04^a	36.17±0.09^a	34.89±0.49^b	0.0204
NDF	62.71±0.20^a	63.29±0.40^a	63.68±0.15^a	59.44±0.96^b	0.0153
Hcel	25.79±0.16^b	27.35±0.45^a	27.52±0.23^a	24.55±0.47^b	0.0110
TC	78.75±0.06^a	76.48±0.15^c	76.78±0.18^c	77.29±0.05^b	0.0007
NFC	16.05±0.25^a	13.20±0.55^b	13.09±0.02^b	17.86±1.00^a	0.0113
NFE	48.77±0.43	47.76±0.16	47.99±0.15	48.25±0.46	0.3008
ADFom	29.91±0.13^a	28.31±0.07^b	28.69±0.02^b	27.45±0.38^c	0.0047
NDFom	55.70±0.04^a	55.66±0.38^a	56.20±0.22^a	52.00±0.85^b	0.0100
TSM	26.20±0.78	25.40±0.37	25.65±0.39	25.15±0.90	0.7079

DM: Dry matter (g/kg), OM: Organic matter (%), CP: Crude protein (%), EE: Ether extract (%), CF: Crude fiber (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%), Hcel: Hemicellulose (%), TC: Total carbohydrates (g/kg), NFC: Nitrogen free carbohydrates (g/kg), NFE: Nitrogen free extract (g/kg), TSM: Total soluble matter, C: Control, LAB^6: Rye + *Lactobacillus plantarum* 1 x 10^6, LAB^8: Rye + *Lactobacillus plantarum* 1 x 10^8, LAB^9: Rye+ *Lactobacillus plantarum* 1 x 10^9. [1]ADFom = ADF – ash, NDFom=NDF – Ash, [2] $^{a, b, c, d}$ Mean values within the same column with no common letters differ significantly (P<0.05)

Table 2. DCP and energy contents of rye silages

Group	C	LAB^{6}	LAB^{8}	LAB^{9}	P
DCP	5.09±0.08^{c}	6.43±0.06^{a}	6.20±0.07^{a}	5.78±0.08^{b}	0.0009
TDN	58.46±0.12^{c}	60.08±0.07^{a}	59.81±0.08^{a}	59.31±0.13^{b}	0.0013
DE	2.58±0.01^{c}	2.65±0.00^{a}	2.64±0.01^{a}	2.62±0.01^{b}	0.0010
ME	2.12±0.01^{c}	2.17±0.00^{a}	2.17±0.00^{a}	2.15±0.00^{b}	0.0028
NE_L	1.32±0.01^{b}	1.35±0.00^{a}	1.35±0.01^{a}	1.34±0.01^{a}	0.0162
NE_M	1.36±0.01^{c}	1.31±0.00^{a}	1.31±0.01^{a}	1.29±0.01^{b}	0.0028
NE_G	0.69±0.00^{d}	0.74±0.00^{a}	0.73±0.00^{b}	0.72±0.01^{c}	0.0006

DCP: Digestible crude protein (%), TDN: Total digestible nutrients (%), DE: Digestible energy (Mcal/kg), ME: Metabolic energy (Mcal/kg), NE_L : Net energy-lactation (Mcal/kg), NE_M : Net energy-maintenance (Mcal/kg), NE_G : Net energy-gain (Mcal/kg), C: Control, LAB^{6}: Rye + *Lactobacillus plantarum* 1 x 10^{6}, LAB^{8}: Rye + *Lactobacillus plantarum* 1 x 10^{8}, LAB^{9}: Rye+ *Lactobacillus plantarum* 1 x 10^{9}. $^{1\ a,\ b,\ c,\ d}$ Mean values within the same column with no common letters differ significantly (P<0.05)

Table 3. Feed quality characteristics of rye silages

Group	C	LAB^{6}	LAB^{8}	LAB^{9}	P
DDM	60.14±0.02^{b}	60.90±0.03^{b}	60.73±0.07^{b}	61.72±0.38^{a}	0.0200
DMI	1.92±0.01^{b}	1.90±0.02^{b}	1.89±0.01^{b}	2.02±0.03^{a}	0.0154
RFV	89.22±0.30^{b}	89.52±0.53^{b}	88.71±0.12^{b}	96.64±2.15^{a}	0.0201
RFQ	90.96±0.47^{b}	92.62±0.48^{b}	91.63±0.35^{b}	97.38±1.77^{a}	0.0293

DDM: Digestible dry matter (%), DMI: Dry matter intake, RFV: Relative feed value, RFQ: Relative forage quality, C: Control, LAB^{6}: Rye + *Lactobacillus plantarum* 1 x 10^{6}, LAB^{8}: Rye + *Lactobacillus plantarum* 1 x 10^{8}, LAB^{9}: Rye+ *Lactobacillus plantarum* 1 x 10^{9}. $^{1\ a,\ b,\ c,\ d}$ Mean values within the same column with no common letters differ significantly (P<0.05)

Table 4. After opening dry matter, pH_1 and physical analysis results of rye silages

Group	C	LAB^{6}	LAB^{8}	LAB^{9}	P
AODM	47.79±0.03^{a}	45.26±0.00 ^{d}	46.68±0.00^{c}	46.97±0.00^{b}	0.0012
pH_1	6.26±0.04^{a}	5.29±0.03^{b}	5.16±0.02^{c}	4.88±0.01^{d}	<.0001
Temperature	20.88±0.09^{c}	20.90±0.12^{c}	21.63±0.13^{a}	21.23±0.05^{b}	0.0006
L*	45.26±0.64	47.71±1.87	42.97±1.29	45.72±2.31	0.2938
a*	3.29±0.29	4.23±0.32	3.65±0.25	3.92±0.95	0.6512
b*	17.06±0.34	18.52±0.66	16.25±0.41	17.77±0.96	0.1289
C*	17.38±0.34	19.01±0.59	16.66±0.40	18.24±1.13	0.1469
h°	79.13±0.79	77.03±1.29	77.34±0.93	77.9±2.20	0.7346

AODM: After opening dry matter, L*: Lightness, a*: redness, b*: yellowness, h: hue angle, C*: Chroma or saturation, C: Control, LAB6: Rye + *Lactobacillus plantarum* 1 x 10^{6}, LAB8: Rye + *Lactobacillus plantarum* 1 x 10^{8}, LAB9: Rye+ *Lactobacillus plantarum* 1 x 10^{9}. $^{1\ a,\ b,\ c,\ d}$ Mean values within the same column with no common letters differ significantly (P<0.05)

Table 5. Microorganism count results of rye silages

Group	C	LAB^{6}	LAB^{8}	LAB^{9}	P
LAB, log_{10} cfu/g	1.00±0.00	N/A	N/A	N/A	N/A
Yeast, log_{10} cfu/g	1.00±0.00	1.00±0.00	N/A	N/A	N/A
Mold, log_{10}	N/A	N/A	N/A	N/A	N/A

C: Control, LAB^{6}: Rye + *Lactobacillus plantarum* 1 x 10^{6}, LAB^{8}: Rye + *Lactobacillus plantarum* 1 x 10^{8}, LAB^{9}: Rye+ *Lactobacillus plantarum* 1 x 10^{9}, N/A: Not applicable. $^{1\ a,\ b,\ c,\ d}$ Mean values within the same column with no common letters differ significantly (P<0.05)

Table 6. pH_2, CO_2 and microorganism count results of rye silages after aerobic stability

Group	C	LAB^{6}	LAB^{8}	LAB^{9}	P
pH_2	19.24±3.78	5.60±1.83	5.60±1.83	15.65±5.72	0.0851
CO_2	5.97±0.08^{a}	5.14±0.16^{b}	5.02±0.08^{b}	5.00±0.11^{b}	0.0092
AAS Yeast log_{10} cfu/g	50.00±4.16^{a}	20.67±3.71^{b}	N/A	40.67±6.36^{a}	N/A
Mold, log_{10}	N/A	N/A	N/A	N/A	N/A

C: Control, LAB6: Rye + *Lactobacillus plantarum* 1 x 10^{6}, LAB8: Rye + *Lactobacillus plantarum* 1 x 10^{8}, LAB9: Rye+ *Lactobacillus plantarum* 1 x 10^{9}, ASS: After aerobic stability, N/A: Not applicable. $^{1\ a,\ b,\ c,\ d}$ Mean values within the same column with no common letters differ significantly (P<0.05).

CONCLUSIONS

In our study, the effects of homofermentative lactic acid bacteria *Lactobacillus plantarum* on aerobic stability and fermentation microorganism growth in rye silage were determined. Lactic acid bacteria were added to rye silage at doses of 10^{6}, 10^{8}, and 10^{9}. It has been determined that the use of lactic acid

bacteria in rye silage reduces the pH and accordingly, yeast and mold growth. Especially in the LAB8 group, yeast formation did not occur after aerobic stability. The dose in question is effective in preventing deterioration.

ACKNOWLEDGEMENTS

This article was prepared out from the MSc thesis of the first author. The second author was the supervisor of the thesis. This work was supported by Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: ZRT.A4.22.016.

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EFFECT OF *Pediococcus acidilactici* INOKUCULATION AT DIFFERENT CONCENTRATİON ON SILAGE QUALITY AND AEROBİC STABILITY IN BARLEY SILAGE

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Abstract

The present study was carried out to investigate the effects of Pediococcus acidilactici (MF098795 lactic acid bacteria strain isolated from homemade pickles on barley (Hordeum vulgare) silage as an inoculant, silage quality, aerobic stability, and microorganism growth. Working groups, control barley (CB), Pediococcus acidilactici lactic acid bacterial strain groups PA 10^6 (LAB^6), PA 10^8 (LAB^8) and PA 10^9 (LAB^9) were created. Barley plant was harvested dough maturity period and chopped into 1.5 - 2 cm sizes vacuumed into 1 kg bags with 5 repetitions and left for fermentation for 90 days. After fermentation, chemical, physical and microbiological analyzes were performed on silage samples. The application of P. acidilactici lactic acid bacteria to barley silage, especially at a dose of $1*10^8$, decreased the pH value of the silages, provided positive contributions to the silage quality, and improved the aerobic stability of the silage. Accordingly, when all data are evaluated, it may be recommended to add P. acidilactici bacteria at the level of 10^8 cfu/g cfu/g in terms of silage quality and aerobic stability in barley silage.

Key words: *Barley silage, Pediococcus acidilactici, silage quality, aerobic stability*

INTRODUCTION

Ensiling is a widely used method today, which ensures that the feed remains fresh without spoiling under anaerobic conditions and that it is preserved for a long time by being subjected to fermentation (Amanullah et al. 2014). In the livestock sector, plants such as corn, oats, barley, wheat, vetch, sorghum, and alfalfa are the sources of silage that are produced intensively. Silage can be made from most of the grasses and legumes. In this context, there are differences in the nutrient content of the material to be silage. Different types of feed additives are used in silages to improve the feed quality parameters and meet the nutritional needs of the animals (Gül and Tan, 2013; Yolcu and Tan, 2008).

Lactic bacteria play an important role in silage production. These bacteria use the readily fermentable carbohydrates of plants to produce lactic acid which reduces the pH and thus preserves the nutrients and prevents the growth of undesirable microbes responsible for spoilage (Amanullah et al. 2014). In the ensiling method, it is undesirable for the silage to contact with oxygen. Because in the presence of oxygen, yeast and mold growth occurs in the silage, which causes aerobic deterioration of the silage and adversely affects the quality parameters. When the lactic acid bacteria cannot be fed adequately in the silage medium, the development slows down or stops. In this case, the silage deteriorates. As a matter of fact, additives are used to ensure that the feed is preserved for a long time and to improve its quality (Zhang et al. 2009; Kiraz and Kutlu, 2016). Homofermentative bacterial genera commonly used in silages are Lactobacillus plantarum, Pediococcus acidilactici and Enterococcus faecium (Erbil, 2012).

Inoculants used in silages play an active role in the rapid development and increase of lactic acid bacteria. In this way, nutrient losses are reduced, silage fermentation is improved, feed value is increased and quality silage with high aerobic stability is obtained (Okuyucu et al. 2018).

In this study, it was aimed to determine the effects of Pediococcus acidilactici, which is a kind of homofermentative lactic acid bacteria, on fermentation, aerobic stability, and microorganism growth in barley silage.

MATERIALS AND METHODS

In the study, silage barley plant *(hordeum vulgare)* was obtained from the research area of Kırşehir Ahi Evran University Field Crops Department (Latitude: 39.1286"N, Longitude: 34.1078"E). The material was harvested dough maturity period and chopped into 1.5 - 2 cm sizes.

After the chopping process was completed, 1000 g of plant material was placed in 2 kg plastic bags and homofermentative *Pediococcus acidilactici* (MF098795 strain) isolated from pickles was sprayed at 1×10^6, 1×10^8 and 1×10^9 kob/g cfu/g concentrations. After the sowing process, the air in the packages was vacuumed with the help of a vacuum device (Packtech PT-VKM-CPRO). In the study, a total of 20 silages were prepared with 5 replications in each group and left to fermentation in a dark environment at 20-22 °C under laboratory conditions for 90 days. Study groups were formed as control barley (CB), barley + *P. acidilactici* 10^6 (LAB^6), barley + *P. acidilactici* 10^8 (LAB^8) and barley + *P. acidilactici* 10^9 (LAB^9). At the end of the fermentation process, five parallel samples were taken from each group from the silages opened at the end of the fermentation process and physical, chemical, microbiological and statistical analyzes were made on the samples.

Dry matter (DM), crude protein (CP), and ash contents of silages were analyzed according to AOAC (1998). Organic matter (OM) content was calculated as [OM, %, DM basis = 100–ash%]. The acid detergent fiber (ADF) and the neutral detergent fiber (NDF) were analyzed by the methods of Van Soest et al. (1991) with amylase and sodium sulfite, and the results were expressed inclusive of residual ash. The NDF and ADF contents were corrected for residual ash content (NDFom and ADFom, respectively). The hemicellulose was calculated according to Van Soest et al (1991) as [hemicellulose = NDF–ADF]. Ether extract (EE) content using ANKOM XT15 Extraction System according to AOCS (2005).

The pH values were carried out as described in Chen et al. (1994), total soluble matter (TSM) contents were carried out as described in Singh et al. (2020).

The total digestible nutrient (TDN), the digestible dry protein (DCP), and metabolizable energy values of silages were calculated as reported by Filik (2020).

After the silage samples were opened, the *L**, *a**, and *b** color values were measured from three different parts of the silage with the Konica-Minolta CR-410 color meter. Using the a* and b* values, the Chroma (C*, saturation index) and hue angle (*h*°) values were calculated according to describe of AMSA, (2012).

In the study, the lactic acid bacteria, yeast, and mold counts contained in the silages were determined by the method reported by Seale et al. (1990). On the fifth day after open, the CO_2 value and pH values were determined as described by Ashbell et al. (1991).

The relative feed value (RFV) and relative feed quality (RFQ) calculations of the silages were calculated according to the reports of Kılıç and Abdiwali (2016) and Filik (2020).

SAS (2001) package program was used in statistical analysis, and the linear relations between the experimental groups were determined by applying orthogonal polynomial contrast with the General Linear Model (PROC GLM) procedure in accordance with the trial model of the study (random plots trial plan). The difference between the groups was made using the Duncan Multiple Comparison Method (Genç and Soysal, 2018).

RESULTS AND DISCUSSION

The nutrient contents of the silages after fermentation are given in Table 1. Dry matter contents were determined as 929.10, 931.70, 929.45 and 928.20 (g/kg) in CB, LAB^6, LAB^8 and LAB^9 groups, respectively ($P<0,05$). Compared with the control group, LAB^6 inoculant increased the DM content of silages, but did not affect it in LAB^8 and LAB^9 groups ($P<0.05$). Pienaar (2010) reported that the addition of homofermentative lactic acid bacteria to oat silage reduced the DM content of silages. The lowest OM content in silages was determined in the LAB^9 group (%92.75). Inoculant treatment did not affect OM content among other groups. The OM content of the silages was found to be compatible with the findings of Addah et al. (2011). The ash values of the silages were found between 6.68% and 7.25%, and the highest value was determined in the LAB group. Inoculant application to barley silage decreased the CP content of the silage compared to the control ($P<0.01$). Pienaar (2010), in his study, determined the HP content in barley silage to be 9.9% in the control group and 9.5% in the LAB supplemented group. Although these values were lower than the values obtained in this study, the researcher confirmed that the application of inoculant decreased the CP content.

EE contents of CB, LAB^6, LAB^8, LAB^9 groups were determined as 5.14, 5.00, 5.23, 5.57%, respectively. Significant differences were found among the groups in terms of EE content ($P<0.01$). These results were higher than those

of Kiraz and Kutlu (2016). As a matter of fact, Kiraz and Kutlu (2016) determined the EE content of barley silage to be 2.19% in the control group and 1.85% in the inoculant group. This difference may be related to the maturity level of the plant. Crude fiber (CF), ADF and NDF contents in groups CB, LAB^6, LAB^8, LAB^9 were determined as 23.61, 23.77, 23.52, 24.18%, 29.78, 29.47, 29.68, 30.46%, and 53.13, 53.88, 53.46, 54.24%, respectively. Inoculant application affected the ADF content of the silages, while it affected the CF and NDF content. Kiraz and Kutlu (2016) reported ADF content in control and inoculant barley silage as 35.41 and 34.02% (P<0.01), and Addah et al. (2011) reported 22.25 and 22.14% (P<0.001), respectively. The differences among the ADF contents obtained in this study and the literature reports may be related to the degree of maturity of the plants at the time of harvest. ADFom and NDFom contents of silages in groups CB, LAB^6, LAB^8, LAB^9 were determined as 23.08, 22.79, 22.85, 23.21% and 46.43, 47.20, 46.63, 46.99%, respectively. There was no difference among the groups in terms of Hcel content of the silages (P>0.05). The TSM content of the silages was found to be 22.23 and 23.55 brix degrees, and there was no difference among the groups.

The DCP, TDN and energy contents of the silages are given in Table 2. The DCP and TDN contents of the silages in groups CB, LAB^6, LAB^8, LAB^9 were found as 8.56, 8.20, 8.35, 8.13 (P<0.01) and 62.88, 62.45, 62.65, 62.34 (P<0.01), respectively. When the energy contents of the silages were examined, DE, ME, NE_L, NE_M and NE_G values were found as, respectively, 2.77, 2.75, 2.76 and 2.75 (P<0.05); 2.27, 2.26, 2.27 and 2.25 (P<0.05); 1.42, 1.41, 1.42 and 1.41 (P>0.05); 1.41, 1.39, 1.40 and 1.39 (P<0.05); 0.82, 0.81, 0.82 and 0.81 (P>0.05).

The feed quality indicators of silages are given in Table 3. The RFV and RFQ values of the silages were calculated as 115.06, 113.86, 114.47, 111.77 (P>0.05) and 115.48, 113.10, 114.33, 112.14 (P>0.05), respectively. RFV values of all silages in accordance with the calculations II. quality feed (103-124) (Kılıç and Abdiwalli, 2016). The RFQ values of silages were concluded that all silages are feeds (100-120) that can be used in the feeding of heifers and dry cows aged 18-24 months (Filik, 2020).

DM, pH_1, temperature, and color parameter values of silages at opening time are given in Table 4. The DM contents of the silages at the time of opening were found to be between 44.08 and 46.54, and there was no statistical difference between the groups (P>0.05). The pH_1 values of the silages were determined as 6.00, 5.75, 5.35 and 5.76, respectively (P<0.01). The pH_1 values of the LAB^8 group decreased significantly compared to the control group and the other groups (P<0.001). Addah et al. (2011) determined the pH1 value of barley silage without inoculant as 4.51 and as 3.95 in silage with inoculant (P<0.01). This difference in pH_1 value may be due to the individual differences of the inoculant and the vegetation difference of the silage material. Inoculant application to silages did not affect the post-opening temperature, L*, a*, b*, C*, and h^o values of the silages (P>0.05).

Microorganism count results of silages after opening are given in Table 5. LAB formation was not detected in the CB and LAB^6 group. In LAB^8 and LAB^9 groups, it was determined as 2.50, 33 log10 cfu/g, respectively, and the differences between the groups were found to be insignificant (P>0.05). Yeast content in CB, LAB^6, LAB^8 and LAB^9 groups was calculated as 1.67, 2.00, 1.00 and 26.00 (log_{10} cfu/g) (P<0.001), respectively. No significant mold growth was observed in the study.

The results of pH_2, CO_2 and microorganism counts of silages after aerobic stability are given in Table 6. The pH_2 values after aerobic stability (AAS) in CB, LAB^6, LAB^8 and LAB^9 silages were determined as 5.56, 5.16, 5.37 and 4.88, respectively. The pH_2 value of the control group was higher than the inoculant groups (P<0.05). The CO_2 values after aerobic stability (AAS) in CB, LAB^6, LAB^8 and LAB^9 silages were found as 19.24, 5.98, 3.96, and 4.84, respectively (P<0.05). The yeast count in the silages on the 5th day after open was 64.67, 35.00, 2.00 and 6.67 in the CB, LAB^6, LAB^8 and LAB^9 silages, respectively (P<0.01. No mold was detected on the 5th day after silage growth. As with pH, the highest CO_2 value was determined in the control group. According to the analyzes of the 5th day after open of silages, the application of inoculant to the silages decreased the pH, CO_2 production of the silages and decreased the yeast counts.

Table 1. The nutrient contents of the silages after fermentation

Groups	CB	LAB^6	LAB^8	LAB^9	P
DM	929.10±0.10^b	931.70±0.10^a	929.45±0.75^b	928.20±0.40^b	0.0183
OM	93.30±0.02^a	93.32±0.08^a	93.17±0.04^a	92.75±0.00^b	0.0027
Ash	6.70±0.02^b	6.68±0.08^b	6.83±0.04^b	7.25±0.00^a	0.0027
CP	13.58±0.04^a	13.18±0.00^c	13.35±0.00^b	13.10±0.02d	0.0003
EE	5.14±0.04bc	5.00±0.04c	5.23±0.04b	5.57±0.05a	0.0029
CF	23.61±0.04	23.77±0.16	23.52±0.09	24.18±0.13	0.0532

ADF	29.78±0.03b	29.47±0.09c	29.68±0.06bc	30.46±0.09a	0.0024
NDF	53.13±0.45	53.88±0.26	53.46±0.00	54.24±0.14	0.1426
[1]ADFom	23.08±0.05a	22.79±0.01b	22.85±0.02b	23.21±0.09a	0.0139
[1]NDFom	46.43±0.47	47.20±0.34	46.63±0.04	46.99±0.14	0.3773
Hcel	23.35±0.43	24.41±0.35	23.78±0.05	23.78±0.05	0.2043
TSM	23.05±0.57	23.55±0.61	22.23±0.67	22.40±0.17	0.3292

DM: Dry matter (g/kg), OM: Organic matter (%), CP: Crude protein (%), EE: Ether Extract (%), CF: Crude Fiber (%), ADF: Acid detergent fiber (%), NDF: Neutral detergent fiber (%), Hcel: Hemicellulose (%), TSM: Total soluble matter, CB: Control Barley, LAB6: Barley + *Pediococcus acidilactici* 1 x 10^6, LAB8: Barley + *Pediococcus acidilactici* 1 x 10^8, LAB9: Barley + *Pediococcus acidilactici* 1 x 10^9. [1]ADFom = ADF – ash, NDFom = NDF – ash. [2] a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

Table2. DCP, TDN and Energy Contents of silages

Groups	CB	LAB[6]	LAB[8]	LAB[9]	P
DCP	8.56±0.03[a]	8.20±0.00[c]	8.35±0.00[b]	8.13±0.02d	0.0002
TDN	62.88±0.04[a]	62.45±0.01[c]	62.65±0.01[b]	62.34±0.01d	0.0003
DE	2.77±0.00[a]	2.75±0.00[c]	2.76±0.00[b]	2.75±0.00[c]	0.0001
ME	2.27±0.00[a]	2.26±0.00[a]	2.27±0.01[a]	2.25±0.00[b]	0.0190
NE_L	1.42±0.00	1.41±0.00	1.42±0.01	1.41±0.00	0.1208
NE_M	1.41±0.01[a]	1.39±0.00[b]	1.40±0.00[ab]	1.39±0.00[b]	0.0298
NE_G	0.82±0.00	0.81±0.00	0.82±0.00	0.81±0.00	0.1208

DCP: Digestible crude protein (%), TDN: Total digestible nutrients (%), DE: Digestible energy (Mcal/kg), ME: Metabolic energy (Mcal/kg), NE_L: Net energy-lactation (Mcal/kg), NE_M: Net energy-maintenance (Mcal/kg), NE_G: Net energy-gain (Mcal/kg), CB: Control Barley, LAB[6]: Barley + *Pediococcus acidilactici* 1 x 10^6, LAB[8]: Barley + *Pediococcus acidilactici* 1 x 10^8, LAB[9]: Barley + *Pediococcus acidilactici* 1 x 10^9. a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

Table 3. The feed quality indicators of silages

Groups	CB	LAB[6]	LAB[8]	LAB[9]	P
DDM, %	65.71±0.02[b]	65.94±0.07[a]	65.78±0.04[ab]	65.17±0.07[c]	0.0022
DMI, % of Body Weight	2.26±0.02	2.23±0.01	2.25±0.00	2.22±0.01	0.1785
RFV[1]	115.06±1.03	113.86±0.42	114.47±0.09	111.77±0.42	0.0622
RFQ[2]	115.48±1.07	113.10±0.52	114.33±0.03	112.14±0.31	0.0650

DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed value, RFQ: Relative forage quality, CB: Control Barley, LAB6: Barley + Pediococcus acidilactici 1 x 106, LAB8: Barley + Pediococcus acidilactici 1 x 108, LAB9: Barley + Pediococcus acidilactici 1 x 109. 1) According to the roughage classification method, the RFV value "V" (< 75) indicates poor quality to be rejected; (75–86) IV. Quality; (87–102), III. Quality; (103–124), II. Quality; (125–151) "prime" quality; and (> 151) refers to the best quality. 2) According to the RFQ method developed to determine roughage quality for dairy cattle, "140–160" Dairy, 1st trimester dairy calf, "125–150" dairy, last 200 days Heifer, 3 to 12 months stocker cattle, "115–130" Heifer, 12 to 18 months beef cow-calf and "100–200" heifer are described as 18 to 24 months dry cow. a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

Table 4. DM, pH, temperature, and color parameter values of silages at opening time

Groups	CB	LAB[6]	LAB[8]	LAB[9]	P
DM	44.42±0.61	45.84±0.19	46.54±0.03	44.08±0.00	0.6747
pH_1	6.00±0.04[a]	5.75±0.16[a]	5.35±0.01[b]	5.76±0.01[a]	0.0013
Temperature	23.03±0.05	22.85±0.06	22.78±0.26	22.93±0.16	0.7159
*L**	40.38±3.17	44.62±1.63	43.32±1.20	45.17±3.36	0.5575
*a**	3.45±0.27	2.87±0.13	3.65±0.40	3.69±0.33	0.2431
*b**	14.98±0.93	16.78±0.46	15.96±0.51	17.94±1.61	0.2393
*C**	15.37±0.95	17.03±0.44	16.38±0.58	18.33±1.57	0.2462
h^o	77.03±0.66	80.27±0.59	77.23±0.99	78.13±1.55	0.1539

DM: Dry matter, pH1: pH value at the time of opening, L*: Lightness, a*: redness, b*: yellowness, C*: Chroma, or saturation, h°: hue angle. CB: Control Barley, LAB[6]: Barley + *Pediococcus acidilactici* 1 x 10^6, LAB[8]: Barley + *Pediococcus acidilactici* 1 x 10^8, LAB[9]: Barley + *Pediococcus acidilactici* 1 x 10^9. a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

Table 5. Microorganism count results of silages after opening

Groups	CB	LAB^{6}	LAB^{8}	LAB^{9}	P
LAB, log_{10} cfu/g	N/A	N/A	2.50±1.50	9.33±2.03	0.0958
Yeast, log_{10} cfu/g	1.67±0.33^{b}	2.00±1.00^{b}	1.00±0.00^{b}	26.00±0.00^{a}	0.0006
Mold, log_{10}	N/A	N/A	2.00±0.00	10.67±1.76	0.1333

N/A: Not applicable. $^{a, b, c}$ Mean values within the same column with no common letters differ significantly (P<0.05). CB: Control Barley, LAB^{6}: Barley + *Pediococcus acidilactici* 1 x 10^{6}, LAB^{8}: Barley + *Pediococcus acidilactici* 1 x 10^{8}, LAB^{9}: Barley + *Pediococcus acidilactici* 1 x 10^{9}. a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

Tablo 6. The results of pH_2, CO_2 and microorganism counts of silages after aerobic stability

Groups	CB	LAB^{6}	LAB^{8}	LAB^{9}	P
pH_2	5.56±0.07^{a}	5.16±0.21^{b}	5.37±0.15^{b}	4.88±0.03^{b}	0.0190
CO_2	19.24±3.78^{a}	5.98±2.08^{b}	3.96±0.69^{b}	4.84±0.82^{b}	0.0228
AAS Yeast log_{10} cfu/g	64.67±2.67^{a}	35.00±2.65^{b}	2.00±0.00^{c}	6.67±0.67^{c}	0.0001
AAS Mold, log_{10} cfu/g	N/A	N/A	N/A	N/A	N/A

AAS: After aerobic stability, N/A: Not applicable. $^{1\ a, b, c}$ Mean values within the same column with no common letters differ significantly (P<0.05). CB: Control Barley, LAB^{6}: Barley + *Pediococcus acidilactici* 1 x 10^{6}, LAB^{8}: Barley + *Pediococcus acidilactici* 1 x 10^{8}, LAB^{9}: Barley + *Pediococcus acidilactici* 1 x 10^{9}. a, b, c Mean values within the same column with no common letters differ significantly (P<0.05).

CONCLUSIONS

In conclusion, the use of homofermentative *Pediococcus acidilactici* (MF098795) lactic acid bacteria in barley silages provided positive contributions to the nutrient content of the silages and improved aerobic stability. Accordingly, when all data are evaluated, it may be recommended to add *P. acidilactici* bacteria at the level of 10^{8} cfu/g in terms of silage quality and aerobic stability in barley silage.

ACKNOWLEDGEMENTS

The authors would like to thank Associate Professor Gökhan FİLİK from Kırşehir Ahi Evran University, Faculty of Agriculture, Department of Agricultural Biotechnology, for have been using laboratory equipment available.

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SEAFOOD CONSUMPTION PREFERENCES DURING THE COVID-19 PANDEMIC PERIOD IN ISTANBUL

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Abstract

This survey study provides an overview of the impact of the Covid 19 pandemic on consumers' seafood consumption in the city of Istanbul, which has the densest population in Turkey, including economic, social, and transportation wise, environmental challenges that the fisheries sector has had to face during this period the Covid -19 pandemic in 2020. The results showed that 91% of the participants, 45% of whom were female and 54% were male, consumed seafood during the pandemic period. It was also revealed that 64% of the participants did not consume enough seafood in general. It was determined that the income levels of the participants who did not consume enough were in the range of 0-2800₺. The survey results showed that the Covid-19 pandemic did not affect both the availability of seafood in Istanbul and the consumption of seafood. In addition, the results showed that the cafes and restaurants that have been closed for more than a year in Turkey harm the consumption of seafood. The problems experienced in this period can help how to take measures in possible similar situations that may occur in the future for the country's seafood sector and consumption, and how the sector can take environmentally oriented and sustainable steps independently from the outside.

Key words: *seafood consumption, consumer preferences, the Covid-19 pandemic, Istanbul*

INTRODUCTION

Since it was the first description in December 2019, the novel COVID-19 virus has changed our interpersonal and social lives at an incredibly rapid pace. After the summer of 2020, many countries around the world, including Turkey, were forced to implement drastic measures, such as the implementation of 'social distancing' rules, a term recently adopted. They were various practices to reduce the number of physical contact in public places – or temporary closure of restaurants, cultural venues, and non-essential retail stores (Buchholz, 2021; Schreiner and Baier, 2022). For this reason, consumers preferred to do their food shopping online without leaving the house, both to protect their health and due to the mandatory social distance rules (Roggeveen and Sethuraman, 2020).

Consumption of seafood has increased in recent years, mainly due to including a serious source of protein and essential fatty acids and minerals, good for heart health and other important diseases and prevent them (Turan et al., 2006). Although our country is surrounded by seas, compared to the world in general, the consumption of seafood is quite low. Annual per capita fish consumption of the regions is average kg: Africa 12.4 kg, Asia 108.7 kg, North America 8.1, Europe 16.1kg and only Turkey consumes 6.3 kg (FAO, 2020).

Istanbul is the most populous city in Turkey, which hosts 18.49% of Turkey's population. The population of Istanbul is more than that whom of 131 countries in the world. According to official TUIK (2020) data, the number is 15 million 840 thousand 900 people. At the same time, Istanbul is the most developed province of Turkey, which holds the Turkish economy with its production and service sectors (Korfalı et al., 2014). It has also the country's largest employee population with 2 million male and 1 million female employees (Turkish Employment Agency, 2020).

Consumption of seafood is affected by many factors such as socioeconomic status, general food consumption patterns, the personal health status of consumers, and a range of behavioral attitudes (Erdogan et al., 2011). It is aimed to determine the Turkish people's consumption habits and preferences of fresh and processed seafood, and their attitudes and knowledge about seafood consumption during the Covid 19 pandemic, based on the example of Istanbul, the largest metropolis in Turkey.

MATERIALS AND METHODS

In this study, a one-month survey study was conducted between May 2021 and June 2021, when there was full closure, to determine the consumer preferences of the people working in different parts of Istanbul.

The survey study, with questions prepared on the Google Forum, only to people living in the city of Istanbul, using e-mail, messages, and social media tools; It was held with the participation of a total of 162 people, 74 of whom were men and 88 were women.

There were 24 questions in the survey. Seven of these were related to demographic characteristics (gender, age, income, marital status, and education), while others were related to seafood consumption and the effect of the Covid-19 pandemic on seafood consumption.

Results of the survey and analyzes were evaluated with the MS-Excel program.

RESULTS AND DISCUSSION

Of the 162 individuals who participated in the survey, 54% were female and 46% were male. Similar to our study, it has been reported that female individuals are less than male individuals in the number of participants in seafood survey studies conducted in our country (Arslan and İzci, 2016; Aydın and Karadurmuş, 2013; Sağlam and Samsun, 2018). Considering the age distribution of the individuals participating in the survey, it was determined that the highest participation was 75% in the 22-30 age group in the present study. While other (students, housewives, trade, consultants, etc.) took the first place in the survey as the occupational group with 52%, it was followed by the foreign company occupational group with 22%. The least-marked occupational group was tradesmen with 2.4%. Most of the participants consume seafood one meal a week and answered that they consume it because of health (Figures 1 and 2). Similarly, in a few studies conducted in different cities in Turkey, participants stated that they mostly consume seafood once a week and prefer it because they find it healthy (Aydın and Karadurmuş, 2013; Arslan and İzci, 2016).

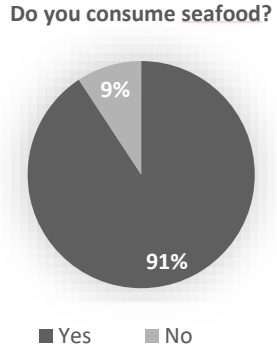

Figure 1. The rate of consumption of seafood by the participants

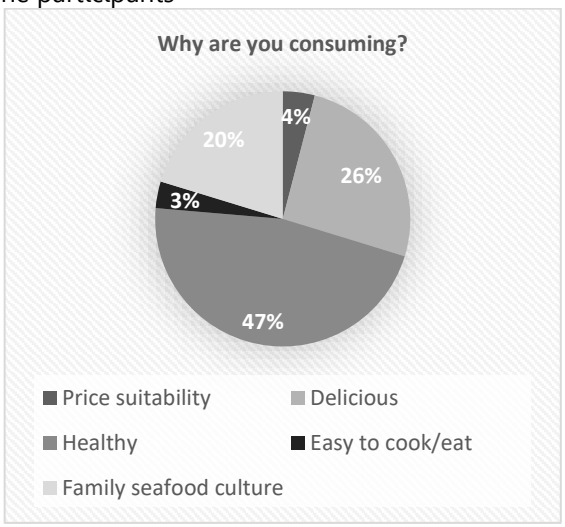

Figure 2. Participants' reasons for consuming seafood

Only 34% of the participants stated the high price as the reason for not consuming seafood (Figure 3). When 32% of them stated that they dislike fishy taste and odor. In another study conducted by Erdogan et al (2011) in Istanbul, the taste and odor is the most frequent reason given by respondents (56.95%) who do not consume seafood. Likewise, Hicks et al. (2008) reported that respondents were not eating seafood that's why the main reason was the odor and taste of fish. In previous studies, having a dislike for the taste and/or smell of fish is a widely reported barrier to seafood consumption, in general, the world (Best and Appleton, 2013; Bostic et al., 2017; Govzman et al., 2021). Furthermore, the attitude of consumers with low fish consumption towards not liking fish is related to the unpleasant taste and smell, its smell while cooking along with its bones (Carlucci et al., 2011; Grieger et al., 2012). Experimental evidence has been provided by researchers that household seafood consumption is negatively affected by pressure from some members who do not like to eat fish, as well (Altintzoglou et al., 1010; Birch and Lawley, 2012; Carlucci et al., 2015). Some studies also reported similar findings for participants who found that seafood is so expensive (Hicks et al., 2018; Wake and Geleto, 2019). On the contrary, Erdogan et al. (2011) reported that Turkish consumers give importance to freshness and quality rather than price. This can be explained by the fact that the prices of fish and seafood increased even more due to the difficult conditions etc. (fishery boats landing in the sea) at the time of the Covid-19 pandemic. This shows that fish consumption is strongly

correlated with the income of families and individuals (Desiere et al., 2018).

In the present study, when the question of "How do you prefer seafood more" was asked to the participants, it was determined that 88% preferred to consume fresh. It has been stated before that the preference for seafood as fresh and living in places where they can be found fresh are a situation that increases seafood consumption more (Appleton, 2016; Neale et al., 2012).

Due to the Covid 19 pandemic, individuals reported that their seafood consumption habits did not change at a rate of fifty percent (Figure 4). Furthermore, the majority answered that the accessibility of fish and seafood was not affected due to the pandemic (Figure 5). However, They reported that the closure of cafes and restaurants due to the pandemic adversely affected their seafood consumption (Figure 6).

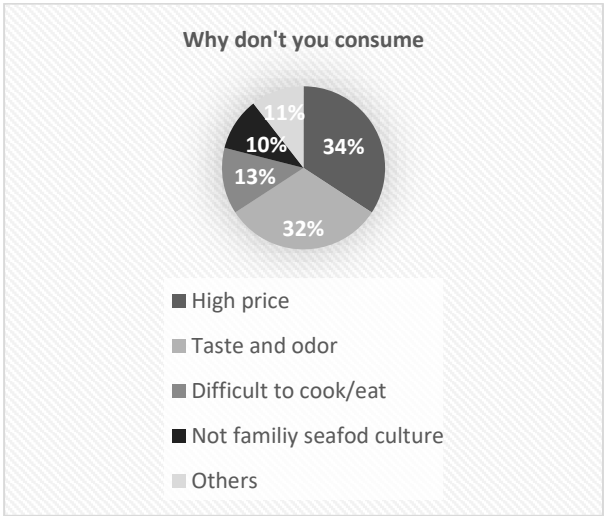

Figure 3. The reason for participants not consuming seafood.

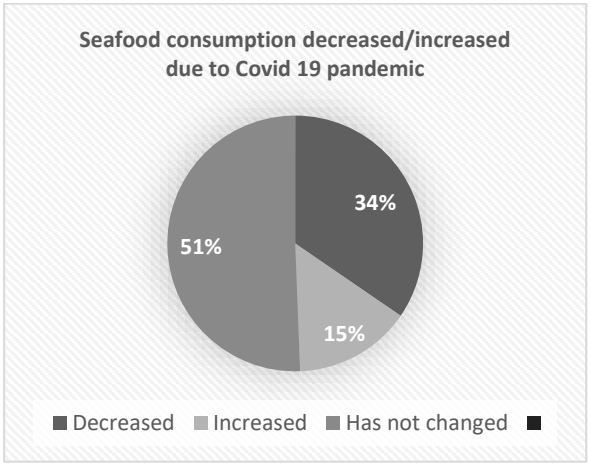

Figure 4. The effect of the pandemic on the consumption of seafood.

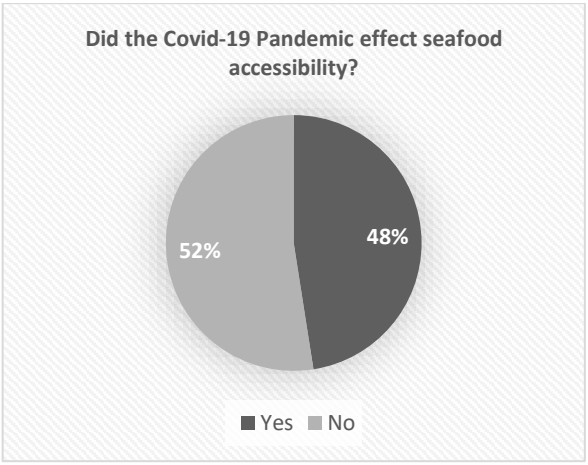

Figure 5. Accessibility of seafood during the Covid-19 pandemic.

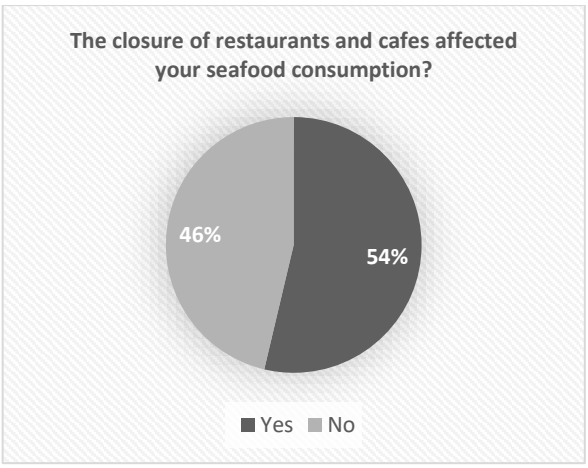

Figure 6. Effect of the closure of restaurants and cafes on seafood consumption.

The results of the present study also demonstrated that participants preferred fish stores the most (43%) when purchasing seafood during the pandemic. According to our results, the consumption and accessibility of fresh seafood are not affected much because the city of Istanbul is located very close to the Sea of Marmara. On the other hand, in the Covid 19 pandemic, consumers in the USA were able to prefer frozen seafood much more due to social distancing measures that have led to widespread restaurant closures reduced seafood market, and reduced foot traffic (White et al., 2020). According to White et al. (2020), it can be said that the places far from the sea considerably reduce the consumption of seafood, therefore; the pandemic restrictions may not affect all places in our country equally like Istanbul. The closure of cafes and restaurants in many places during the pandemic period has considerably reduced or affected the consumption of seafood in our findings (Minahal et al., 2020; White et al., 2020).

When we asked the participants whether the accessibility of fishery products was affected during the Covid-19 pandemic period, it was found that the majority (52%) were not affected. The fact that accessibility to seafood is more comfortable during the pandemic period can be explained that the city of Istanbul is on the coast of the Marmara Sea, and the coastal and angling continues. Some studies presented that the seafood sector (fishing, transportation, accessibility, etc.) in countries around the world was likely affected by the Covid-19 period (Basset et al., 2021; Bennet et al., 2020; FAO, 2020; Fernandez-Gonzalez et al., 2021; Love et al., 2020; White et al., 2021).

CONCLUSIONS

We can say that during the pandemic period in the province of Istanbul, access to fishery products was not very difficult and fresh fish could be consumed. However, these limited results based on a single city may not indicate that pandemic affects other cities in the country in the same way. Therefore, the Covid 19 pandemic has put forward measures that can be taken in such cases for production and consumption. In order to be ready for a possible future scenario, it has become important to ensure the continuation of fisheries, and the protection and sustainability of our seas. Finally, we hope that the results of our study will encourage further research on seafood consumption and deficiencies in our country, based on consumer responses to seafood.

ACKNOWLEDGEMENTS

This work was supported by the Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: ZRT.E2.16.002

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POTENTIAL HEALTH RISKS OF HEAVY METALS VIA CONSUMPTION OF GILTHEAD SEABREAM (SPARUS AURATA LINNAEUS, 1758) FARMED IN TURKEY

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Abstract

Sea bream is one of the main cultured fish, and Turkey is one of the leading producers of this species. As the consumption of cultured fish is increasing day by day, it is important for public health to evaluate the risk of metal transmission to humans through consumption. In this study, Cd, Pb, and Hg concentrations in seabream from three main aquaculture facilities in the Aegean Sea were studied, and potential health risks were determined. The mean concentrations of Cd, Pb, and Hg (mg/kg) were 0.03±0.07, 0.06±0.08, 0.02±0.01, respectively. Even though these mean values were below the permitted levels, 7.14% of the samples contained Cd above the permitted limits. The individual and combined target hazard ratios (THQ and TTHQ) of the elements were well below 1, indicating no potential public health risk to the average consumer. All estimated weekly intake (EWI) values remained well below the established Provisional Tolerable Weekly Intakes (PTWI). It has been concluded that seabream, cultured in the Aegean Sea by Turkey, is safe for the average consumer in terms of Cd, Pb, and Hg intake.

Key words: *Heavy metals, trace elements, seabream, fish, food safety*

EVALUATION OF THE ANTIBACTERIAL EFFECT OF JUJUBE LEAF EXTRACT ON *Listeria monocytogenes*

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Abstract

The extraction of health beneficial plants and their use as antimicrobial agents against foodborne pathogens is a trending issue. The antibacterial activity of jujube (Ziziphus jujuba) tree leaves extract against Salmonella Enteritidis and Listeria monocytogenes (ATCC 7644) was studied both in vitro and on fish steaks. Dried leaves were grinded for the treatment either by water infusion or methanol extraction methods. Aqueous infusion was carried out at 90ºC for 60 min by using a magnetic stirrer. Meanwhile, 80% methanol was used for extraction with a stirrer at 250 rpm for 24 h. Leaf to solvent ratio was 1:10. After the stirring processes, extracts were filtered. Aqueous extract was used as obtained. On the contrary jujube leaf methanolic extract (JME) was gained by evaporating the solvent under controlled vacuum conditions. In vitro assay was performed with disc diffusion method. There were no inhibition zones formed in the plates of both bacteria exposed to aqueous extract. Only L. monocytogenes was inhibited with 10 mm diameter zones in the JME treated plates, while no zones were observed for Salmonella Enteritidis. Chloramphenicol and ciprofloxacin were used as reference antibiotics. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of L. monocytogenes were determined in JME containing broth. MIC and MBC were 61.9 mg mL^{-1} and 247.5 mg mL^{-1}, respectively. The second part of the study was conducted by using rainbow trout steaks with the marketing name of Black Sea salmon. There were no significant inhibition of L. monocytogenes in JME treated and non-treated salmon steaks at the concentration which gave less greenish plant extract color to the flesh. To conclude, the complex cell membrane of Gram-negative bacteria such as Salmonella makes it more resistant to external factors than Gram-positive bacteria such as L. monocytogenes. In order to investigate the antibacterial effect of jujube tree leaf extract in food, future studies should be conducted on extracts with lower solvent and higher leaf content.

Key words: *Jujube leaf extract, Listeria monocytogenes, Salmonella, rainbow trout, antibacterial effect, minimum inhibitory concentration, minimum bactericidal concentration.*

INTRODUCTION

Jujube tree is an ancient plant which has been cultivated for more than 7000 years. Having nutritious and delicious fruit, high crop yields, economic benefits, easy pest control, and resistance to abiotic stress, jujube responds the demands of consumers, growers, and marketers. Because of these features it is suggested as a super fruit for the future (Liu et al., 2020). Not only the fruit, but also the leaves are beneficial and have been used for medicinal purposes. Jujube leaves are known to have sedative and hypoglycemic effects and can be used for their anti-obese and anti-allergic properties (Kemeç Hürkan, 2019). The main phenolic compounds in the leaves are determined to be catechin, caffeic acid, rutin, apigenin-7-glucoside, chlorogenic acid, syringic acid, p-coumaric acid, ferulic acid, eriodictyol and quercetin (San & Yıldırım, 2010). Yet the knowledge on antimicrobial effects are limited.

The addition of plant extracts to food in order to inhibit the bacterial growth is a trending process. Consumers prefer natural antimicrobials like plant extracts over synthetic agents to ensure the elimination of bacteria (Smid & Gorris, 1999). It is stated that Gram-positive bacteria are more sensitive to antimicrobial agents than Gram-negative bacteria (Burt, 2004). *Listeria monocytogenes* is a Gram-positive bacterium that is responsible for listeriosis infections (Farber & Peterkin, 1991). *Salmonella* is a Gram-negative bacterium causing gastroenteritis (Doyle & Cliver, 1990). These rod shaped and motile, non-spore forming bacteria are the pathogenic bacteria that mainly cause food poisoning and isolated from a wide variety of raw, processed, cooked or ready-to-eat foods (Wagner & McLauchlin, 2008; Üçok Alakavuk et al., 2021).

Large size rainbow trout cultivated in the Black Sea, or Black Sea salmon as its marketing name, is one of the important aquaculture species

which was produced 31509 tonnes in 2021 (TUIK, 2022). Rainbow trout is mostly marketed as IQF frozen steaks evoking Atlantic (Norwegian) salmon in color and shape. It has a brand value and besides other benefits, a recent study indicated that it has higher omega-3 values than Atlantic salmon (Keskin et al., 2022).

Contamination during processing is a serious problem and further growth of bacteria in raw fish possesses health risk to consumer. The aim of this study was to reveal the antibacterial effect of jujube leaf extract on *L. monocytogenes* and *Salmonella* Enteritidis both *in vitro* and on fish steaks.

MATERIALS AND METHODS

Jujube (*Ziziphus jujuba* Mill.) leaves were air dried and grinded into small pieces. Grinded leaves were treated by water infusion or methanol extraction methods with 1 to 10 leaf to solvent ratio. Aqueous infusion was carried out on a magnetic stirrer for 60 min at 90ºC. In the meantime, methanolic (80%) extraction was performed with a stirrer at 250 rpm for 24 h. Both solutions were filtered. Aqueous extract was used as obtained. Jujube leaf methanolic extract (JME) was obtained by evaporating the methanol under vacuum at 40 °C.

Stock cultures (100 µL) of *L. monocytogenes* ATCC 7644 and *Salmonella* Enteritidis were separately transferred into 10 mL Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE), and incubated at 30ºC for 24 hours. A 100 µL of culture taken from the tube was transferred to 10 mL TSBYE again and incubated for another 24 h at 30ºC. After incubation, the culture solution was centrifuged at 4000 rpm for 10 minutes. The supernatant was then discarded. Remaining pellet was washed twice with 10 mL of 0.1% peptone water (PW) (Lin et al., 2004). The inoculum was prepared by mixing the obtained precipitate with 10 mL of 0.1% PW.

In vitro assay was carried out according to disc diffusion method. Extract-impregnated paper disks were put on Tryptic Soy Agar (TSA) plates inoculated with bacteria. After incubation at 35ºC for 24-48 h, inhibition zones were measured in diameters. Chloramphenicol and ciprofloxacin were used as reference antibiotics. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined in serially diluted extract containing Mueller Hinton Broth (MHB).

For fish assay, *Oncorhynchus mykiss* (Walbaum, 1792) steaks were laterally cut to obtain ten gram pieces. Bacterial inoculum (100 µL) was spread onto the surface of fish and allowed to penetrate for 20 min. Half of the fish were kept non-treated and the other half were treated with 250 µL JME dissolved with 750 µL methanol (80%).

Under aseptic conditions, 25 g fish sample was diluted with 225 mL Maximum Recovery Diluent (MRD) and homogenized. Serial dilutions of 9 mL from each group were prepared in MRD. Palcam Listeria Selective Supplement added Listeria Identification Agar Base was inoculated with 0.1 mL dilutions and incubated at 35°C for 24 hours. Two petri dishes were inoculated from each dilution. After incubation, the number of bacteria was calculated as log CFU/g by counting the gray-green typical colonies (Hitchins et al., 2022).

RESULTS AND DISCUSSION

According to disc diffusion assay, aqueous extract of jujube leaves had no inhibitory effect on either *L. monocytogenes* or *Salmonella* Enteritidis. It was assumed that water lacks the ability to dissolve antimicrobial compounds in leaves. On the other hand, inhibition zone occurred around the JME-impregnated discs only for *L. monocytogenes* (Table 1). It was reported that Gram-positive bacteria were more vulnerable to antimicrobials than Gram-negative bacteria (Burt, 2004). The less susceptibility of Gram-negative bacteria is explained by the fact that their cell walls are surrounded by a lipopolysaccharide outer membrane, which restricts the diffusion of antimicrobial compounds into the cell (Vaara, 1992). This could be why there was no inhibition achieved for *Salmonella* in this study. Similar to this study, *Ziziphus spina-christi* leaf extracted in methanol/water: 50/50 (128 mg/mL) had antibacterial effect on *L. monocytogenes* with an inhibition of 11.17mm diameters. However, it showed no effect on *Salmonella typhimurium* during disc diffusion tests (Gheith & El-Mahmoudy, 2018). Yahia et al. (2020) similarly extracted *Ziziphus lotus* and *Ziziphus mauritiana* leaves with methanol (70%) and tested the antibacterial activity (10 mg/mL) against *L. monocytogenes* and *S. typhimurium* which formed 10.0 – 12.2 mm and 11.2 – 12.2 mm zones, respectively. In this study, these zones were achieved at very high concentrations for *L. monocytogenes.*

It was also reported that both the metanolic extract and essential oil of jujube seeds exhibited antimicrobial effect against *L. monocytogenes* with the inhibition zones of 17.2 and 17.3 mm, respectively (Al-Reza et al., 2010).

Table 1. Antibacterial effect of jujube leaf methanol extract on *Listeria monocytogenes*, (Lm) or *Salmonella* Enteritidis (SE)

	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)
Lm	11.0	61.9	247.5
SE	ni	-	-

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration
ni = no inhibition, - = not tested.

Çoban & Biyik (2010) determined that 100 mg/mL ethanolic jujube leaf extract had antibacterial effect on *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Bacillus thrungiensis*, and *Proteus* spp. with inhibition zones ranging between 10 - 20 mm.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) should be determined to investigate the effectiveness of antimicrobial compounds in various foods (Ukuku et al., 2015). In the MIC assay, the antibacterial activity was tested in a growing medium where *L. monocytogenes* was active and it was found to be 61.9 mg/mL. Accordingly, MBC was determined as 247.5 mg/mL (Table 1). MIC and MBC were not specified for *Salmonella* since JME had no inhibitory effect in disc diffusion tests.

Abdulla et al. (2016) reported that aqueous or ethanolic extract (100µg/ml) of jujube leaves demonstrated antibacterial effect on various pathogens such as *Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa, S. aureus* and *E. faecalis* according to agar diffusion method.

It was also stated that both aqueous and ethanolic *Z. jujuba* leaf extracts showed antibacterial effect on *E. coli*, *Klepsiella* spp. and *S. aureus*, and the MICs were at range of 11.7 to 8.7 mg/mL and 14.8 to 8.2 mg/mL, respectively (Alhassan et al., 2019). On the contrary, jujube leaf extract used as reducing agent of gold nanoparticles was shown to have no antimicrobial effect on *E. coli* (Aljabali et al., 2018).

In the food matrix study, rainbow trout steaks, also known as Black Sea salmon marketing name, were inoculated with 7-8 log CFU/g bacteria. No significant inhibition of *L. monocytogenes* was observed in JME treated and non-treated fish steaks (Figure 1). So, higher concentrations of extracts could be added in order to achieve successful elimination of bacteria. Nonetheless, it should be kept in mind that most plant extracts cause greenish color in food matrices altering the natural color.

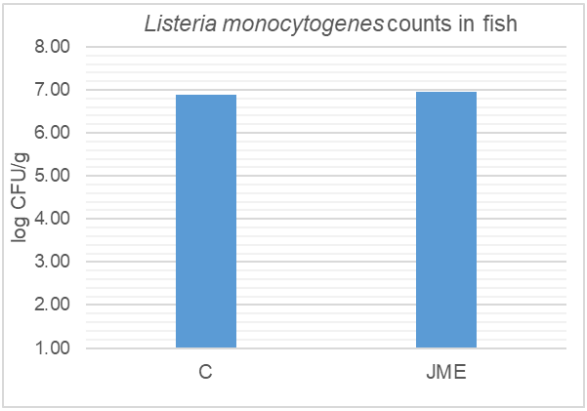

Figure 1. *Listeria monocytogenes* counts in non-treated (C) and jujube extract (JME) treated fish samples.

One of the important reasons why the same extract in food matrix is not as effective as in synthetic laboratory medium is the protective effect of food compounds. Nutrient composition, complex structure and pH of the food play an important role in the efficacy of antimicrobials to inhibit or eliminate the bacteria (Ukuku et al., 2015). Another reason of antimicrobial effectiveness may be the dependence on plant extraction methods' effectiveness. It is essential to obtain and purify the active compounds (Gyawali et al., 2015).

CONCLUSIONS

Considering *in vitro* assays, JME had inhibitory effect on *L. monocytogenes* at high concentrations. Yet, no significant inhibition was observed in JME treated and non-treated fish steaks. Future studies should focus on jujube tree leaf extract with lower solvent and higher leaf content in order to achieve antimicrobial success in food matrices.

ACKNOWLEDGEMENTS

I would like to thank to the Departments of Food Safety and Processing Technology, Faculty of Aquatic Sciences, Istanbul University, for allowing this preliminary research to be carried out.

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STUDY ON INFECTION WITH SOME ECTOPARASITES OF GUPPY (POECILIA RETICULATA) AS AN ORNAMENTAL FISH IN TEHRAN, IRAN

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Abstract

The guppy (Poecilia reticulata) is one of the most popular ornamental freshwater fish and nowadays is kept as a common pet fish in Iran and other countries. The aim of this study was to determine prevalence rate of infestation with ectoparasites in guppy (P. reticulata) in Tehran, Iran. In this study 100 guppy fish in different ages were collected randomly from 10 ornamental fish supply centers of Tehran, Iran in autumn of 2021. All of these fish were cultured in Iran. They were transferred to the ornamental fish clinic of Faculty of Veterinary Medicine, University of Tehran. After that, these fish were examined by preparing a wet mount form their skin and gills. After 48 hours fish were rechecked. Wet mounts were studied under the light microscope. Results showed that most of the fish (78%) at least were infected with 1 type of ectoparasites. Trichodina sp. (16%), Dactylogyrus spp. (10%) and Gyrodactylus spp. (52%) were seen in infected fish. 8% of fish had been infected with 2 parasites. Simultaneously, 4 fish (4%) were infected with Gyrodactylus sp. and Dactylogyrus sp. And when the fish were rechecked, it showed that severity of infection had been increased and after one week, mortality was seen. Mortality of fish showed that when fish are kept in their environment for long time, maybe they do not have any mortality in despite of that they are infected with some parasites, but when they are displaced, mortality is being started, because stress of transportation cause to depressing the immune system of fish and increase propagation of parasites, So it cause to mortality of fish. In conclusion for prevention of high mortalities, it is better to check the ornamental fish in their farm and treat the infected fish.

Key words: *ectoparasites, guppy, Iran, Gyrodactylus*

REPRODUCTIVE BIOLOGY OF SALEMA, *Sarpa salpa* (Linnaeus, 1758) AROUND NORTH AEGEAN SEA, TURKEY

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Abstract

The aim of this study is to reveal the first data on the reproductive biology of Salema in Turkish waters. For this purpose, 418 Salema individuals were obtained from commercial fishermen between August 2020 and July 2021 in the North Aegean Sea. The individuals varied from 10.2 cm to 38 cm in total length and ranged between 15.94 g and 752.42 g in weight. In total, the female to male sex ratio was determined as 0.81:1. The length at first sexual maturity (L50) of Salema was determined as a 31.23 cm TL and 27.89 cm TL for female and male individuals, respectively. The Gonadosomatic index value (GSI) varied between 0.05 and 8.85 for female and ranged from 0.03 to 2.22 for male individuals. The reproduction took place between October and November and peaked in October. It was determined that the fecundity of Salema individuals varied between 656 512 and 991 171 eggs, with a mean of 854341±33211 eggs. As a result, it was determined that Salema is a protandric hermaphrodite species and all individuals larger than 31.6 cm TL at length and 6 years at age were found to be a female.

Key words: *Sarpa salpa, GSI, CF, spawning season, first sexual maturity length, fecundity*

INTRODUCTION

A knowledge on reproductive biology of fish species is an important tool for fisheries management authority. The spawning seasons, spawning fraction, fecundity, sexual maturity etc. are basic components of reproductive biology. Thus, valid and current information on these components should be offer to fisheries managers by species based (Millar and Kendall, 2009).

Salema, (Linnaeus, 1758) is a herbivorous bony fish species with medium economic value, which is distributed in sea and brackish water areas at a depths between 5 and 70 meters in rocky and sandy areas with algae growth. It has a wide geographical distribution from Bay of Biscay to South Africa (Bauchot and Hureu, 1990).

Varied reproduction types such as gonochorism or hermaphroditism for Salema were identified (Allsop and West, 2003; Sadovy de Mitcheson and Liu, 2008). The detailed information on reproduction biology was revealed some published literature around South Africa (van der Walt and Mann, 1998), Mediterranean (Criscoli et al., 2006; El-Etreby et al., 2015), Atlantic coasts (Mendes-Villamil et al., 2002; Paiva et al., 2018). A knowledge on population biology and reproduction is scarce around Aegean Sea and Turkish coasts.

In this study, the components of reproduction such as sex ratio, length at first sexual maturity, condition factor, gonadosomatic index, spawning season and sex reversal was determined. Thus, we aimed to contribute management issues with reveal this mandatory and missing data.

MATERIALS AND METHODS

Salema individuals were obtained from commercial catches of gill net fisheries around North Aegean coasts of Turkey between August 2020 and July 2021. Miminum 30 specimen was selected as random from total commercial catch for each month during study period. Sub-sampled fish individuals were transported from the vessel to laboratuary with inside the icebox, immidiatelly. In the laboratory all specimens were measured to the lowest millimeter and weighed to the lowest 0.1 g.

Stages of maturity were determined by Holden and Raitt (1974): immature, maturing, ripening, ripe, and spent. The gonadosomatic index (GSI) was calculated using the formula developed by Gibson and Ezzi (1980):

GSI= (Gonad weight/(Body weight−Gonad weight))×100

The length at first maturity (L50) was estimated by fitting a logistic function using the Newton algorithm which is defined as:

P(1)=1/1+e–(a+b1)

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

After microscopic examination, 28 of 185 female gonads were selected as mature for

fecundity analysis, which had hydrated oocytes. The gonads were dried on drying paper, their total weight was measured and 0.05 g subsamples from three parts (front, middle and back) were collected from each ovary. The gravimetric method according to Bagenal (1978) was used to calculate fecundity. Diameters of oocytes were measured using the Q-Capture Pro image analysis tool. Ripening oocytes were determined according to the precursor of vitellogenesis (oocytes larger than 50 µm). Absolute fecundity was calculated using Bagenal's (1978) formula: $F = n \times (G/g)$ where n is the mean number of eggs in each gonad, G is the total weight of gonad, and g is the weight of the female individual. The total length–fecundity and age-fecundity relationships were estimated for females using linear and exponential regression according to which the equations had the best fit.

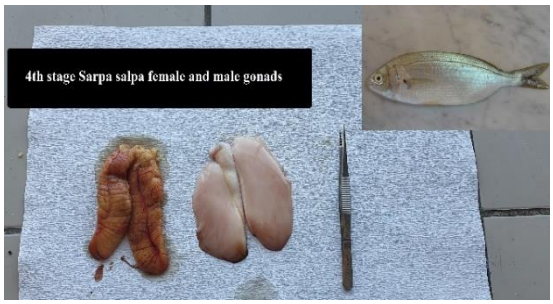

Figure 1. Mature female and male gonads of Salema, *Sarpa salpa*.

RESULTS AND DISCUSSION

A total of 418 specimen of *S. salpa* were obtained from the commercial catchs of gillnet fishery around North Aegean coasts of Turkey. The individuals varied from 10.2 cm to 38 cm in total length and ranged between 15.94 g and 752.42 g in weight. 6 of 418 individual were not sexed due to immature gonads. Between the remaining 412 individual, 185 individual were determined as a female and 227 were male. Thus, the female:male ratio was found as a 0.81:1. The length at first Sexual maturity (L_{50}) of Salema was determined as a 31.23 cm TL and 27.89 cm TL for female and male individuals, respectively. It was found that females reached sexuality at larger sizes. Condition factor (C) of Salema individuals was ranged between 1.21 and 1.37. Lowest Condition Factor (CF) values were determined between August and October, and highest CF values were determined between in February and March for all individuals. CF varied from 1.18 to 1.34 and ranged from 1.14 to 1.39 for males and females, respectively. There was no statistically difference was found for CF values of Salema between the sexes ($p>0,05$). The Gonadosomatic index of Salema individuals were ranged between 0.06 and 4.49, with a mean of 0.48±0.08 at all data set. The Gonadosomatic index value (GSI) varied between 0.05 and 8.85 for female and ranged from 0.03 to 2.22 for male individuals. The mean GSI for females and males was determined as a 0.71±0.15 and 0.31±0.08, respectively. It was determined that the highest GSI values were seen in October for both sexes.

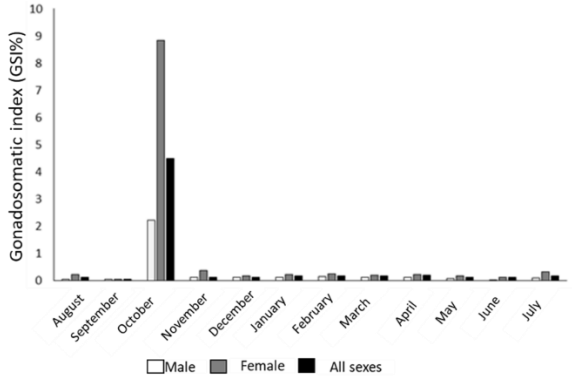

Figure 2. The temporal variation of GSI values for males, females and all sexes of Salema individuals.

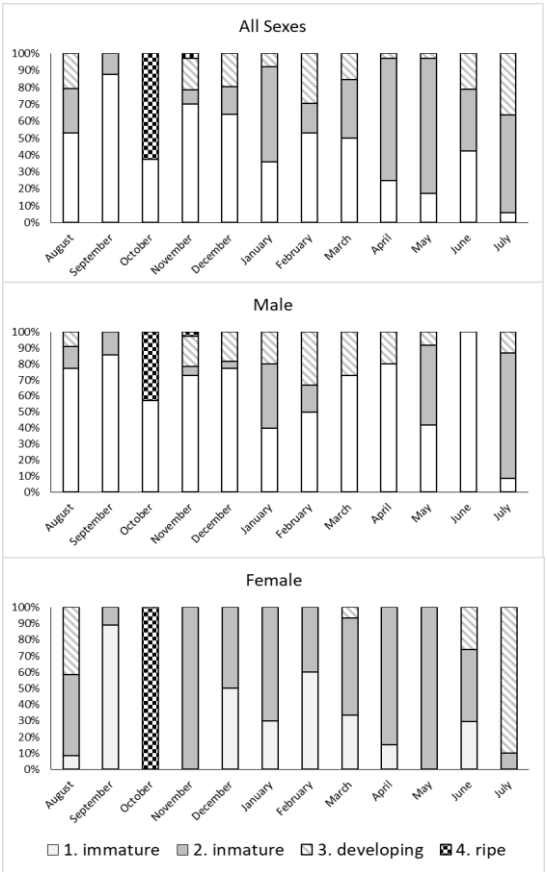

Figure 3. Sexual maturity stages of Salema individuals

The sexual maturity stages of Salema was showed at Figure 3. The 4th stage ripe gonads were found only in October for females and October and November for males. All female gonads were found as ripe in October. The sexual

maturity of females were low from November to June. The observation of non-mature individuals in September was may be a result of smaller length distribution in September.

When monthly variation of the CF, GSI and sexual maturity stages were evaluated togather, it was estimated that the spawning period of Salema took place between July and November, and sharply picked in October.

It was determined that the fecundity of Salema individuals varied between 656512 and 991171 eggs, with a mean of 854341±33211 eggs. Both lineer relationships were found between fecundity and length (F=47.964TL-810.02) and between fecundity and weight (F=1.0098TW+270.58). Whereas higher statistical significance level was observed for the relationship between fecundity and weight (r^2=0.80) rather than fecundity and length (r^2=0.69).

The spawning period of Salema was found between October and November in Adriatic (Pallaoro et al., 2008), from October to December in Mediterranean (El-Etreby et al., 2015) and from December to January in Canary Islands (Mendes-Villamil et al., 2002). Criscoli et al. (2006) were determined that Salema spawned twice per year as from March to May and from September to October around Mediterranean. It can be said that the results mostly coincide with the findings of Pallaoro et al. (2008) and Criscoli et al. (2006), whereas spawning took place only once a year. The findings on spawning period of Mendes-Villamil et al. (2002) was differ, due to particular geographical location, correspondingly varied sea water characteristics.

The length at first sexual maturity (L_{50}) of Salema was found as 19.51 cm for males (Criscoli et al., 2006), 24.5 cm for males (Paiva et al., 2016), 22.6 cm for males and 29.4 cm for females (Mendes-Villamil et al., 2002) and 22.5 cm for males and 28.5 cm for females (El-Etreby et al., 2015). In this study, larger L_{50} sizes were found for both males and females. This may be stemmed from low individual number with ripe gonads, and/or may be exposed lower fishing pressure in our study area.

Protandric hermaphroditism and sex reversal from male to female was observed. In the data set, all individuals larger than 31.6 cm TL were observed as female. Similar situation was stated by Criscoli et al. (2006), Pallaoro et al. (2008) and Paiva et al. (2016).

Mean absolute fecundity of Salema was estimated as a 854341±33211. Fecundity was found as 1063297±563054 (Paiva et al., 2016) and 2636616 (El-Etreby et al., 2015) around Mediterranean. Relatively lower fecundity may be a result of smaller length distribution of mature females via previous studies, which were used to calculate fecundity.

CONCLUSIONS

As a result, it was determined that Salema is a protandric hermaphrodite species and all individuals larger than 31.6 cm TL at length and 6 years at age were found to be a female. It has been determined that the Salema caught below the first sexual maturity length, therefore, the mesh sizes of gillnets should be increased and the minimum landing size should be specified as 31 cm TL in the circular.

ACKNOWLEDGEMENTS

This work was supported by the Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit. Project Number: FBA-2020-3295.

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REPRODUCTIVE BIOLOGY OF TUB GURNARD *Chelidonichthys lucerna* IN THE SEA OF MARMARA

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Abstract

This study indicated that the reproductive biology of C.lucerna in the Sea of Marmara. Individuals were obtained with monthly trawl surveys between March 2017 and December 2018 from 34 stations located in the Sea of Marmara, Turkey. A total of 799 individuals were examined. It was determined that 50.6% of individuals were female and 34.4% were male. The female:male ratio was calculated as 1:1.5. The length at first maturity (L_{50}) was calculated as 19.3 cm in females, and 17 cm in males. Monthly GSI values were changed between 0.36-10.58. The GSI value was maximum in March 2017, Sempember 2017, and Semptember 2018 in females. When these results are considered the spawning period of C.lucerna was determined in two period that is spring and autumn.

Key words: *Tub gurnard, first reproductive length, GSI*

INTRODUCTION

Chelidonichthys lucerna (Linnaeus, 1758), the tub gurnard, is a species of high commercial value belonging to the Triglidae family. The species is distributed in the Turkish seas (Mater et al., 2003), and is found in the region extending from the East Atlantic (Norway to Cape Blanc) to the Mediterranean (Froese and Pauly, 2008). The life span of the tub gurnard, which can reach a maximum length of 75 cm, is 15 years. It generally survives at depths of 20–350 m with a sandy muddy bottom (Mater et al., 2003).

The relationship between size and weight of tub gurnard analyzed by Abdallah (2002) in Egypt, Stergiou and Moutopoulos (2001) in Greek waters, Olim and Borges (2006) in Portugal. Pauly (1978) calculated length and growth parameters, Faltas and Abdallah (1997) studied the growth, mortality and annual stock participation, in Egypt. Biological properties were determined by Booth (1997) in South Africa, Stergiou et al. (1997) in Greek waters, and Serena et al. (1998). Ismen et al. (2004) examined the age, growth and reproduction characteristics of individuals obtained from the Iskenderun Bay, Eryılmaz and Meriç (2005) examined some biological characteristics, Uçkun (2005) examined the age and growth characteristics of species belonging to the Triglidae family. İlhan (Uçkun) and Toğulga (2007), and Kınacıgil et al. (2008) studied the age, growth and reproduction characteristics of the tub gurnard in Izmir Bay.

This study indicated the reproductive period, first reproductive length and maturity stages of the *C. lucerna* in the Sea of Marmara. This study is the first detailed study in the whole area of the Sea of Marmara.

MATERIALS AND METHODS

Samplings were conducted by TAGEM (General Directorate of Agricultural Research and Policy of Turkey) project number TAGEM/HAYSÜD/2014/05/01. Monthly trawl surveys were realized between March 2017 and December 2018 from 34 stations located in the Sea of Marmara (Fig. 1). Sampling stations were determined to represent varied depth structures (20-50 m, 50-100 m and 100-200 m depths). MEDITS's standards were implemented for trawl tows. Each tow was conducted with 3 miles tow speed and 30 m duration.

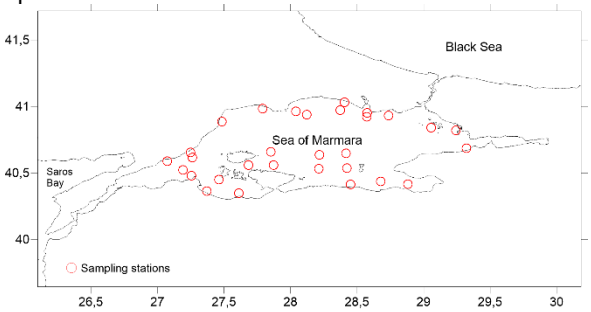

Figure 1. Sampling stations in the Sea of Marmara

The spawning season of the species was estimated by assessing the sexual maturity stages and based on the Gonadosomatic Index Value (GSI). The GSI was calculated using the formula developed by Gibson and Ezzi (1980):

GSI= (Gonad weight/(Body weight−Gonad weight))×100

The length at first maturity (L50) was estimated using the following formula (King 1995) for both sexes:

P=1/(1+exp[−rm(L−Lm50)]) 100

where rm is the slope of the curve, Lm is the mean total length (cm) at sexual maturity, L is the mean total length(cm) and P is the probability of the presence of mature fish.

RESULTS AND DISCUSSION

The gonadomomatic index and condition factor value monthly calculated for *C. lucerna*. The GSI values were between 0.36-10.57 and KF values were between 0.007-0.01. The maximum GSI values were determined in March and September 2017, the minimum GSI values were determined in February 2018 in females and the minimum and maximum GSI values were calculated in February 2018 and November 2018, respectively in males. The monthly sexual maturity stages and GSI values were evaluated together the spawning period of *C. lucerna* was determined in two periods that is Spring (September and October) and Autumn (March and April). The lengths at 50,% maturity of *C. lucerna* were calculated as 19.3 cm in females and 17 cm in males (Figure 2).

There are previous studies on the reproduction of the species (Table 1). Only three studies were examined the reproduction of the C. lucerna in the Aegean Sea, Mediterranean Sea and the Sea of Marmara. Ismen et al. (2004) presented the reproductive season of C. lucerna in December and May in the Iskenderun Bay, and detected the first reproductive lengths as 18 and 20 cm in females and males, respectively. Eryılmaz and Meriç (2005) stated that the spawning period continues throughout the year in the Marmara Sea and calculated the first reproductive length as 17.7 cm in females, and 19.9 cm in males. Ilhan and Togulga (2007) determined the spawning period between December to March and the first reproductive length as 18.5 cm in females, and 19 cm in males in the İzmir Bay.

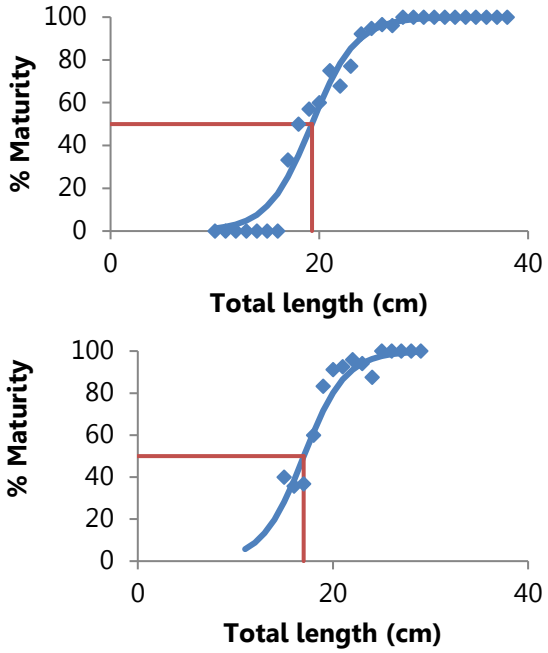

Figure 2. First reproductive length of *C. lucerna* in females (a) and males (b)

Table 1. Reproductive parameters of *C.lucerna* in the different areas

Author	Area	Sex	Reproductive period	Lm (cm)
Papaconstantinou 1983	Thermaikos Gulf, Greece	M F	January-May / October-July	31.7 26.0
Baron 1985	Brittany, France	M F		40.1 35.5
Faltas and Abdallah 1997	Alexandria, Egypt	M F		17.0 15.6
Işmen et al. 2004	İskenderun Bay, Türkiye	M F	December-May	20.0 18.0
Eryilmaz and Meriç 2005	Sea of Marmara, Türkiye	M F	Throughout the year	19.9 17.7
İlhan ve Toğulga 2007	İzmir Bay, Türkiye	M F	December -March	19.0 18.5
Boudaya et al. 2008	Gabes Bay, Tunusia	M F	October-April	19.2 21.6
Vallisneri et al. 2012	Adriatik Sea	M F		22.1 24.3
McCarthy and Marriott 2018	Northwest Wales Coast, England	M F M+F		29.1 27.7 28.0
This study	Sea of Marmara	M F	September –October March -April	17.0 19.3

CONCLUSIONS

In conclusion, the results of the present study indicate the first reproductive length and reproductive period of *C.lucerna* in the Sea of Marmara. The first reproductive length was calculated as 19.3 cm in males and 17 cm in

females. However, the minimum landing size of the *C.lucerna* is 18 cm in total length. According to the results of the present study, we recommend increasing the minimum landing size to 19 cm total length.

ACKNOWLEDGEMENTS

This study is supported by (TAGEM) General Directorate of Agricultural Research and Policy with the Project Number: TAGEM/ HAYSÜD / 2014/05/01. The authors would like to thank Haşim İnceoğlu, Alpaslan Kara, Engin Kocabaş, Habib Bal, Adnan Ayaz, Cahide Çiğdem Yığın, Ahmet Öktener, Güzin Gül, Koray Cabbar, Gürkan Ali Yazıcı, G. Erman Uğur, and Mine Çardak for their contributions in the field samplings.

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SIGNATURES OF POSITIVE SELECTION IN SAHIWAL AND CHOLISTANI CATTLE BREEDS

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Abstract

This study explores the genome structure of Sahiwal and Cholistani cattle breeds found in Pakistan. Illumina HD Bovine HD BeadChip Genotypes were used on 127 individuals of four different breeds including Holstein (60), Angus (40), Sahiwal (14), and Cholistani (13). PCA and neighbor-joining (NJ)-tree revealed a separation of Pakistani indicine from European taurine. Signature of selection analysis (Fst) reveals some genomic regions under positive selection and harbored some important candidate genes which are involved with coat color, eye area pigmentation, milk fat percentage, fat composition, milk yield, lactation persistency, reproduction, heat tolerance, temperament, Innate Immune, and bovine tuberculosis susceptibility. Identifying these candidate regions provides a foundation to improve future dairy production in sub-tropical areas of Pakistan through genome-assisted breeding strategies of indigenous breeds.

Key words: *Genome, Cattle structure, Genes*

RESULTS OF A 20-YEAR SELECTION BASED ON THE OBSERVATION OF THE VARROA SENSITIVE HYGIENE BEHAVIOUR OF INDIVIDUAL BEES

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Abstract

Breeding Varroa-resistant bees is one of the most important goals in breeding programs for both beekeepers and scientists. The bee parasite Varroa destructor threatens bee populations worldwide. Particular attention is paid to a selection for an increased Varroa sensitive hygiene (VSH) behaviour. By using infrared video technology, it was possible to demonstrate that also Apis mellifera, albeit extremely rarely, shows VSH. For about 20 years, the hygiene behaviour of individually marked worker bees towards Varroa-parasitized cells has been used as a selection trait. In the course of the selection program, there was a significant increase in VSH behavior and, by using phenotyped bees from the selection line, some significant SNPs for VHS could be found. To test comparatively the Varroa resistance and other traits of the selection line, selection line colonies (Sel x Sel), unselected control line colonies (Con x Con) and colonies originated from control line queens mated to drones of the selection line (Con x Sel) were studied in an elaborate, strongly standardized comparison test with regard to the described VSH behaviour, various Varroa resistance characteristics usually measured by breeders, survival without Varroa treatment and usual beekeeping characteristics (honey yield, gentleness, calmness on comb). The evaluation of the comparative video recordings of the hygiene behaviour towards Varroa-parasitized brood showed a very clear superiority of the selection line. The results for the control line were significantly worse. The results of the Con x Sel group were about halfway between the two groups, tending towards the selection line. These results demonstrate that individual detection and uncapping of Varroa-parasitized brood has a sufficiently high heritability. The superiority of the selection line was also shown for other Varroa resistance characteristics, but the survival of the colonies was only significantly correlated with the percentage of individual hygienic bees of the colonies in the video assay. The relationship of field-recorded Varroa resistance traits to colony survival was not significant. Regarding the beekeeping traits, the selection line was only significantly inferior regarding calmness on comb, the differences in gentleness and honey yield were not significant.

Key words: *Varroa Resistance, Varroa sensitive hygiene, Individual behaviour observations*

EFFECT OF CLİMATE CHANGE AND OTHER FACTORS THAT LEAD TO HONEYBEE COLONY LOSSES (1)

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Abstract

The Western honey bee (Apis mellifera L.*, Hymenoptera: Apidae) is a very important species for a variety of reasons, including economics, agriculture and ecology. The lack of a clear explanation for the incidents in the United States, numerous European countries, Asia, Africa and the Middle East has attracted a lot of media interest. Because of honeybees' complex social behavior, pinpointing the root causes of colony collapse has proven difficult. Additionally, they come into contact with several environmental chemicals and are subjected to a wide range of human actions and their outcomes in the course of their daily routine. Recent studies have established some of the most important ones, including pests and diseases, bee management (such as beekeeping methods and breeding), the change in climatic conditions, agricultural practices, and the use of pesticides, among others, as contributors to honey bee losses. The ectoparasitic mite Varroa destructor stands out as a global cause of colony collapse. Microsporidian parasites, especially Nosema ceranae, are a further factor. As a result, it's clear that honey bee colony losses can be attributed to a variety of causes. The causes of the current decline in honey bee colonies are unclear, but increased monitoring and scientific research should shed new light on the matter. The current research focuses on the most important things that have been shown to contribute to the alarming rise in honey bee colony losses.*

Key words: *climate change, abiotic factors, agricultural practices, honeybees, pesticides, interaction between diseases, mites, pests, honeybees, colony losses*

INTRODUCTION

Honey bees (*Apis mellifera* L., 1758) are extremely valuable pollinators, contributing greatly to the health of both natural and man-made ecosystems. Global pollination services are projected to be worth around €153 billion per year, making honeybees important players in international trade. Honeybees play a crucial role in the modern economy by harvesting and processing a variety of products such as honey, beeswax, propolis, royal jelly, and even bee venom, which are used in numerous industries including the food and cosmetics industries. Many people around the world rely on beekeeping as their primary means of subsistence, making it an effective tool in the fight against poverty (Garrido et al., 2016; Hristov et al., 2020b; Tihelka, 2018)**.**

As far as agriculture and wild flower species are concerned, managed honeybees are the most crucial pollinators. The Western honeybee, *Apis mellifera*, is crucial to the commercial pollination of specific crops in many nations across the world. Honeybee colony numbers have been constant in China and Japan over the past decade, despite reports of bee losses in those countries as well. According to data compiled from around the world, no major honeybee colony losses have been documented in Africa, Australia, or South America. The Western honeybee, *Apis mellifera*, is crucial to the commercial pollination of specific crops in many nations across the world, especially in the northern hemisphere. However, there has been a rise in losses in managed honeybee colonies in several regions of the world in recent years. Initial reports of colony collapse disorder (CCD) emerged in the United States in 2006 (Beyer et al., 2018; Hristov et al., 2020b; Stanimirović et al., 2019; Tihelka, 2018)**.**

Climate change is one of the most significant problems honeybees face. It is a global phenomenon with many different parts that has a big effect on the distribution and number of many habitats and animals, including plants and pollinators (Drossart&Gérard, 2020; López Uribe&Simone-Finstrom, 2019; Sperandio et al., 2019; Vercelli et al., 2021).

Climate change poses a huge threat to honeybees, as it has a substantial impact on other aspects that are tightly related. These factors have a significant impact on the behavior, physiology and distribution of honeybees. In this setting, beekeeping management practices are

essential for the preservation of honeybee colony health, overwintering success, and crop yield (Vercelli et al., 2021).

Many species have seen rapid range declines as a result of modern climate change. The degree to which species' ranges grow or shrink in response to climate change is determined by the relative risks of extinction and colonization (Kerr et al., 2015; Miller-Struttmann et al., 2015; Pashalidou et al., 2020; Soroye et al., 2020).

A recent study indicates that insects are declining eight times more rapidly than mammals, birds or reptiles. Currently, the reduction of pollinators is receiving considerable attention, partly due to the critical ecological services they provide (D'Alvise et al., 2019; Dimov et al., 2021; Hubert et al., 2017; Ptaszyńska et al., 2021).

For a combination of reasons, mass species extinctions are occurring. Pollinators, food security, and climate change are all interconnected. Even though the environmental movement has been going strong, these problems keep coming back because of the "cooptation and reproduction of existing mechanisms of exploitation and political oppression (Bartomeus&Dicks, 2019; Hall& Martins, 2020; Kevan & Menzel, 2012; Marshman et al., 2019).

The purpose of this overview is to gain insight into the primary causes of honeybee declines, the approaches taken to remedy these declines, and the potential future directions of the influence of climate change research on honeybees.

1. Climate Change

1.1. The Effect of Climate Change and Variability on Apiculture

Agriculture and climate change are intricately intertwined. Increasingly severe weather patterns brought on by climate change have already had a negative effect on agriculture in many parts of the world. Honeybees are protected in Europe and elsewhere for the sole purpose of maintaining viable honey markets. In 2011, the international trade of natural honey was worth US$ 3.3 billion. Crop production, livestock productivity, horticultural crops, aquaculture (fish production) and apiculture are all negatively impacted which has far-reaching consequences for people's livelihoods, food production and national economies, especially in the developing world where agriculture is a primary economic driver.

Climate change is having a negative impact on agriculture in general and beekeeping in particular. Pollinators, such as honeybees, are in danger as a result of climate change. Honeybee activity appears to be primarily controlled by temperature and to a lesser extent, precipitation. The extent to which climate change may affect honey output is poorly understood. The observed fluctuation in honeybee numbers and honey yields over climatic gradients implies that climate change is among the environmental factors that may alter the provisioning services provided by honeybees. The economy of one country and the people whose lives depend on honey production are in jeopardy as a result of a decline in honey production and productivity, as well as a drop in honey income (Berhane, 2018; Durodola, 2019; Lemi, 2019; Neenu et al., 2013; Sengar&Sengar, 2014; M. Singh et al., 2017; V. K. Singh, 2012; Uprety et al., 2019).

1.2. Effect of Climate Change in Bees Losses

Pollinators, like the honeybee, are crucial to today's agricultural system *(Apis mellifera)*. Research into the possible causes of honeybee mortality is crucial because of the threats to our food supply posed by the problems honeybees face. Illness and parasites, pesticides, diet, habitat change and population growth are the top four threats to biodiversity. The *Varroa destructor* mite is by far the most common and devastating parasite affecting honeybee populations worldwide. Honeybees' foraging efficiency can be negatively impacted by neonicotinoid insecticides, among other non-lethal impacts. The nutritional needs of an organism are contingent on the availability and quality of nectar and pollen. Agriculture, landscape design, climate and the elements all play a role in the availability of these materials. The effects of weather and climate fluctuation on colony health, as well as the role of resource availability and biodiversity, have been the subject of habitat dynamics research. Higher air temperatures have been shown to increase colony net gain rates and increase the efficiency of honey storage rates. On the other hand, especially rainy days have been shown to change how bees behave in their nests. The ambient air temperature affects how often honeybees do waggle runs, how fast they lay eggs, how much honey they store and how they keep themselves warm. In the United States, beekeepers frequently cite "weather" or "bad winter" as the leading cause of honey bee colony losses. There hasn't been enough research to establish a solid link between long-term weather or climate and colony health. The links between climatic change, climate variability, and colony mortality were discussed qualitatively. First, we identify the most important monthly climate variables in determining colony mortality (Brodschneider et al., 2019; Clarke & Robert, 2018; Genersch et al., 2010; Van Dooremalen et al., 2012).

The varroa mite is responsible for the demise of most *Apis mellifera* colonies worldwide, with the exception of a few African countries and Australia. It is also important to discuss how beekeepers might adjust their techniques in the face of declining supplies (food supplementation or colony transhumance). For this reason, beekeepers can employ monitoring equipment to better predict how rapid changes in landscapes will affect their colonies' productivity and control colony transhumance. We should increase local floral resources for bees, making them suitable for both honeybees and wild bees, as a rise in colony transhumance would have a negative impact on the carbon footprint of beekeeping activity (Sperandio et al., 2019; Drossart&Gérard, 2020; LópeUribe&Simone-Finstrom, 2019; Schatz et al., 2021; Sperandio et al., 2019).

1.3. Efeect of Climate Change in Abiotic Factors

1.3.1. Impacts of Temperature and Relative Humidity

The importance of pollination services by honeybees (*Apis mellifera* L.,) and their products is well-known. However, honeybee colonies currently face many challenges. These challenges include both biotic and abiotic factors. In this article, the impacts of biotic factors (mainly temperature and relative humidity) on honeybee activities are reviewed. The suitable ranges of these two factors and the potential impacts of atypical minimal or maximal limits are presented. Social homeostasis of honeybees and activities inside and outside the colony that are influenced by these two factors are included, followed by a suggestion of additional studies (Abou-Shaara et al., 2012, 2017; Boes, 2010; Tan et al., 2012).

1.3.2. Impact of High Tempreture

Greenhouse gas emissions and, by extension, climate change have risen steadily since the Industrial Revolution due to human activities like the burning of fossil fuels, the expansion of industrial processes and the intensification of agricultural practices. A number of components of the climate system are altered as a result of climate change. These include minimum, maximum, and average temperatures; rainfall patterns and extreme weather events like floods and droughts. Temperature may also affect bees' immune systems which could have repercussions for their spread and longevity as a species as a result of climate change (Blasco-Lavilla et al., 2021; Butolo et al., 2021; Medina et al., 2020).

Honeybees from Italy have a higher heat tolerance than Carniolan bees, according to research by Kovac et al., (2014). Alqarni and Alghamdi (2006), discovered that Yemeni bees are more tolerant of the high temperatures experienced during the summer in KSA. Italian bees could only withstand temperatures up to 66 °C but Yemeni bees could withstand temperatures as high as 50.7 °C (Abou-Shaara et al., 2017).

Three honeybee subspecies were studied for their ability to survive in two ecological zones of Saudi Arabia: the desert environment of Riyadh and the semiarid climate of Albaha. Of the 420 colonies initially included in the study, only 101 were viable throughout the evaluation. Based on these findings, it is imperative that the native honeybee race bees prioritized for conservation and selection (Alattal&Alghamdi, 2015; Alqarni et al., 2019).

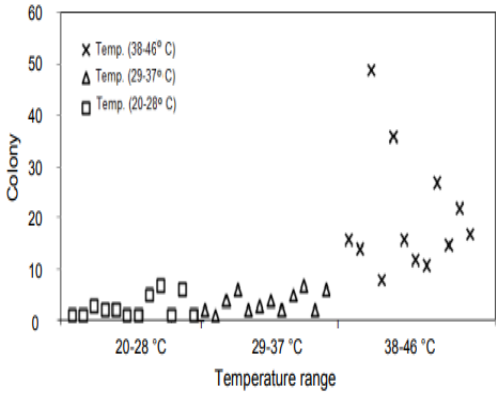

Figure 1. depicts the frequency of honeybee colony losses across three temperature classes (20-28 °C, 29-37 °C and 38-46 °C) (Alattal&Alghamdi, 2015).

In each class, for two consecutive years (N = 12), the number of lost colonies of each honeybee subspecies in each location was recorded. Colony losses are strongly correlated with various temperature ranges.

1.3.3. Impact of High Humidity RH

Another biotic element, air humidity, may not have a significant effect in a temperate region, but it is essential in dry and hot conditions. Alqarni (2006) discovered that in the summer of Saudi Arabia, when temperatures were high and humidity was low, Yemeni bees lost significantly less weight than Carniolan bees.

Honey bees are able to endure uncomfortable temperatures because of the unique properties of their cuticle. Due to their water-resistant qualities, the lipids in the cuticle help the body conserve fluids. On the antennae of honeybees, thermal sensors capable of detecting temperature variations of at least 0.2 °C have been found. Specific molecular transient receptor channel (AmHsTRPA) implicated in temperature sensation was discovered by Kohno et al., (2010);

it is expressed in the antennal flagellum and becomes activated at a temperature threshold of 34 °C (Abou-Shaara et al., 2017).

1.3.4. Unfavorable Weather Conditions

Unfavorable climatic conditions and extreme variations in weather have been cited by a number of current authors as contributing reasons to the decline of bee colonies. Historical records lend credence to the conclusions drawn from historical records. There is a link between long periods of cold and wet or hot and dry weather and the loss of bees and the destruction of their colonies.

The best way to prevent the hive from being emptied in this way is to drink warm beverages, according to beekeepers. About a third of the hives died during the cold season of 2012-2013, thus the same holds true for that year. In years when temperatures increase early in the spring, frequently before the snow melts, beekeepers report seeing a pattern of symptoms similar to the "empty hive." (Belsky&Joshi, 2019; Gregorc, 2020; Neov et al., 2019).

1.3.5. Impact of Low Tempreature on Honeybees

Apis mellifera, the western honey bee, must cope with drastic changes in weather and food supply throughout the year. As a result, summer bees and winter bees exist separately. The average lifespan of a summer bee is just a few weeks whereas winter bees can live for up to six months (Dainat et al., 2012; Desai&Currie, 2016; Steinmann et al., 2015).

Honeybees are negatively impacted by cold stress, which reduces their ability to live and develop. Recent years have seen unacceptably high colony losses throughout the winter, according to beekeepers. In light of this, it would be useful to learn how honey bees' physiology and lifespan react to cold stress. The effects of low temperature stress on brood mortality have recently been studied by Ramirez et al., (2017). In that study, we showed that in-vitro-reared honeybees were negatively affected by cold stress and had a lower chance of surviving. As a result, bee physiology may be significantly impacted by cold stress (Dostálková et al., 2021b; Negri et al., 2017, 2019; Prado et al., 2022a; Ramirez et al., 2017)**.**

Cold temperatures can upregulate expression of immune-related genes in honey bee capped brood (larval and pupal stages) grown at less-than-ideal temperatures. It's possible that this upregulation caused by cold has ecological significance. In comparison to control populations, honeybee larvae and workers whose diets included abscisic acid (ABA) fared better in the winter and could withstand colder temperatures (Abou-Shaara et al., 2017).

Abscisic acid (ABA), found in nectar, pollen, and honey, has been shown to improve honeybee colony overwintering survival (Negri et al., 2015, 2017, 2020; Ramirez et al., 2017).

1.3.6. Impact of Warmer Winters on Honeybees

As a result of climate change, it is expected that high-latitude winters will warm more rapidly than their summer counterparts. Insects rely heavily on cold temperatures to slow their metabolism and conserve energy during diapause. We then tracked the effects of cold weather on a range of honeybee overwintering physiological variables, including glycerol levels (a cryoprotectant), immune gene expression, antioxidant gene expression and the expression of genes encoding multifunctional proteins like vitellogenin (which extends bee life). However, certain insects, such as honeybees, are quite active all year round and increase their metabolic rate to produce endothermic heat when the weather gets cold. Warm temperatures will likely boost honeybee viability as the climate warms (Dostálková et al., 2021a; Prado et al., 2022b; Ramirez et al., 2021; Steinmann et al., 2015).

1.3.7. Effect of Worker Age and Climate on the Hindgut Microbiota in Term of the Overwintering

Manipulating honey bee populations for crop pollination and honey production is a global industry. Colonies that have successfully overwintered are essential for pollinating crops in the spring yet beekeepers often record colony losses during the winter. In this study, we compare the effects of mild and cold winters on the microbiota of honeybees' digestive systems. Overwintering gut microorganisms were stable in both climates. Microbiota shifts in hotter climates, on the other hand are indicative of a host with weakened physiology. Increased by a factor of two fungi were significantly linked to pathogenic bacteria in warmer environments. In warm climates, worker bees had a shorter life expectancy than those in colder regions. Our findings suggest that overwintering in warm climates can exacerbate underlying conditions of disease, parasites and inadequate nutrition, increasing winter colony loss (Anderson et al., 2018, 2022; Bosmans et al., 2018; Ferguson et al., 2018; Gregory et al., 2022; Maes et al., 2021; Tauber et al., 2019).

2. Agricultural Practices

The United States pioneered the cultivation of genetically modified crops in 1996 and since then they have seen widespread application in other

nations. In 2007, genetically modified crops were planted on 113 million ha across the globe (with the European Union being an exception). Concerns have been raised concerning the well-being of honeybees and other pollinators as the amount of land planted with these crops continues to grow. It has been argued that there is no proof that honeybees suffer any harm from eating genetically engineered plants. Technological advancements are a contributing factor to the decline of wild and domesticated honeybee populations according to Johnson et al., (2010) and Johnson (2015) (Le Conte et al., 2011; Maggi et al., 2013, 2016; Meixner&Le Conte, 2016; Neov et al., 2019, 2021).

2.1. Diet and Nutrition

When hives are too crowded, the honeybees may not be able to go out foraging as often as they need to and when the weather is too cold and wet, they may not find enough food to survive. In regions with high rates of agricultural output, where "monofloral" diet stress is said to be more noticeable, poor feeding practices are prevalent. Honeybees gather nectar and pollen from mass-flowering crops cultivated over expansive areas. Low-nutrient pollen and nectar from plant species contain natural but poisonous to honeybee chemicals (Neov et al., 2021).

2.2. Breeding of the Honeybees

The intricacy of the stressors that might affect honeybee health is either directly caused by or exacerbated by beekeeper management practices. Artificial, unbalanced pollen diets; hive-applied antibiotics, acaricides and insecticides; environmental hazards; parasites and diseases; overuse of honeybee products; and most importantly, migratory management are all factors that threaten bee populations. Unintended consequences of selective breeding for only one trait include a loss of genetic diversity in the honeybee population and a lack of resistance to infectious diseases, mites, beekeeping acaricides used in beehives, etc (Neov et al., 2021).

2.3. Changes in Honeybee Colonies (Influence of some Stressors on the Health Status of Honeybee Colonies)

After 2006, a great deal of research concluded that the Colony Collapse Disorder (CCD) syndrome is characterized by the absence of adult dead bees in a hive in which few workers and a queen remain. The ratio of brood quantity to the number of workers is heavily skewed in favor of the former, and there is an abundance of food, which plays a significant role in global colony losses. However, scientists and specialists examining the issue note two types of honeybee colony decline: yearly due to unsuccessful wintering and seasonal. Researchers discovered that winter losses varied between 7% to 30% in Europe and between 16% and 25% in Canada and 4% in China.

The second type involves a gradual but steady decline in honeybee populations across many years and geographical areas. The number of bee colonies in Europe has declined by 25% since the mid-1980s. The same decline can be seen in the United States where the number has dropped by 50% to 60%. The number of honeybee colonies worldwide has increased by 45% since 1961. Long-term decreases in the U.S and certain European countries are the main outliers to this global increase. But this decline is compensated by an increase in honeybee populations everywhere in the world.

While the latter finding is encouraging, it must be read with caution. For instance, it is well-documented that the number of bee colonies in Europe has been reduced by 25% since the mid-1980s and that the same trend can be seen in the United States where the number has dropped by 50% to 60% (Neov et al., 2021).

Extreme colony losses in four different parts of Turkey were blamed on the weather in 2006 and 2007. Colony losses were severe for all regions exhibiting CCD-like symptoms. This is distinct from what we see in the United States which could point to a unique set of factors at play, such as the interplay between illness and host (De Jongh et al., 2022; Giray et al., 2007, 2010; Maggi et al., 2013, 2016; Meixner&Le Conte, 2016; Neov et al., 2019, 2021).

2.4. Habitat Loss and Landscape Changes

When natural areas are destroyed or fragmented due to urbanization or agricultural intensification, honeybees and wild bees suffer. Over the course of the twentieth century and into the present day, natural vegetation (such as grassland or tree-filled areas) has seen a steady decline in its habitats. It is thought that raising honeybees in an urban setting has benefits, such as access to year-round food supplies. However in highly populated areas, flowering plants may not be enough (Neov et al., 2021).

When both nesting and foraging resources are taken into account, it is possible that nesting qualities are a more significant predictor of wild bee diversity and sensitivity to disturbance. Natural nesting death rates of over 80% have been observed, suggesting that careful thought given to how and where a nest is built could go a

long way toward enhancing bee conservation and guaranteeing pollination benefits. This absence of direct evidence is remarkable given that wild bees spend the vast majority of their lives either within the nest or in close proximity to nesting materials throughout the five main stages of nesting (initiation, construction, development, overwintering and emergence).

In general, nest availability and nest site conditions are likely impacted by risks (including habitat disturbances and climate change which in turn impacts the success of bees in starting new nests, expanding existing ones and surviving the winter (Antoine&Forrest, 2021; Harmon-Threatt, 2020; Kandemir, 2007).

2.5. Pesticides as a Factor Associated with Honeybee Health

Beekeepers use chemicals to combat pests and diseases in and around hives, exposing honeybees to pesticides in a targeted but unintended way. French researchers, Chauzat et al., (2006), found 19 of the 36 chemical pesticides they were looking for in a variety of experimental bee colonies around the country. After further analysis, Chauzat and Faucon, (2007) discovered 14 different pesticide residues in the honeybee wax samples they had collected. The authors claim that the residues are due to both the direct use of pesticides in beekeeping and environmental pollution. Honeybee products have been shown to be tainted due to pesticide contamination and there are numerous entry points for these chemicals into the hive (Neov et al., 2021).

Honeybees have been found to be particularly vulnerable to the effects of pesticides. The presence of pesticide compounds in beeswax negatively impacts the health of honeybee colonies. Numerous studies have examined the dangers of particular pesticide compounds to bees and many have concluded that these chemicals pose a threat to insect pollinators. Imidacloprid (a neonicotinoid) is one example that has been demonstrated to have an effect on both honeybees and wild bees. Woodcock et al., (2017), found that honeybee health declines in corn-growing areas due to exposure to field-realistic levels of pesticides (Authority, 2014; Uhl & Brühl, 2019; Valk et al., 2012; van der Valk et al., 2011; Van der Valk et al., 2013; Yasrebi-de Kom et al., 2019).

European legislation recognizes that flower-visiting insects (FVIs) represent a rich and unique ecosystem and should be safeguarded against the negative impacts of pesticides. FVI species are in steady decline, in part due to the use of pesticides in agriculture. Exposure of FVIs to pesticides at ecologically relevant levels can have major negative consequences on populations. Using this information, the European regulatory framework for exposure and effect assessment was analyzed and criticized. This is very important if we want to learn more about FVIs and make a more secure FVI risk assessment (Berényi et al., 2006; Dittes, Aupperle-Lellbach, et al., 2020; Dittes, Schäfer, et al., 2020; Kalayci et al., 2020; Karapınar et al., 2018; Šimenc et al., 2021; Tlak Gajger, Bičak, et al., 2014; Tlak Gajger, Kolodziejek, et al., 2014).

Also there are other factors which lead to colony losses are analyzed: infestation with parasites, primarily with *Varroa destructor*, and mixed virus infections; bacterial infections (American and European foulbrood); fungal infections (nosemosis and ascosphaerosis); trypanosomal infections (lotmariosis) and finally general management of the apiary (Currie et al., 2010; Ellis et al., 2010; Pohorecka et al., 2011; Stanimirović et al., 2019; Topolska et al., 2010; van der Zee et al., 2012; vanEngelsdorp et al., 2010).

2.6. Effect of Stressors on Honey Bees

The ecological services provided by wild and farmed bees have come under jeopardy. The reduction of bee populations is now generally understood to be complex. Biological and chemical agents are major players in this scenario. Bees are already declining due to loss of habitat, poor beekeeping methods, climate change and a reduction in the number of flowers available for pollination.

Mass mortality is the most obvious effect of stressors on bees, but even non-lethal effects can result in substantial losses. Standard regulatory procedures for risk assessment do not analyze the impacts of pesticides and other stresses on bee health. One theory for the periodic collapse of honeybee colonies postulates that lost bees can no longer locate their hives. Therefore, stressors' impact on bee health is not just confined to deadly effects but also includes changes in behavior, decreased cognitive processes and sensory capacities and alterations at the physiological, molecular and genetic levels. The current search for better risk assessment techniques and methodologies is justified by the economic and ecological difficulties posed by the bee population's reduction (Gregorc, 2020; Havard et al., 2019; Wood et al., 2020)

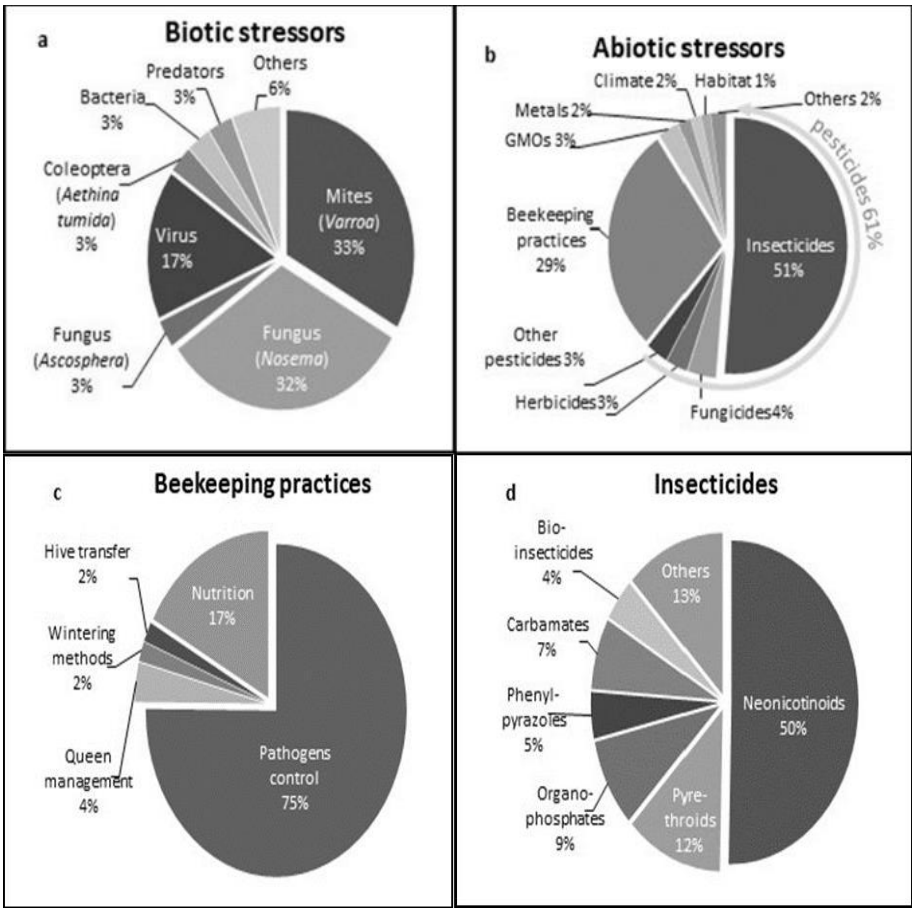

Figure 2. Proportion of the various biotic (a) and abiotic (b) stressors, beekeeping methods (c), and pesticides (d) identified in studies on the influence of stressors on *Apis mellifera* published between 2007 and 2017. b indicates that "Other pesticides" refers to adjuvants, inert components, pesticide residues in fields, and wood preservatives. The "other" sections are described in full in the supplemental materials (Havard et al., 2019).

2.7. Beekeeping and Climate Change

To survive, bees and beekeepers will need to quickly adjust to the effects of climate change and the resulting shifts in resource availability. The potential for introducing new illnesses or pests into an area must be taken into account when deciding whether or not to test an ecotype or subspecies that is not native to that area (Decourtye et al., 2019; Drossart&Gérard, 2020; Kluser et al., 2010; Schatz et al., 2021)**.**

Table 1. Environmental Factors Associated with Honeybee Colony Losses (Hristov et al., 2020a)

Anthropogenic Direct Drivers that Cause Honey Bee Decline	*Impact on Honeybee*
Pesticides	High rate of mortality, alteration of different biological processes.
Climate Change	Alteration of honeybee behavior, physiology and distribution, induced changes in flora for honeybees vitality.
Introduction of Alien Species	Competition for food resources, decline of indigenous species, alteration of the new habitat.
Genetically Modified Organisms (GMOs) Crop	Alteration bees foraging behavior.
Land use and Management	Habitat and forage loss, honeybee and wild bee competition.
Bee Management	Hybridity of honey bees, migratory pollination.
Environmental Pollution	Imbalance in homeostasis, weakening of the immune system.
Interactions between Drivers	In many cases poorly studied.

CONCLUSIONS

In conclusion, one of the greatest threats honey bees face is climate change. The distribution and abundance of many ecosystems and creatures, such as plants and pollinators, are profoundly affected by this complex worldwide

phenomenon. As a result of its profound effects on other, interconnected factors, climate change presents a serious danger to honeybees. Honeybees' behavior, physiology, and dispersion are all significantly affected by these elements. Good beekeeping management in this area is important for the health of honeybee colonies, their ability to survive the winter, and the amount of crops that can be grown.

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EFFECT OF CLİMATE CHANGE AND OTHER FACTORS THAT LEAD TO HONEYBEE COLONY LOSSES (2)

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Abstract

The Western honey bee (Apis mellifera L., Hymenoptera: Apidae) is an economically, agriculturally, and ecologically significant species. The lack of explanation for incidences in the United States, several European nations, Asia, Africa, and the Middle East has piqued the curiosity of the media. Because of the complexities of honey bee social behavior, it is difficult to pinpoint what causes colony collapse. And in the course of their everyday lives, they are exposed to a broad variety of human acts and the results of those actions, as well as a number of environmental pollutants. Recent research has pinpointed a number of factors, including pests and illnesses, bee management (including beekeeping techniques and breeding), climate change, agricultural activities, and pesticide usage, as potential causes of honey bee declines. The ectoparasitic mite Varroa destructor is a major culprit in colony failures all over the world. Parasites from the genus Microsporidia, namely Nosema ceranae, should also be considered. As a result, it is clear that honey bee colony losses can be attributed to a variety of factors. More study and monitoring should help us better understand what's causing the present drop in honey bee populations. This study looks at the main things that have been shown to be causing the worrying rise in honey bee colony losses.

Key words: *climate change, biotic factors, pesticides, interaction between diseases, honeybees, colony losses*

INTRODUCTION

Apis mellifera L., 1758, often known as honey bees, is vital to the success of many ecosystems, both natural and artificial. Honeybees play a significant role in global commerce since their pollination services are estimated to be worth roughly €153 billion annually. Honeybees are essential to the contemporary economy because of the honey, beeswax, propolis, royal jelly, and even bee venom that they produce and process. These products are utilized in a wide range of sectors, including the food and cosmetics industries. Beekeeping is a main source of income for many people around the globe, making it a powerful weapon in the battle against poverty (Garrido et al., 2016; Hristov et al., 2020b; Tihelka, 2018)**.**

Managed honeybees are the most important pollinators in terms of both agricultural crops and wild flower species. It is well acknowledged that the Western honeybee, *Apis mellifera*, is vital to the commercial pollination of various crops in many countries throughout the globe. Despite similar allegations of bee losses in China and Japan, the number of honey bee colonies in these two nations has remained stable over the previous decade. The global survey of data shows that honeybee colonies are safe in Africa, Australia, and South America. Many countries, particularly those in the northern hemisphere, rely on the Western honeybee, *Apis mellifera* for the commercial pollination of certain crops. However, in recent years, losses in managed honeybee colonies have increased in various places worldwide. In 2006, colony collapse disorder (CCD) was first documented in the USA (Beyer et al., 2018; Hristov et al., 2020b; Stanimirović et al., 2019; Tihelka, 2018)**.**

Climate change is one of honey bees' most serious challenges. The distribution and abundance of many ecosystems and creatures, such as plants and pollinators, are profoundly affected by this complex worldwide phenomena (Drossart & Gérard, 2020; López-Uribe & Simone-Finstrom, 2019; Sperandio et al., 2019; Vercelli et al., 2021). Because of the far-reaching effects of climate change on interconnected systems, honeybees face a grave danger. Honey bees' behavior, physiology, and dispersion are all significantly affected by these elements. Honeybee colony health, overwintering success, and crop output are all dependent on good beekeeping management methods in this environment (Vercelli et al., 2021).

Modern climatic changes have caused rapid range decreases in several species. Whether a

species' range grows or shrinks because of climate change depends on how extinction and colonization threats balance out (Kerr et al., 2015; Miller-Struttmann et al., 2015; Pashalidou et al., 2020; Soroye et al., 2020).

A recent study found that insect populations are dropping eight times faster than those of mammals, birds, or reptiles. Incorrect pesticide use, increased use of fertilizers and intensive agronomic operations, extremely intensive farming, insect starvation due to farmed monocultures, parasites, long-term drought, long-term lack of sunlight, especially when temperatures are low, and viral, bacterial, and fungal illnesses are among the leading causes of the decline in insect populations. Due in part to the crucial ecological services they offer, the current decline of pollinators is garnering increasing attention (D'Alvise et al., 2019; Dimov et al., 2021; Hubert et al., 2017; Ptaszyńska, Latoch, Hurd, Polaszek, Michalska-Madej, Grochowalski, Strapagiel, Gnat, Załuski, Gancarz, et al., 2021).

Multiple factors are contributing to the current rate of extinction. Climate change, pollinators, and food insecurity all have mutually interdependent relationships. Although the environmental movement has been effective, these issues keep resurfacing because of the "cooptation and reproduction of existing structures of exploitation and political oppression (Bartomeus & Dicks, 2019; Hall & Martins, 2020; Kevan & Menzel, 2012; Marshman et al., 2019).

Insight into the root reasons of honeybee reductions, current efforts to reverse the trend, and future avenues for studying the impact of climate change on honeybees are the goals of this review.

1. Climate Chanage on Biotic Factors

1.1. New Diseases for Honeybees

Bee exchanges have led to an increase in procedures (chemical treatments) to combat new pests. This has had negative effects on the economy, the environment, and the value of goods (honey, wax, royal jelly, etc.,). This highlights the need for research on the risks associated with bee exchanges. This could lead to regulations and the creation of new practices to combat pests (Abou-Shaara et al., 2012, 2017; Tan et al., 2012).

1.2. Role of Pests and Diseases as Drivers Leading to Honeybee Colony Losses

Ascosphaera apis is one of the main pests and diseases that can damage honeybees. Mites, numerous viruses, microsporidia, bacterial infections, and fungi are just some of the problems happening to the current drop in honeybee colonies around the world Table 1. Scientists' understanding of the consequences of these pests and illnesses has only improved in recent years (Hristov et al., 2020a).

1.3. Parasitic Mites

Varroa destructor, *Acarapis woodi*, *Varroa jacobsoni* and *Tropilaelaps clareae* are four of the honeybee mite species that can cause serious economic damage. Western honey bees have so far proven to be more vulnerable than their Asian counterparts, the *Apis cerana*. African honey bees and their Africanized counterparts in South America appear to be thriving. Although Varroa mites have been present in New Zealand since the

year 2000, they have not yet made their way to Australia. *V. destructor* consumes the fatty bodies of adult bees but not their hemolymph. Drones' flight performance and fertility have been shown to suffer after losing mass due to *V. destructor*. Foraging bees with mite infections have been shown to have poor orientation and homing skills, taking longer to return to the colony or failing to do so at all (Hristov et al., 2020b).

1.4. Effect of Climate Change on Honeybee Fungi

Global warming it is well acknowledged that climate change poses a threat to biodiversity due to its biological implications. Due to the fact that plant and pollinator phenology can be controlled by separate environmental cues or thresholds, climatic shifts can cause plant-pollinator mismatches. Generalist species are thought to be less vulnerable to climate change and may even benefit from it. The effects of climate change on host-pathogen dynamics are evident (e.g. temperature, precipitation, and seasonality). When it comes to the spread and success of infections, the weather can play a significant role (Figure 1., arrow 4a). The capacity of infections to complete their life cycle can also be affected by temperature. *Nosema ceranae*, which infects both Apis and Bombus species, has spores that lose viability very quickly

when exposed to subfreezing temperatures. The difference in the epidemiology and impact of *N. ceranae* and *Nosema apis* on honey bees, where the latter is better adapted to a cold environment, may be at least in part

explained by the lower germination of *Nosema ceranae* spores at low temperatures. Temperature also plays a role in the life cycle

within the honey bee host with *N. ceranae* being more suited than *N. apis* to finishing its life cycle in honeybees at a variety of temperatures. Wild

bees and their parasites are likely to experience similar outcomes. The most noticeable effect of climate change is on bees' capacity to forage, with worse nutrition leading to lower disease resistance (Figure 1., arrow 4b) (Meeus et al., 2018).

Domesticating honeybees has a lengthy history. *Apis mellifera* from Africa, Asia, and Europe with genetically diverse lineages have been transferred to various regions. The decrease of diversity and number of wild bees, brought on by habitat destruction, exacerbates the pollination gap. The success of an invasive species is correlated with the landscape composition. Invader complexes, in which the invading plant and pollinator interact, results in mutually beneficial feedback. Morales and Aizen (2006) identified such complexes, with a substantially closer relationship between invasive flower visitors and invasive plants than with native plants (Figure 1., arrow 1c).

Climate change has interaction chain effects on the other factors influencing the loss of wild bees (Figure 1., arrow 4c). It has been reported that climate change will impact plant (effect on habitat) and pollinator communities (influence on invasive species).

Negative effects on wild solitary bees diminished as the proportion of semi-natural habitat increased. Climate change has interaction chain effects on the other factors influencing the loss of wild bees (Meeus et al., 2018).

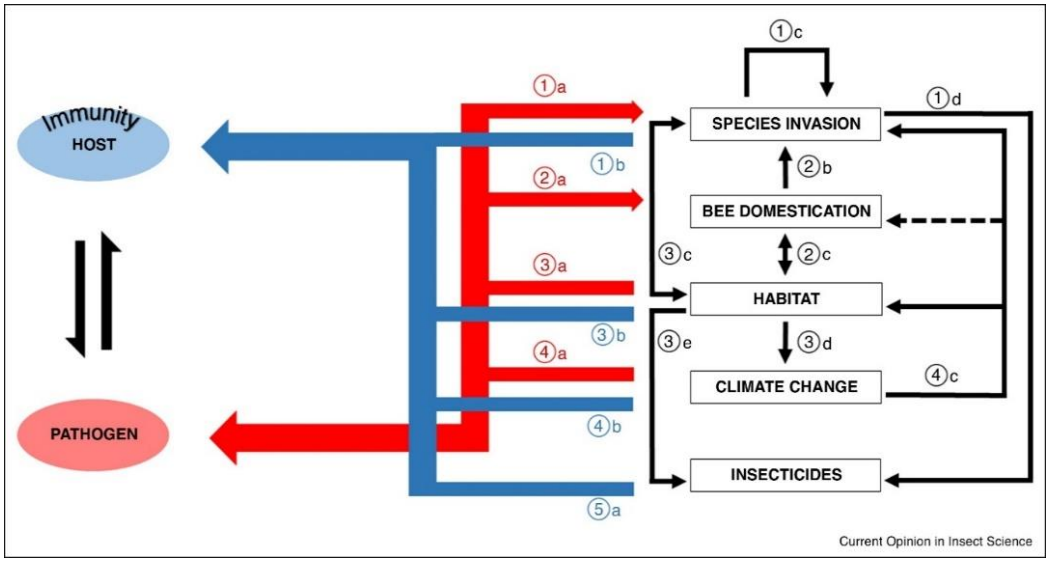

Figure 1. The influence of drivers of wild bee decline on host–pathogen dynamics (Meeus et al., 2018)

1.5. Effect of Climate Change on Honeybees Virus

Viral infections are an essential component of ecosystems, but their presence is generally overlooked. Biotic and abiotic factors play a role in the propagation of viruses and the harm they cause to their hosts. The SARS-CoV-2 pandemic has shown that host-host interactions can be disrupted when the natural dynamic interactions between hosts and their viral infections are disrupted (Bee et al., 2020; Galbraith et al., 2018; McMahon et al., 2015; Piot et al., 2022; Singh et al., 2010).

The ecology of viruses in bees is especially complicated because many viruses seem to be shared by different types of bees. Viruses have been shown to spread from managed *A. mellifera* or bumble bee (*Bombus spp)* colonies to wild bee populations. According to studies, viral prevalence is higher in regions with a higher density of infected managed colonies. These include the deformed wing virus, Israeli acute paralysis virus, acute bee paralysis virus, and Kashmir bee virus. These include the deformed wing virus, Israeli acute paralysis virus, acute bee paralysis virus, and the Kashmir bee virus, Lake Sinai virus, and chronic bee paralysis virus. bee populations in Asia, Africa, the Middle East, Australia, and South America (Dolezal et al., 2016; Galbraith et al., 2018; Remnant et al., 2017).

Varroa destructor, which feeds on growing honeybee larvae and adult bees and spreads deadly viruses, has caused major honeybee losses since transferring hosts from the Asian honeybee *(A. cerana)* to *A. mellifera* in the middle of the 20th century. Additionally, the introduction of *V. destructor* has drastically altered the worldwide viral landscape in honeybee populations by enhancing virus transmission and resulting in the selection of more virulent virus strains. The combination of *V. destructor* and viruses is now regarded as the leading cause of global colony losses, but determining the significance of viruses alone remains a formidable problem due to the pervasiveness of *V. destructor*.

Viruses normally survive as latent infections in honeybee populations, but outbreaks can develop when colonies experience stress or are exposed to particular environmental circumstances. The proliferation of V*. destructor* has greatly exacerbated colony stress and raised the significance of viruses in colony losses (Roberts et al., 2017, 2020; Ryabov et al., 2017). Viruses are infectious creatures that can only replicate within cells and must use their host's resources to do so (i.e., transcription, translation and replication). They can cause severe damage to their hosts and display a wide variety of symptoms. Honeybees are susceptible to viral infections, which have been related to alterations in a variety of features, including their appearance, functionality, and behavior. In extreme cases, viruses can cause both individuals and colonies to die off more quickly (Bee et al., 2020; McMenamin& Flenniken, 2018; Steinmann et al., 2015).

The global climate has an ever-present effect on the interactions between bees and diseases. Several studies have demonstrated that climatic variables can affect honeybees as hosts and their interactions with their (viral) diseases, but these studies have only been conducted at the local scale; no study has yet examined these consequences at the global level. If we want to know how viruses affect bee populations, we need to know how many different kinds of viruses interact with the many different kinds of bees that make up the pollinator community and how the weather affects those interactions on a global scale. A host's way of life can be directly impacted by weather, and the spread of disease can be indirectly impacted as well. Since most bee pathogens spread by the fecal-oral pathway, which allows for cross- and intra-species transmission via shared flowers, environmental factors including UV-exposure, temperature, and precipitation may affect pathogen survival on flowers and in turn, spread. Additionally, climatic variables influence vegetation phenology, flower attractiveness, and diversity, which may modify the transmission network via flowers and the quality and amount of forage resources, which may influence host immunity, and therefore, disease susceptibility and transmission (Piot et al., 2022).

1.6. Effect of Climate Change on Honey Bees Bactria

The pollen reserve flora, which is dominated by bacteria of the genera *Pseudomonas* and *Lactobacillus* and fungi of the genera *Saccharomyces*, Candida, and *Cryptococcu*s, greatly outnumbers the microflora of honey. Surprisingly, the gut flora of healthy, free-flying bees contains very few yeasts, and a rise in yeasts is caused by illnesses, starvation, antibiotics, and insecticides. Therefore, an increase in the amount of yeast colonies isolated from the bees' stomachs may serve as an indicator of stress. However, new research by Tauber et al.,(2019). reveals that yeasts are necessary throughout the early stages of a young bee's existence, which includes feeding the hive, but become less important after the honeybee begins foraging. Social immune defenses, natural homeostatic mechanisms, microbiome diversity and function play a significant influence in disease resistance in honeybee colonies. However, little is known about the links and variance between bee diseases, bee microbiota, and anthropogenic environmental changes (Hubert et al., 2017; Ptaszyńska, Latoch, Hurd, Polaszek, Michalska-Madej, Grochowalski, Strapagiel, Gnat, Załuski, & Gancarz, 2021).

The indigenous gut microbiota of *A. mellifera* is found to be altered by environmental stresses. Increases or declines in the major microbial taxa or the presence of transitory bacteria not typically associated with the host are frequently observed in diseased bees exhibiting dysbiosis. Certain taxa (e.g., Bifidobacterium) have decreased in CCD-affected hives, while the abundance of other key taxa has increased (e.g., Firmicutes). Changes in the microbiome's diversity and relative abundance can have serious effects on health and make *A. mellifera* more likely to get infection when dysbiosis happens (Anderson et al., 2013; Corby-Harris et al., 2014; Mattila et al., 2012; Moran et al., 2012; Subotic et al., 2019).

Table 1. Some Honeybee Pests and Diseases Correlated with Colony Losses (Hristov et al., 2020a)

Type of Pathogen	*Kind of Relationship*
Varroa destructor	Ectoparasitic mite
Acarapis woodi	Tracheal mite
Varroa jacobsoni	Ectoparasitic mite
Tropilaelaps clareae	Ectoparasitic mite
Deformed Wing Virus A	Viral pathogen
Deformed Wing Virus B (VDV1)	
Acute Bee Paralysis Virus	
Kashmir Bee Virus	
Israeli Acute Paralysis Virus	
Chronic Bee Paralysis	
Sacbrood Virus	
Black Queen Cell Virus	
Nosema ceranae	Intestinal parasites
Nosema apis	
Nosema neumanni	
Ascosphaera apis	Fungal pathogen
Aspergillus spp.	
Aethina tumida	Beekeeping pest

1.7. Anthropogenic Direct Drivers Associated with Honeybee Colony Decline

As many pests and illnesses act as direct natural drivers, there are numerous more anthropogenic drivers that result in colony losses. These variables often work together to cause illness, death, and colony collapse.

The most crucial aspects will be highlighted in this analysis (Hristov et al., 2020a). Currently, the most influential characteristics in industrialized nations are anthropogenic landscapes, which comprise those formed either directly by human activity or indirectly by natural processes driven by human activity. In addition to influencing geological characteristics, human activity has also had a significant impact on flora and wildlife. The loss of biodiversity is sometimes called the sixth mass extinction, and the dramatic drop in the number and types of insects has led to the word "Insectaggedon" to describe the event (Ptaszyńska, Latoch, Hurd, Polaszek, Michalska-Madej, Grochowalski, Strapagiel, Gnat, Załuski, & Gancarz, 2021).

2. Interaction Between Abiotic and Biotic Factors

2.1. Interactions between Pesticides and Pathogen Susceptibility in Honeybees

Poor queen quality, shifting cultural and commercial beekeeping practices, and exposure to agricultural and apicultural pesticides are only some of the factors that threaten the health and survival of managed honeybee colonies. Parasites and pathogens are becoming increasingly common. Poor management of hives by beekeepers may promote parasite populations and disease transmission (Belsky & Joshi, 2019; Bird et al., 2021; Collison et al., 2016; Harwood & Dolezal, 2020; O'Neal et al., 2018, 2019; Sánchez-Bayo et al., 2016).

Bee-disease interactions affect bee survival, pathogen burdens, and immunity. Researchers should make it a top priority to learn more about how pesticides affect the antimicrobial peptide (AMP) part of the immune response (Collison et al., 2016). There is no clear comparison of the effects of pesticides and viruses on honeybee colonies. The complexity of such interactions may explain why there is so little research evaluating what at first glance seems to be a clear comparison. The social structure of bee colonies, the huge variety of chemical classes of pesticides, and the pairing of several bee viruses with ectoparasitic Varroa mites are all examples of such intricacies (Harwood & Dolezal, 2020; Hsieh et al., 2020; McMenamin et al., 2016).

Three major topics about the effects of pesticides and pathogen infections on bee health are addressed in this article. Does exposure to pesticides and infection with pathogens enhance the load of the pathogens? Do pesticides and pathogens weaken bees' immune systems? Is it possible that bees might not live as long if they are exposed to both pesticides and pathogens? (Bird et al., 2021; Collison et al., 2016; Czerwinski & Sadd, 2017; Grassl et al., 2018; Harwood & Dolezal, 2020; O'Neal et al., 2018; Sánchez-Bayo et al., 2016; Straw et al., 2022).

2.2. Individual-Level Effects

Even sublethal concentrations of pesticides have deleterious effects on honeybee behavior, foraging, lifespan, and olfactory learning and memory. Honeybees can have their detoxification pathways compromised by pesticide exposure. The effect of pesticide exposure is a source of concern in light of the growing importance of diseases in colony loss.

2.3. Colony-Level Effects

Field investigations of pesticide impacts on honeybee colonies are common. Biotic and abiotic stresses make it challenging to control for all possible outcomes. Differences in assessing pesticide exposure, toxicity and risk among the various castes can be brought about by social bee behaviors including age-based divisions of labor.

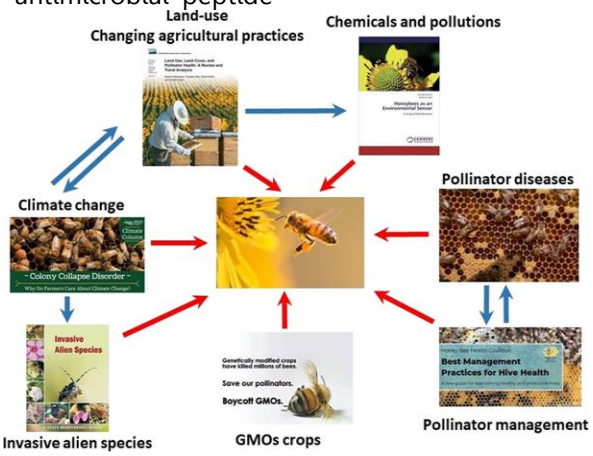

Figure 2. Depicts the Primary Causes of Change in Honeybee Colony Declines (Neov et al., 2021).

2.4.Community-Level Effects

High pesticide residues have been found in honeybees. Honeybees are exposed to a wide variety of pesticides. As Varroa mites can act as vectors for several viruses, it is possible for viruses to be spread between honey bees in various apiaries through the phoretic movement of mites. It is possible for bees to spread pathogens and pesticide residues from one hive to another by stealing from or stealing from weaker colonies (O'Neal et al., 2018).

2.5. Interactive and Cumulative Effects

Biotic and abiotic stressors in action opinions are divided on what exactly causes bee colony collapse. Advocates for each possible stressor claim to be the primary reason for the occurrence. It is also taken into account that in real-world field studies, data on the impacts on the defensive capacities of an individual cannot be immediately extended to the entire colony.

Recent research has revealed that when pesticides and diseases interact, it can have a negative impact on bee colonies' health. Bees become more susceptible to the intestinal parasite *N. ceranae* after exposure to neonicotinoid insecticides. When both mite-harboring mites and imidacloprid are present in the hive, the infection rate with *Nosema spp.* rises sharply, as does the death rate. Death rates were also increased when both fipronil poisoning and *N. ceranae* infection were present.

Pesticides and diseases aren't the only stresses that can interact with one another. Beehive malnutrition is frequently cited as a contributing factor to bee parasite-caused losses. If wild bees have access to all the nutrients they need, it will reduce fatality rates. The LD50 values (lethal dose, 50%) for specific bee hazardous chemicals may be affected by dietary stress. A case in point: the parasites of the genus Crithidia (Belsky & Joshi, 2019; Gregorc, 2020; Neov et al., 2019).

2.6. Interaction of Pesticides and Honey Bee Viruses

Many studies have shown that pesticides exert additive or synergistic effects on virus-induced mortality and replication in honey bees. Honeybees, like other insects, have evolved mechanisms to detoxify xenobiotics like pesticides and fight disease threats like viruses. The next question is whether or not being exposed to pesticides makes viruses more likely to spread. while others have shown little to no effect of pesticide exposure on viral infections in honey bees and bumble bees. This difference is probably because bees encounter different pesticides and diseases in different places (Harwood & Dolezal, 2020).

2.7. How Pesticides Can Impact Antiviral Pathways

Insect hemocyte differentiation and function can be impacted by pesticide exposure. In a variety of studies, pesticides have been shown to alter the expression of genes involved in detoxification and immunity. Imidacloprid, for instance, has been shown to reduce the activity of genes that control the DUOX pathway in D. melanogaster. Some pesticides can directly make insects more susceptible to viruses by blocking ion channels that help them fight viruses (Harwood & Dolezal, 2020; McMenamin et al., 2016).

Studies have shown that eating a "monofloral" diet might be stressful. Honey bees gather nectar and pollen from mass-flowering crops cultivated over expansive areas, such as sunflowers, rapeseed, and acacia, for the purposes of making honey or just pollinating the plants. Low-nutrient pollen and nectar from plants contain natural but poisonous to honey bee compounds. The amygdalin glycoside in almond blooms fits the description (Neov et al., 2021).

2.8. Interaction between Fungi and Bacteria

Apis mellifera L., or honeybees, plays a vital role in pollinating several commercially significant crops. However, infection is a factor in their demise. Most bee diseases, like Nosema and American foulbrood (AFB), develop in the honeybee digestive tract. This work used 454-pyrosequencing to look at how gut-propagating pathogens like *Nosema ceranae* and *Paenibacillus larva*e affected bacterial populations in the digestive tract of *A. mellifera*. The bacterial populations of *P. larvae* infected larvae were unaffected by the infection, but pyrosequencing studies demonstrated that *N. ceranae* was involved in the eradication of Serratia and the substantial rise in Snodgrassella and Bartonella in the guts of adult bees. The results showed that only *N. cerana*e affected some core bacteria in the gut of *A. mellifera*, making core gut bacteria grow and causing dysbiosis (Diaz et al., 2019; Huang et al., 2018; Naree et al., 2022; Panjad & Yongsawas, 2021; Xing et al., 2021; Zhang et al., 2021).

According to research examining gut bacteria at various life stages and locales, honeybees' health and susceptibility to ecological changes were shown to be significantly affected by the dynamics of bacterial communities. But there isn't enough information yet about how the spread of diseases changes the bacteria and how

that affects honeybee health (Panjad & Yongsawas, 2021).

2.9. Interaction Between Climate Change and Environmental Pollutants

Due to their inseparable symbiosis with the determinants of environmental health, honey bees can serve as a model to examine the relationships between environmental change and AMR. Pollutants in the air, water, and soil, for instance, can have an adverse effect on honey bee health by seeping into the pollen and honey that they eat. Furthermore, rising temperatures and other climatic factors associated with climate change can amplify the prevalence and spread of honey bee diseases while simultaneously reducing the efficacy of antimicrobials in combating pests and pathogens. Years of unrestricted antibiotic usage have led to an uptick in multidrug-resistant bacteria, further undermining medication efficacy. Colony losses in apiaries throughout the world are at an all-time high. Most of these losses, which have been dubbed "colony collapse disorder," are thought to be the result of complex interactions between a variety of environmental, pathogenic, and climatic variables. Therefore, it is essential and highly relevant to honey bee health that interdisciplinary research investigates these interactions (De Jongh et al., 2022).

3. Approaches to Limiting the Consequences and Solving the Problem Associated with Honeybee Colony Decline

a. Dealing with the CCD syndrome of honey bees and honeybee colonies requires the construction of qualitatively different production systems. Developing nonchemical methods of controlling harmful populations and pathological phenomena in crops is a step in the right direction. Any steps taken to cut back on the use of plant protection products and reduce agriculture's reliance on pesticides would reduce the amount of honey bees and honeybee colonies that are poisoned. Improving honeybee selection, reducing stress, and exploiting honeybee colonies are among the measures to be taken to improve honeybee health (Neov et al., 2021).

b. Some additional precautions are suggested, including: (1) ensuring that the colony has access to sufficient quantities of high-quality forage and clean water; (2) avoiding sugarisation, i.e. the excessive use of sugar syrup; (3) catering to the colony's nutritional requirements; (4) being mindful of both the timing and composition of the diet when feeding bees; (5) in the event of a shortage of natural feed, supplementing with sugar syrup; (6) organized control of *V. destructor* in the colonies is obligatory due to its vector role, and (7) compliance with hygienic and sanitary measures and principles of good apiculture practice and management in Apiaries (Stanimirović et al., 2019).

c. Future monitoring could be improved by comparing the losses of a particular operation to the usual losses in a region and by comparing the weather to long-term statistics. Long-term monitoring and locally occurring pollutants or diseases tend to be priorities in the field for study into causes of bee losses. Future monitoring could be improved by comparing the losses of a particular operation to the usual losses in a region. Because losses in regions fluctuated throughout the years, beekeeping inputs were not implicated (Giray et al., 2010).

d. The use of *Varroa destructor* as a vector of bee viruses is emerging as a significant factor in the losses of honeybee colonies reported internationally. Bee variety, beekeeping techniques, and the bees' feeding habitat affect bee health. Places with established honeybee parasitic Varroa mite populations have consistently higher colony losses. The prospective pesticide risk maps could aid in the reduction of honeybee colony losses. The maps could identify places with relatively high pesticide pressure in a species-specific manner, enabling conservation measures on a local scale. This could help restore vulnerable bee species and minimize honeybee colony losses (Neov et al., 2019).

e. Due to parasites, diseases, pesticides, and habitat loss, Western honey bees *(Apis mellifera)* are in severe decline despite their importance as pollinators in natural and agricultural settings. The FlowTM hive is a new type of bee hive that was made in 2015. It makes it possible to get honey from the hive without first opening it, which makes the bees less stressed (Subotic et al., 2019).

f. Focus on nutrition's potential as a modifiable mechanism to reduce the harmful effects of stressors like pesticide and pathogen exposure. Bernklau and colleagues (2019), test the effects of feeding infected bees nectar solutions containing caffeine, gallic acid, kaempferol, and p-coumaric acid. They conclude that oxidative stress caused by varying doses of clothianidin may be alleviated by providing more calories (López-Uribe & Simone-Finstrom, 2019).

4. Future Directions

Researching how pesticides and viruses affect honeybees has been hard, but it is important work if we want to make sure food security stays high as climate change continues to affect agroecosystems. First, we need to learn more about how the Toll and IMD pathways of the

innate immune system work to fight viruses. Mechanistic explanations for how viruses are destroyed or prevented from replicating are still lacking, despite our knowledge that these pathways are engaged upon viral infection. There are several facets to this problem that require more research. To forecast how exposure will influence host antiviral responses, we must continue to discover immune and detoxification pathway components that are directly affected by pesticides. Here, the latest in machine learning and metabolic modeling could improve the search (Harwood&Dolezal, 2020).

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THE EFFECT OF USING EGG YOLK PLASMA ENRICHED WITH Β-CAROTENE ON FROZEN SEMEN IN ARABIC STALLIONS

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Abstract

Horse semen cryopreservation is one of the most important methods for genetic preservation of rare and endangered genotypes. The current study was conducted to evaluate the effect of β-carotene -enriched egg yolk plasma (EYP) as an antioxidant supplement to the INRA-96 extender in relation to sperm freezing in Arabian stallions. For this purpose, β-carotene was used in various concentrations as a supplementary ingredient in the formulation of the feed for laying hens. The birds were randomized into four groups fed 0 (control), 500, 1000 and 2000 mg/kg of a β-carotene supplemented diet. Subsequently, different variants of enriched extenders (INRA-96 + 2.5% glycerol (G)) were obtained by adding 2% EYP from four treatment groups. Sperm properties, including motility, viability, morphology, lipid peroxidation (MDA), plasma membrane integrity (HOS test), acrosome integrity, and DNA fragmentation were assessed post-thaw. The results showed that an overdose of β-carotene has an adverse effect on sperm characteristics. In the current study, we came to the conclusion that the optimal concentration of β-carotene in layer feed (500 mg/kg) could give the best results in terms of sperm quality. This study demonstrated that the EYP from the β-carotene (500 mg/kg)-fed dietary supplement improved sperm motility, viability, plasma membrane integrity, and acrosome integrity. In addition, lipid peroxidation and the frequency of sperm abnormalities and DNA fragmentation were reduced using the treatments mentioned. According to this research, β-carotene- enriched EYP acts as a valuable natural and safe supplement material that could be used to improve the sperm quality of stallions under cryopreservation conditions.

Key words: *Arabic stallion, β-carotene, Cryopreservation, DNA fragmentation, Egg yolk plasma*

COMPARISON OF MEAT AND MILK PRODUCTION AMOUNTS AND PRICES IN TÜRKIYE AND EUROPEAN UNION COUNTRIES USING CLUSTERING ANALYSIS

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Abstract

In this study, it was aimed to compare the quantity of beef and cow milk produced in Türkiye and the other EU-28 nations, their pricing, and the amount of beef and cow milk that can be purchased given per capita income by using cluster analysis. The material of the study consists of the production amounts (tonne) and prices (USD) of beef and raw cow milk and the per capita income levels (USD) of the countries, published by the Food and Agriculture Organization (FAO) in 2020. In the study, Türkiye and EU-28 countries were clustered according to the amount and prices of meat and milk production and the amount of products that can be purchased with per capita national income with a agglomertive hierarchical clustering using Ward´s method. The Euclidean distance was used as the distance measure tool. Results showed that Türkiye was in the same cluster with the EU-28 countries in terms of beef and milk production amounts in 2020. However, it has been determined that the amount of meat and milk per capita was on the average of EU-28 countries. It was showed that Türkiye´s beef prices were about twice as expensive as the average for the EU´s 28 member states, and that it falls into the same group as Greece, where the cost of meat is the highest. It has been observed that the price of milk is comparable to that of the other 28 EU member states. According to research, the amount of beef that can be purchased with per capita income (tons) in Türkiye was roughly 8 times lower than the average of the EU´s 28 member states. Greece, Romania, Bulgaria, and the nations with the lowest amount have all been found to be in the same group. In terms of cow milk, Türkiye was in the same group as the nations with the lowest amounts of cow´s milk, while sharing similarities with many of the EU-28´s other members. As a result, although the amount of meat and milk production, which are the main food products in Türkiye´s candidacy process, was higher than EU-28 countries, the amount of meat and milk that can be purchased with per capita income was considerably lower than EU-28 countries. It is crucial to take measures to overcome price downsides in Türkiye, since meat production is highly dependent on milk production. In order for these sectors to maintain their competitiveness, production per animal and the efficiency in productivity should be increased.

Key words: *Cluster analysis, European Union, Meat, Milk, Türkiye*

SURGICAL INTRAUTERINE INSEMINATION USING FRESH URETHRAL SEMEN IN DOMESTIC CAT (FELIS CATUS)

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Abstract

The objective of this study was to compare pregnancy rates in domestic cats using fresh semen for bilateral intrauterine insemination (BIUI) method and novel uterine body insemination (UBI) method. Queens received a single injection of eCG (200 IU; im) to induce ovarian follicular development and, after 83 h, an injection of hCG (100 IU; im) for final oocyte maturation and ovulation induction. Thirty-four hours after hCG administration, fresh semen collected following urethral catheterization (UC) from a single tom cat was injected by the BIUI (n = 8 queens) and UBI (n = 7 queens) techniques. Pregnancy rates were 75.00% (6/8) by BIUI and 42.85% (3/7) by UBI method. Mean Litter size was 3.00 ± 0.86 by BIUI, and 2.00 ± 1.00 by UBI method. Our findings showed that BIUI of queens with fresh semen resulted in higher pregnancy rates compared with the novel UBI method, also acceptable pregnancy rates following BIUI with fresh semen in the domestic cat was achieved.

Key words: *intrauterine insemination, assisted reproductive techniques, fresh semen*

INTRODUCTION

Since most feline species are endangered and the domestic cat is a useful biomodel for wild felids research; many studies on assisted reproductive technologies (ARTs) have been done in order to save these threatened species (Farstad 2000). In domestic cats, follicular development can be stimulated with exogenous follicle stimulating hormone (FSH) or equine chorionic gonadotropin (eCG), and ovulation can be induced with gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) (Roth et al. 1997).

The first artificial insemination (AI) in the domestic cat was conducted more than 50 years ago using 10-50 × 10^6 fresh sperm inseminated intravaginally in natural estrus queens (Sojka, Jennings, and Hamner 1970). It is reported, in fact, that intrauterine insemination requires lesser fresh spermatozoa than intravaginal or transcervical insemination in order to achieve comparable pregnancy rates (Tsutsui et al. 2000; Zambelli and Castagnetti 2001). This is especially important when the quality of the semen is poor. Tsutsui et al. (Tsutsui et al. 2000) achieved a pregnancy rate of 80% by inseminating 8 × 10^6 fresh sperm into one uterine horn.

Semen collection is more difficult in cats when compared to domestic animals; either the cat is anesthetized for electroejaculation, or an estrous queen or phantom is needed as teaser for the artificial vagina (AV) method (Chatdarong et al. 2007; Ackermann and Lopes 2020). Urethral catheterization (UC) of tom cats after treatment with an alpha-adrenergic agonist has recently been developed as an alternative method of semen collection, this approach has allowed the recovery of high sperm number in domestic cats without causing erection or ejaculation (Zambelli et al. 2008).

Since prior findings demonstrate significant discrepancies in the procedure, such as inseminating dose, fresh semen quality, time of AI, and so on, it is difficult to make reliable comparisons between AI methods for domestic cats. There are few data on the efficiency of the UC-collected sperm in terms of the pregnancy rate of queens, including Zambelli et al. and DAŞKIN et al. (Zambelli et al. 2015; DAŞKIN, Totan, and OLĞAÇ 2022; Zambelli, Bini, and Cunto 2015), which made us even more obliged to test the efficiency of the urethral semen in the two deposition sites (BIUAI and UBI). The objective of the present study was to

determine pregnancy rates following BIUAI and UBI with fresh semen collected via UC technique.

MATERIALS AND METHODS

Fifteen mixed breed queens between 1 and 3 years of age and body weight of 3 ± 0.4 kg were enrolled in this experiment. A 4y old mixed-breed tom cat, with body weight of 4.5 kg was used as the source of semen. All animals were exposed to 12 hours of artificial fluorescent light.

General anesthesia was induced in the tom with medetomidine (150 µg/kg; im) (Dorbene vet®, Royan Daru Co, Tehran, Iran) and urethral semen was collected each time just before the AI procedure by UC technique, as previously described by Zambelli et al. (Zambelli et al. 2008). The semen sample was placed into an Eppendorf tube containing prewarmed *hepes Hams f10 (*BIO-IDEA®, Iran) medium.

Queens received a single injection of eCG (200 IU; im) (Gonaser® 5000 Hipra laboratories Co, Amer Spain) to induce estrus and, after 80 h, an injection of hCG (100 IU; im) (PD Preg® 5000, Pooyesh Darou Biopharmaceutical Co., Ltd., Tehran, Iran) to induce ovulation. Before insemination, ovaries were exposed via ventral midline laparotomy to visually examine the ovaries and to determine whether ovulation had occurred.

General anesthesia was induced in the queens 34 h after the hCG treatment with xylazine (3 mg/kg; im) (Xyla® Interchemie werken "De Adelaar" BV, Metaalweg 8 Venray The Netherlands) and ketamine (10 mg/kg; im) (Bremer Pharma GMBH Warburg Germany). Insemination was performed 34 h after the hCG treatment. A ventral midline laparotomy was done and the ovaries were exposed, females with positive ovarian response (presence of preovulatory follicles or fresh CLs) were inseminated. An insulin needle was inserted either into the luminal region of the uterine body (bifurcation region) (UBI) or into the luminal region of both uterine horns (BIUI).

Cats were examined with a real-time, B-mode ultrasonographic scanner (Emperor - V9 EV 5 MHz, EMP Co., China) 30 d after AI. The number of kits was counted on the delivery day.

Continuous dependent variables including gestation length and litter size were analyzed using GLM procedure. Further, binary dependent variables including rates of conception, abortion and mummification were analyzed using logistic regression analysis by GENMOD procedure considering function link logit in the model. All analyses were performed in SAS version 9.4 (SAS Institute Inc., Carry, NC, USA). Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Characteristics of the semen were similar ($P > 0.05$) between the two groups. In order not to affect the pregnancy rates, semen of one tom cat (confirmed semen quality) was used in both groups. In this study semen was collected by UC, this technique is appropriate in practice since no expensive equipment and specific permissions are required (Zambelli et al. 2008)

Vaginal AI typically requires 50–80 $\times 10^6$ fresh spermatozoa for consistent conception rates (Tanaka et al. 2000), whereas transcervical AI requires 10–30 $\times 10^6$ fresh spermatozoa to obtain similar fertility (Zambelli and Cunto 2005). Because AI time in relation to ovulation is less dependent on sperm viability when the semen is freshly collected and frozen-thawed semen yields to less satisfactory results (Platz, Wildt, and Seager 1978), in this study, each queen was inseminated with fresh sperm following the BIUI or novel UBI method.

Combining artificial insemination with estrus induction allows for greater control over the parameters that can affect insemination success, such as the precise timing of the procedure (Zambelli et al. 2015).

The total ovulation induction rate In our study was 73.33% (11/15), and anovulation in those queens could be attributed to anesthetics or even handling stress, which could lead to ovulation suppression (CARTER et al. 1984).

In this study, the urethral semen yielded a pregnancy rate of 75% (6/8) and 42.85% (3/7) after BIUI and UBI, respectively (Figure 1). The impregnated queens in BIUI group gave birth to 1–6 kittens (Mean±SE, 3.00 ± 0.86 kittens) during a period of 63–66 days (Mean±SE, 64.50 ± 0.43 days) after AI and the impregnated queens in UBI group gave birth to 1–3 kittens (Mean±SE, 2.00 ± 1.00 kittens) during a period of 65–67 days (Mean±SE, 66.00 ± 1.00 days) after AI.

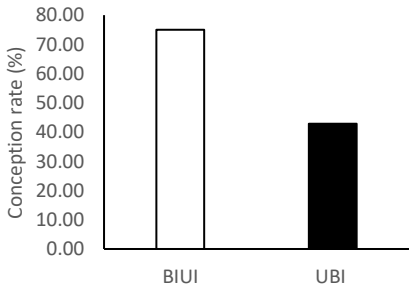

Figure 1. conception rate between the two groups ($p<0.05$)

Despite the observation of fresh CLs and the use of the same male spermatozoa for

insemination, AI results in the BIUI method were considerably more efficient than in the UBI method. The queens who failed to conceive after BIUI or UBI could have been affected by several factors, such as the negative impact of anesthesia on sperm transport inside female genital tract (Howard et al. 1992), the circulatory persistence of hCG, in combination with residual eCG, stimulates the development of secondary follicles and CLs, which can disturb the postovulatory endocrine milieu and impair conception, embryo migration, and implantation (Graham, Swanson, and Brown 2000). It is also likely that the invasiveness of the surgery may interfere with successful fertilization and embryo development.

CONCLUSIONS

The present study offers a novel approach to AI in felids by using laparotomy to deposit semen into the uterine bifurcation region. In this experiment we have obtained 75.00% and 42.85% pregnancy rate following BIUI and UBI respectively. Although the UBI method did not yield a high pregnancy rate, but it can be improved in the future by increasing the semen dose and quality as well as using a progesterone analogue as a pretreatment. In conclusion, BIUI with fresh semen resulted in higher pregnancy rates compared with UBI.

Table 1. Gestation length, conception rate and litter size in cats of BIUI and UBI groups. Data are presented as percentages or mean ± standard error. Values in parenthesis are actual numbers.

	BIUI (n = 8)	UBI (n = 7)
Litter size	3.00 ± 0.86	2.00 ± 1.00
Conception rate (%)	75.00 (6/8)[a]	42.85 (3/7)[b]
Gestation length (day)	64.50 ± 0.43	66.00 ± 1.00

[ab]Various letters indicate tendency for significant difference ($0.05 \leq P < 0.10$).

ACKNOWLEDGEMENTS

We would like to thank Dr Ali Hoshyarkhah, Dr Behzad jalalifar, Dr Alireza Jafari and Dr Ali Rezazadeh for their overall cooperation in this project.

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UNILATERAL INTRAUTERINE INSEMINATION IN DOMESTIC CAT USING FRESH URETHRAL SEMEN (A CASE REPORT)

Behrang EKRAMI [1], *Amin ROSHANI [2]*, Hamid GHASEMZADEH-NAVA [3], Vahid AKBARINEJAD [3], Homa MOHAMMADIFARD[2], Amin ZAMANPOOR[4]

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Abstract

The objective of this study was to assess the fertility of the urethral semen in domestic cats by surgical unilateral intrauterine insemination (UIUI) method. The queen received a single injection of eCG (200 IU; im) and, after 83 h, an injection of hCG (100 IU; im) to induce ovulation. Thirty-four hours after hCG administration, fresh semen collected from a single tom cat with 2.16 × 10^6 sperm was injected by the UIUI technique. Thirty days after AI, Pregnancy was confirmed via ultrasonography. The impregnated queen gave birth to three kittens during a period of 63 days. Our results showed that urethral semen with low sperm dose can lead to pregnancy.

Key words: *unilateral intrauterine insemination, urethral catheterization, fresh semen*

INTRODUCTION

The domestic cat serves as a useful biomodel for the conservation of endangered felids (Rijsselaere and Van Soom 2010).

Domestic cats are induced ovulators and in the case of wild felids, eCG followed by hCG has become the regimen of choice (Roth et al. 1997; Rijsselaere and Van Soom 2010).

Semen collection is more difficult in cats than in other species; either an estrous queen is needed as teaser, or the cat is anesthetized for electroejaculation, furthermore ovulation must be induced in the queen (Chatdarong et al. 2007). Urethral catheterization (UC) after treatment with an α_2 agonist is a new and promising method which was first described by Zambelli et al. (Zambelli et al. 2008). The main advantages of UC method is that it can be repeated in the same animal, and no special equipment or expertise is required (Prochowska et al. 2015).

The first report of artificial insemination (AI) in the domestic cat dates back to more than 50 years ago (Sojka, Jennings, and Hamner 1970) and since then many improvements have been made, including the description of different methods for intrauterine insemination with surgical (Howard et al. 1992; Tsutsui et al. 2000) and nonsurgical methods (Chatdarong et al. 2007). It is reported, in fact, that intrauterine insemination requires less spermatozoa than intravaginal or transcervical insemination in order to achieve comparable pregnancy rates. (Tsutsui et al. 2000; Zambelli and Castagnetti 2001). This is especially important when the quality of the semen is poor.

Because anesthesia can negatively affect smooth muscle contraction, it compromises sperm transport during intravaginal and transcervical insemination (Swanson 2019). One advantage of intrauterine insemination over intravaginal or transcervical insemination is that, because the cervix has already been bypassed, sperm can be deposited closer to the site of fertilization (ampulla) (Swanson 2019). However, there may still be a problem with sperm transport via the uterotubal junction and into the oviducts (Swanson 2019).

Tsutsui et al. achieved a pregnancy rate of 80% by inseminating 8 × 10^6 fresh sperm into one uterine horn and Howard et al. achieved a pregnancy rate of 50% by inseminating 2.4-19.2 × 10^6 fresh sperm into both uterine horns (Tsutsui et al. 2000; Howard et al. 1992).

There are just a few studies, including transcervical AI by Zambelli et al. and intravaginal AI by DAKIN et al., evaluating the effectiveness of the UC-collected sperm in terms of the conception rate of queens (Zambelli et al. 2015; DAŞKIN, Totan, and OLĞAÇ 2022; Zambelli, Bini, and Cunto 2015). To our knowledge, this is the first study assessing the fertility of the

urethral semen surgically deposited into one uterine horn. Since surgical insemination of urethral semen into one uterine horn had not previously been documented in the literature, the objective of current study was to obtain pregnancy using the UIUAI method.

MATERIALS AND METHODS

A 1y old short haired queen with body weight of 2.4 kg was used in this study. A 4y old mixed-breed tom cat, with body weight of 4.5 kg was used as the source of semen.

General anesthesia was induced in the tom with medetomidine (150 µg/kg; im) and urethral semen was collected just before the AI by UC technique, as previously described by Zambelli et al. (Figure 1) (Zambelli et al. 2008). The semen sample was placed into an Eppendorf tube containing prewarmed *hepes Hams f10 (*BIO-IDEA®, Iran) medium.

The queen received a single injection of eCG (200 IU; im) (Gonaser® 5000 Hipra laboratories Co, Amer Spain) to induce estrus and, after 80 h, an injection of hCG (100 IU; im) (PD Preg® 5000, Pooyesh Darou Biopharmaceutical Co., Ltd., Tehran, Iran) to induce ovulation. Before AI, ovaries were exposed via ventral midline laparotomy to evaluate ovarian response to exogenous gonadotropins and to determine ovulation state at AI.

At AI, general anesthesia was induced in the queens 34 h after the hCG treatment with xylazine (3 mg/kg; im) (Xyla® Interchemie werken "De Adelaar" BV, Metaalweg 8 Venray The Netherlands) and ketamine (10 mg/kg; im) (Bremer Pharma GMBH Warburg Germany). Insemination was performed 34 h after the hCG treatment. An insulin needle was inserted into the luminal region of the tip of the left uterine horn, and fresh semen with 2.16×10^6 sperm was inseminated (Figure 1). After insemination was complete, bleeding was controlled by placing a gauze pad over the insemination site and applying direct pressure to it.

The queen was examined with a real-time, B-mode ultrasonographic scanner (Emperor - V9 EV 5 MHz, EMP Co., China) 30 d after AI. The number of kits was counted on the delivery day.

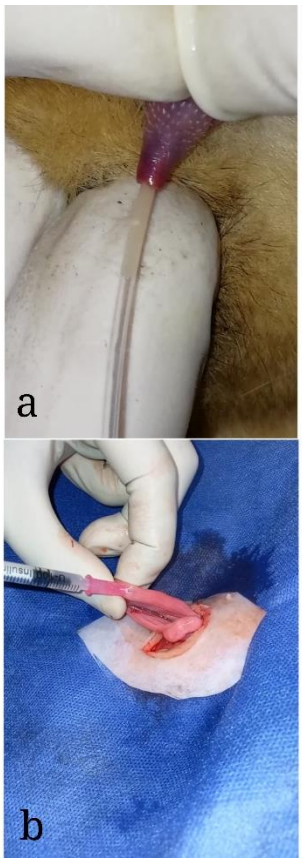

Figure 1. Semen collection by UC method from tomcat (a). Unilateral intrauterine insemination in queen (b).

RESULTS AND DISCUSSION

The domestic cat is a good animal model for practicing AI techniques before applying them to felids that are endangered or threatened with extinction. Most populations of captive wild felid species and domestic cats used as biomodel could benefit from ARTs, particularly in terms of male and female behavioral issues, gene transfer across institutions and physical limitations of animal transfer.

In this study semen was collected by UC, this technique is appropriate in practice since no expensive equipment and specific permissions are required (Zambelli et al. 2008; Lueders et al. 2012).

The synchronization protocol in this study successfully induced ovulation in the queen and the number of follicles and fresh CLs were recorded and are shown in table 1.

Estrus was assessed by vaginal cytology and observing behavioral patterns. Combining artificial insemination with estrus induction allows for greater control over the parameters that can affect insemination success, such as the precise timing of the procedure (Zambelli et al. 2015).

The first successful AI in domestic cats was reported more than 50 years ago following the

intravaginal deposition of fresh semen into naturally estrual, hCG treated females (Sojka, Jennings, and Hamner 1970). Although single insemination had a high pregnancy rate (50%), this method required high sperm counts (10–50 million sperm) for consistent conception, as well as the need to inseminate females during their natural estrous period. Tsutsui et al. reported a conception rate of 80% after surgical Intrauterine AI (IUAI) with 8×10^6 fresh sperm (Tsutsui et al. 2000). In the current study, 2.16×10^6 sperm was injected by the UIUI technique, which was approximately one quarter of the dose that Tsutsui et al. used (8×10^6 fresh sperm) and successfully led to birth of three kittens. The results of the synchronization protocol and insemination are shown in table 1.

Table 1. The results of the synchronization protocol and insemination.

No. of follicles		No. of fresh CLs		Gestation Length	Litter Size
L[a]	R[b]	L	R	63	3
1	0	6	0		

a: Left ovary, b: Right ovary

Our results showed that urethral semen deposited unilaterally in uterine horn can lead to pregnancy and healthy offspring may be carried to term.

CONCLUSIONS

In conclusion, low sperm dose UIUI with fresh urethral semen can lead to pregnancy. This case report is important since it is the first to report a successful pregnancy (three healthy kittens) after UIUI using urethral semen.

ACKNOWLEDGEMENTS

We would like to thank Dr Ali Hoshyarkhah, Dr Behzad jalalifar, Dr Alireza Jafari and Dr Ali Rezazadeh for their overall cooperation in this project.

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THE EFFECTS OF TWO EXOGENOUS GONADOTROPINS ON ESTRUS AND OVULATION İNDUCTİON İN DOMESTIC CAT (FELIS CATUS)

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Abstract

The objective of this study was to induce estrus and ovulation in domestic cats using standard synchronization protocols. Nineteen queens received a single injection of eCG (200 IU; im) to induce ovarian follicular development and, after 83 h, an injection of hCG (100 IU; im) for final oocyte maturation and ovulation induction. Thirty-four hours after hCG administration, ovaries were exposed via ventral midline laparotomy to visually examine the ovarian response. Behavioral estrous signs were observed in 13 out of 19 queens, and in vaginal cytology, all queens showed more than 60% of superficial cells and were considered to be in estrus. The total ovulation induction rates were 68.42% (13/19). Five queens showed signs of ovarian hyperstimulation, and one queen had a follicular cyst at laparotomy. Our findings showed that the standard protocol of eCG followed by hCG is effective in ovulation induction while having side effects including ovarian hyperstimulation and ovarian cysts.

Key words: *estrus synchronization, equine chorionic gonadotropin, human chorionic gonadotropin*

INTRODUCTION

To develop assisted reproductive methods for wild, threatened felids, the domestic cat is widely applied as useful experimental model (Rijsselaere and Van Soom 2010).

Domestic cats are described as seasonally polyestrous species and long-day induced ovulators (Kutzler 2007). So, in the absence of male mating stimuli, ovulation must be induced with the aid of exogenous gonadotropins.

In domestic cats, the routine protocol for inducing estrus and ovulation includes equine chorionic gonadotropin (eCG) followed 80–85 h later by human chorionic gonadotropin (hCG) (Howard et al. 1992b). For non-domestic felids, eCG followed by hCG has become the preferred regimen to reduce animal stress brought on by several FSH injections (Roth et al. 1997). The interval between hCG administration and ovulation in domestic cat is 25–27 h (Sojka, Jennings, and Hamner 1970).

However, estrus and ovulation induction following exogenous gonadotropins in queens can lead to abnormal hormonal profiles that may interfere with fertility and disturb maternal endocrine milieu since both eCG and hCG have prolonged half-lives (Pelican et al. 2006).

In 1992, howard et al. treated domestic cats with eCG and hCG and the queens gave birth to kittens following artificial insemination (Howard et al. 1992a). Since then, different scientists have proposed different protocols, including the use of pLH as an alternative for hCG to avoid hCG-related side effects (Swanson 2019).

As a result, since there are different reports on the efficiency of the routine eCG/hCG protocol, we applied the same procedure to assess its efficiency. The objective of the present study was to evaluate the efficiency of the eCG and hCG on estrus and ovulation induction, as well as to determine their effects on ovarian structures, in an effort to enhance AI synchronization procedures in domestic cats for the preservation of wild and endangered felids.

MATERIALS AND METHODS

Nineteen mixed breed queens between 1 and 3 years of age and body weight of 3 ± 0.4 kg were enrolled in this experiment. All queens were exposed to 12 hours of artificial fluorescent light (from 8 a.m. to 8 p.m.) as soon as they arrived on our private property.

Cats were maintained at 22 to 23 °C. The animals were given a commercial cat food (Fidar®, Iran) twice daily, and had ad libitum access to water. All institutional and national guidelines for the care and use of laboratory animals were followed.

Queens received a single injection of eCG (200 IU; im) (Gonaser® 5000 Hipra laboratories Co, Amer Spain) to induce estrus and, after 80 h, an injection of hCG (100 IU; im) (PD Preg® 5000, Pooyesh Darou Biopharmaceutical Co., Ltd., Tehran, Iran) to induce ovulation.

In order to verify estrus, vaginal cytology was performed in all of the queens 34 h after the hCG treatment. A swab wetted with saline was used to collect exfoliated vaginal cells; the swab was gently rolled onto a clean and dry glass slide, the slide was then stained with Wright-Giemsa (Asia Pajhohesh®, Tehran, Iran). Detection of more than 60% cornified superficial cells was considered to indicate follicular development and estrus.

Queens were also observed for estrous behaviors after pharmacological induction of estrus with eCG. Estrous behaviors were defined as vocalization, rubbing against soft objects, rolling, and crouching with tail deviation.

General anesthesia was induced in the queens 34 h after the hCG treatment with xylazine (3 mg/kg; im) (Xyla® Interchemie werken "De Adelaar" BV, Metaalweg 8 Venray The Netherlands) and ketamine (10 mg/kg; im) (Bremer Pharma GMBH Warburg Germany). A ventral midline laparotomy was done, and the ovaries were exposed, and the number of preovulatory follicles, fresh CLs, or other ovarian structures were recorded for each queen. Follicular development was confirmed by visualization of preovulatory follicles (2 to 4 mm in diameter, clear in appearance, and generally flattened or only slightly raised above the ovarian surface) or postovulatory corpora lutea (CLs) (each approximately 4 mm in diameter, dark red and distinctively raised 2-3 mm above the ovarian surface) as described by Wildt and Seager (Wildt and Seager 1978). Queens with at least one fresh CL, were classified as post-ovulatory phase, regardless of the number of follicles present.

RESULTS AND DISCUSSION

Behavioral estrous signs were observed in 13 out of 19 queens. Also, all queens showed more than 60% of superficial cells in the smear of exfoliated vaginal cells.

A single small hCG dose (100 IU) appears to be equally effective for inducing ovulation in queens while reducing the negative effects of high exogenous gonadotropin doses (Swanson et al. 1996; Graham, Swanson, and Brown 2000), hCG doses higher than 250 IU may have a detrimental effect on oocyte quality (Goodrowe, Wall, and Wildt 1988). As a result, in the current study, 250 IU of hCG was used in order to avoid the unwanted side effects.

In most inseminated cats (n=13), ovulation had occurred at the time of surgery, given an ovulation rate of 68.42% (13/19) after a single hCG injection.

Ovulation is considered to occur 25–27 h after hCG administration (Sojka, Jennings, and Hamner 1970), and the total ovulation induction rate in our study was 68.42% (13/19), and anovulation in those queens could be attributed to anesthetics or even handling stress, which could lead to ovulation suppression (CARTER et al. 1984). And according to Howard et al., it is also likely that performing anesthesia prior to ovulation and the invasiveness of the surgery may interfere with ovulation induction and lead to a decrease in fertility (Howard et al. 1992a). The results of the synchronization protocol are shown in table 1. and the number of follicles and fresh CLs were in the normal range.

One queen developed a follicular cyst at laparotomy, and five queens exhibited signs of ovarian hyperstimulation and we reckoned that it was related to eCG/hCG treatment (Figure 1.). Even though all queen received the same dose of eCG and hCG, only six of the queens had aberrant ovarian structures, demonstrating the individual variations in response to exogenous gonadotropins. Also, the circulatory persistence of hCG, in combination with residual eCG, stimulates the development of secondary follicles and

It is commonly believed that a quiescent ovary is more likely to respond consistently to synchronization protocols (Howard and Wildt 2009). Therefore, in synchronization protocols, pretreatment with a progesterone analogue appears to be beneficial for achieving a more consistent ovarian response and improved oocyte quality (Pelican et al. 2001; Pelican et al. 2008). Absence of CLs after implant removal appears to improve the ovarian response to exogenous gonadotropins (Pelican et al. 2006), so it is better to consider it in the future studies.

Table 1. The results of the synchronization protocol.

Queen No.	No. of follicles		No. of fresh CLs	
	L[a]	R[b]	L	R
1	3	1	1	2
2	0	0	4	7
3	5	2	0	2
4	1	2	3	3
5	2	0	3	4
6	2	3	2	4
7	1	0	6	0
8	1	0	4	5
9	0	0	1	3
10	0	5	2	3
11	4	5	0	0
12	0	0	4	6
13	3	3	4	4
14	0	1	4	2
15	Ovarian cyst			
16	Ovarian hyperstimulation			
17	Ovarian hyperstimulation			
18	Ovarian hyperstimulation			
19	Ovarian hyperstimulation			

a: Left ovary, b: Right ovary

CLs, which can disturb the postovulatory endocrine milieu and impair conception, embryo migration, and implantation (Graham, Swanson, and Brown 2000).

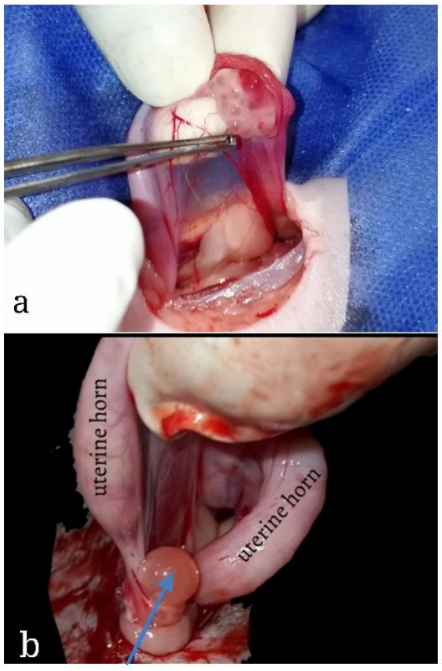

Figure 1. Ovarian hyperstimulation (a) and follicular cyst (b).

CONCLUSIONS

Our findings clearly showed that the combination of eCG and hCG is capable of inducing estrus and ovulation. The total ovulation induction rate in our study was 68.42% (13/19), but it can be improved in the future by reducing animal handling stress, using laparoscopic techniques for observing ovaries, as well as using a progesterone analogue as a pretreatment.

ACKNOWLEDGEMENTS

We would like to thank Dr Ali Hoshyarkhah, Dr Alireza Jafari and Dr Behzad jalalifar for their overall cooperation in this project.

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INTESTINAL LEIOMYOSARCOMA IN A GUINEA PIG: A CASE REPORT

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Abstract

In recent years, guinea pigs (Cavia porcellus) have becoming increasingly common pet animals that are useful in animal modeling studies. Tumors in Guinea pig have been reported rarely; therefore, little is known about these disease conditions. Reports concerning to guinea pigs have described cutaneous carcinoma, ovary teratoma and mammary gland adenocarcinoma. In September 2021, a 4-year-old female guinea pig was referred to veterinary hospital with a history of severe hair loss, depression, anorexia, pasty and malodorous feces. Before treatment, the animal died. On gross necropsy examination, a large and well-demarcated mass was observed on the serosal surface of intestine. Macroscopically, the mass was white to grey and approximately 10 × 8 × 5 cm in size. The cut surface was firm with trabecular structures. No abnormality was observed in other organs. Tissue samples of the mass were fixed in 10% neutral buffered formalin and routinely processed, dehydrated and embedded in paraffin wax, sectioned at 5 µm in thickness and stained with hematoxylin and eosin. Immunohistochemical studies of the mass sections were performed using vimentin and a-smooth muscle actin. Microscopically, the neoplasm was composed of spindle cells with elongate nuclei and abundant eosinophilic cytoplasm, forming broad interlacing fascicles. Mild cellular pleomorphism and some multinucleated cells were present. Mitotic figures were also rare (mitotic index 1 per 10×40 objective fields). Immunohistochemically, the neoplastic cells were negative for desmin and positive for vimentin and α-smooth muscle actin. Based on histopathological findings and the expression of vimentin and α-SMA, well-differentiated leiomyosarcoma was diagnosed. Leiomyosarcomas are malignant tumours of smooth muscle cells. Until now, leiomyosarcoma has been reported in the myometrium, heart and ovary. To our knowledge, the present case represents the first report of intestinal well-differentiated leiomyosarcoma in a guinea pig.

Key words: *guinea pig, intestine, leiomyosarcoma, pathology*

AURAL TUMOR IN A DOG: HISTOPATHOLOGICAL FINDINGS

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Abstract

Squamous cell carcinoma is malignant cutaneous tumor. In all species, SCCs may occur in young animals, but the incidence increases with age. This tumor occurs on the head, abdomen, neck, legs, scrotum, lips, and nail bed. A 3-year-old male Shih Tzu terrier mix dog (8 kg) with a raised mass at the base of left ear was referred to the veterinary hospital. On gross examination, the lobulated mass was approximately 2×2/5×1 cm in size. Based on owner information, within the previous 2-month period the mass had become evident and grew larger. Finally, complete surgical removal was selected. The dog was anesthetized with intravenous injection of ketamine (0/5 mg/kg) and diazepam (0.5 mg/kg) and maintained with 1%-2% isoflurane in oxygen. Analgesia was provided by subcutaneous injection of ketoprofen (2 mg/kg-1, SID for 3 days). On section, the mass was well circumscribed, ulcerated and white to grey. Tissue samples of the mass were fixed in 10 % neutral buffered formalin and stained with Hematoxylin and Eosin. Immunohistochemical studies of the mass sections were performed using primary antibodies against cytokeratin AE1/AE3. The patient was administered meloxicam (0.20 mg kg-1, IM, SID for 4 days), and Amoxicillin (15 mg/kg-1, IM, q 48h for 6 days). Microscopically, the mass was composed of neoplastic cells with eosinophilic cytoplasm, intercellular bridges, and concentric laminated masses of keratin, the keratin pearls. Nuclear pleomorphism and mitotic figures was minimal. Tumor had an infiltrating growth pattern and invasion of the dermis was seen. Immunohistochemically, the neoplastic cells were positive for cytokeratin AE1/AE3. Based on histopathological findings and the expression of cytokeratin AE1/AE3, well-differentiated squamous cell carcinoma was diagnosed. No clinical signs of recurrence were apparent 4 months after cessation of surgery. Similar to this case, in squamous cell carcinomas, surgical excision is the primary treatment option in dogs. Wide surgical excision, with margins of at least 2 cm around the tumor, is usually curative.

Key words: *Dog, ear, Scc, Pathology*

EFFECT OF DIFFERENT LIGHTING PROGRAMS ON HATCHING RESULTS OF THE EGGS OF TURKISH NATIVE GEESE

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Abstract

Lower productivity of native geese has afforded researchers to find ways to increase egg production. Lighting is the most common practice applied to increase egg production of poultry species. On the other hand, only increasing egg production is not solitary enough. The eggs also have to be fertile to produce goslings. And, problems in lighting programs could worsen welfare conditions which could result in fertility problems due to stress. In this study, the effects of different lighting programs on fertility, hatchability and hatchability of fertile eggs were executed on native Turkish goose eggs. Four different programs were used in four different windowed barns between November-June of 2019. In two of barns a fixed 14 and 20 hour lighting was applied, while one has no lighting as control group. In the control group windows of the barn allowed daylight to enter inside. Variable lighting program was used for the last group. Geese start laying in March, so we tried to make the lighting program of March artificially in November. From the first day of November, artificial lighting was applied between the sunrise and sunset of the first day of March in the region and changed every day according to the changes of March. Each lighting group had four replicates containing 3 females and one male at two years of age. The eggs were daily collected and numbered according to treatments. After 7 days of storage, eggs were placed in incubation machine for 27 days and then transferred to hatching machine for days of 28 to 30. For each treatment, mean of different hatching sets were given. Highest fertility (95%) was obtained in the eggs of variable lighting program group, control group had a fertility of 81%, while 14 and 20 hours lighting groups had fertility 52% and 58%. Similar results were obtained in the hatchability of fertile eggs. Variable lighting program group had 90% hatchability. Control group had 79%, 14 hours and 20 hours group 83% and 72%, respectively.

Key words: *Goose, incubation, hatchability, native, lighting program*

THE RELATIONSHIPS BETWEEN SOME EGG CHARACTERISTICS BY DIFFERENT FLOCK AGES IN COTURNIX CHINENSIS, THE CHINESE PAINTED QUAIL

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Abstract

Egg characteristics are used to estimate the weight of the chicks and egg hatchability in the poultry industry. However, it is primarily the subject of basic biological studies in determining population values. In this study, it was aimed to assess the effect of flock age on the egg weight (EW), albumen weight (AW), yolk weight (YW) and eggshell weight (SW) and their ratio to egg (albumen:egg (AE), yolk:egg (YE) eggshell:egg (SE), shape index (SI) and eggshell thickness (ST), and determine the relationships between the egg characteristics of Chinese painted quails. For this purpose, 73 eggs of 18-19.3 weeks old quails (young flock) and 81 eggs of 31-32.3 weeks old quails (old flock) were used in the study. The monogamous quails were housed as 1 male/1 female per cage of 33x40 cm and reared under the same conditions as in the natural enlightenment period and fed with 17% protein and 2750 kcal ME/kg energy. After weighing the freshly laid egg and yolk, the eggshell weight was weighed after drying at room temperature for one day and the albumin weight was found by subtracting the sum of the yolk and shell weights from the egg weight. The eggshell thickness was measured together with the shell membranes and was calculated as the average of the pointed, equatorial, and blunt ends. With quail age, EW (P<0.001), AW (P<0.001), and YW (P<0.001) increased and SI (P=0.002) and SE (P=0.039) decreased, however, SW (P=0.181), AE (P=0.927), YE (P=0.362) and ST (P=0.336) remained unchanged. EW, AW, YW, SW, SI, AE, YE, SE, and ST values in the young and old flock were found as 5.54 and 5.84 g, 3.36 and 3.55 g, 1.72 and 1.82, 0.46 and 0.47 g, 77.43 and 76.31 %, 60.64 and 60.62 %, 30.96 and 31.24 %, 8.40 and 8.14 % and 0.174 and 0.172 mm, respectively. The linear correlations (Pearson-Moment) between some egg characteristics were determined by age of the flock. There were significant positive correlations (P<0.001) between EW and YW (0.790), AW (0.956), and SW (0.582) while the correlations between EW and ST (0.110) and SI (0.102) were insignificant (P=0.356 and 0.390, respectively) in the young flock. The correlations between the SI and YW, SW, AW, and ST were found to be insignificant at 0.021 (P=0.861), 0.022 (P=0.850), 0.134 (P=0.259), and 0.042 (P=0.727). Moreover, there were positive correlations between YW and SW (0.259, 0=0.027) and AW (0.587, P<0.001), while the negative correlation between YW and ST was low (-0.168) and insignificant (P=0.157). Similarly, positive correlations (P<0.001) were found between SW and AW (0.570) and ST (0.733). In addition, there was an insignificant correlation (P<0.222) between AW and ST (0.145). There were significant positive correlations between EW and YW (0.750), AW (0.950), and SW (0.381) (P<0.001), while the negative correlation between EW and SI (-0.332, P=0.002) in the old flock. The correlation between ST and EW (-0.171) was low and not significant (P=0.127), as well as with YW (-0,086, P=0.446). While there was a negative correlation between SI and AW (-0.370, P=0.001), there was a positive correlation between ST (0.341, P=0.002). In addition, the correlation between SI and YW (-0.199) was found to be insignificant (P=0.075). Moreover, significant positive correlations were obtained between YW with SW (0.270, P=0.015) and AW (0.517, P<0.001). There was a significant positive correlation between ST and SW (0.662, P<0.001), while a negative correlation between AW (-0.264, P=0.017). There was a low and insignificant correlation between SW and SI (0.151, P=0.179), while a significant (P=0.014) positive correlation was found between SW and AW (0.273). In addition, the effect of SI, EW, SW, and flock age on ST, which takes relatively more time and requires many measurements, was investigated. For this purpose, a general linear model was used. In order for the model to consist of only significant independent variables, the step-wise method was used according to the 5% significance level. As a result of the analysis, ST = 0.11711 - 0.01331 EW + 0.2817 SW (R2 = 66.73%) equation was obtained.

Key words: *Blue-breasted quail, Egg weight, Shape index, Albumen, Yolk, Eggshell thickness*

PRACTICAL IMPLICATIONS IN OPTIMIZING IN-VITRO ANTIMICROBIAL ASSAYS OF PROBIOTICS AGAINST SALMONELLA STRAINS OF POULTRY: A CASE STUDY

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Abstract

Salmonellosis is a gastrointestinal (GI) infection commonly found in poultry species that can eventually be transmitted to humans leading to food safety issues associated with poultry products. Along with the banning of antimicrobial growth promotors in feed, the use of probiotics is increasingly becoming an alternative approach to control GI infections in poultry. However, the success of the use of probiotics largely depend on the in vitro methods used to screen the probiotics for their antimicrobial potential. This work describes the practical implications and considerations on screening probiotics for antimicrobial activity against Salmonella strains of poultry. In-vitro antimicrobial assays namely: soft agar overlay method, well diffusion method and growth curves with absorption at optical density 600nm, were used to screen Lactic acid bacteria and Bacillus strains for antimicrobial properties against poultry strains of Salmonella. The visibility of inhibition by the probiotic bacterial colony spots overlaid by soft agar with the indicator Salmonella strains largely depends on the type of media use to grow the probiotics and overlay containing the indicator strain. When the BHI agar was used for both base medium (for probiotic bacterial spots) and overlay (with Salmonella strains), given the faster nature of grow, Salmonella can become overgrown in the base media also hindering the visibility of slight inhibitions of probiotics. When MRS agar was used as the base media, the inhibitions were slightly visualized. Well diffusion assays also might have several constraints where the volume of the cell free supernatants (CFS) of probiotics and thickness of the gel can largely affect the visibility of the inhibition. On the other hand, the lack of inhibition might not exactly indicate that there are no bacteriocins produced, where some bacteriocins can be cell associated and lost along with cell pelleting during centrifugation. Therefore, lack of concentration of the bacteriocins in the CFS might be insufficient to visualize a clear inhibition of fast growers like Salmonella strains. Therefore, in such cases, alternative methods of processing probiotic overnight cultures, such as lysis of bacterial cells to release cell associated bacteriocins, precipitation and concentration of proteins in the CFS etc. might be necessary for visualisation. The growth curves of Salmonella strains showed increased growth when treated with CFS of Lactic acid bacteria. As a possible explanation, it can be suggested that CFS of some probiotics may contain metabolites that aid the growth of Salmonella (cross feeding) as opposed to the inhibition expected. Therefore, in such cases, co-culturing of live probiotics with pathogenic Salmonella and selective enumeration of Salmonella could be the best way to realize the accurate effects of probiotics on Salmonella strains. In conclusion, intensive and careful optimization is necessary to overcome practical implications and realize correct antimicrobial properties of probiotics against pathogenic strains of Salmonella in-vitro.

Key words: *Bacteriocins, Growth curve analysis, Pathogenic inhibition, Soft agar overlay assay, Well diffusion assay*

HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF WEST AFRICAN DWARF RAMS FED ENSILED ELEPHANT GRASS AND GMELINA ARBOREA LEAVES

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Abstract

In a study to evaluate the haematological, serum indices and cholesterol levels of rams fed ensiled Elephant grass and Gmelina arborea leaves, 16 WAD rams were randomly assigned to four dietary treatments for 4 weeks. Each treatment was replicated thrice in a Completely Randomized Design (CRD). The data obtained was subjected to analysis of variance (ANOVA) using procedure of SAS (2002) and where the analysis indicated significant treatment effect, the significant means were compared using the least significant different (LSD) method. Water and feed were given ad-libitum. Major parameters measured included: RBC, PCV, WBC, Hb, lymphocytes, neutrophils, monocytes, TP, ALT, AST, albumin, glucose, urea, creatinine, cholesterol, HDL and LDL. The four dietary treatments were 80% Elephant grass+ 20% cassava peels+0% Gamhar (T1), 60% Elephant grass+ 20% cassava peels+20% Gamhar (T2), 20% Elephant grass+ 20% cassava peels+60% Gamhar (T3), and 0% Elephant grass+ 20% cassava peels+80% Gamhar (T4).

With the exception of red blood cell (RBC), white blood cell (WBC), monocytes, creatinine, urea and LDL, all the haematological and biochemical parameters measured were significantly ($p<0.05$) different across the dietary treatments.

It was observed that the ensiled diets offered to the rams did not have deleterious effect on the haematological and serum biochemical indices as the values registered across the dietary treatments fell within the normal ranges. It was concluded that all four test diets were suitable for dry season ram feeding and that Gmelina arborea could be included in ram diets up to 80% without any harmful effect on their haematological and serum biochemical profile.

Key words: *haematology, serum, cholesterol, dietary treatment, West African Dwarf rams*

INTRODUCTION

Small ruminants such as rams play important role in the livestock subsector of the Nigerian agricultural economy (Lakpini *et al*., 2002). The West African dwarf rams are well adapted to the environment i.e. West African humid zone (Gali, 1996) and trypanotolerant (Steele, 1996).

Decline in nutritive value of vegetation resulting from senescence combine to make it difficult for livestock to meet their nutritional requirement during the dry season. Such a situation has long been recognized to result in cyclic body weight gain in the rainy season and weight loss in the dry season (Annor *et al*., 2007). To break this cycle, animal nutritionists have recommended feed supplementation.

However, the use of staple cereal, grains as supplements leads to competition between humans and animals and increases the cost of feed supplementation, making supplementation unprofitable or unsustainable, especially in poor communities.

The need, therefore, exists to find reliable and sustainable sources for feed supplementation with the view to helping to improve the profitability of livestock production during periods of inadequate and/or poor quality herbage supply.

According to Ranjhan, 2001 crop residues (straws and stovers) and agro-industrial by-products will remain important sources of feed for livestock production.

Madubuike and Ekenyem (2006) reported that haematological and serum chemistry assay in livestock could indicate the physiological response of livestock to their nutrition. Esonu et al. (2001) had earlier come to the same conclusion that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environment. The objective of this work was, therefore, to evaluate the nutritional value of four diets formulated from locally available feedstuffs in Ejigbo district of Osun as feed for rams. The test diets were ensiled mixture of elephant grass and cassava peels with different levels of *Gmelina arborea* leaves. West African dwarf rams were used for the dietary testing and the data collected and evaluated included haematological and serum biochemical indices.

Blood is an important index of physiological and pathological changes in an organism (Mitruka and Rawnshey, 1977). The primary function of blood is to transport oxygen from respiratory organs to body cells (Duke, 1975) distributing nutrients and enzymes to cells and carrying away waste products (Slaker and Suverton, 1982) thereby maintaining homeostasis of the internal environment (Bentrick, 1974). The various functions of blood are carried out by the individual and collective actions of its constituents- the haematological and biochemical components (Akinmutimi, 2004).

Haematological tests have been widely used for the diagnosis of various diseases and nutritional status of animal. The information gained from the blood parameters would substantiate the physical examination and together with medical history provide excellent basis for medical judgment (Schalm *et al.*, 1975).

In general, blood examination is performed for several reasons as a screening procedure to assess general health (Jain, 1993). Glucose, cholesterol, calcium, total protein, alkaline phosphates, uric acid, sodium, potassium, chloride levels are diagnostic values for diabetes mellitus, liver disease, hyperparathyroidism, chronic hepatopathy and liver disease, gout, kidney disease, chronic diarrhea and dehydration respectively. It had been reported that biochemical changes as a result of toxins have effects on haematological parameters (Karnish, A. R., 2003).

A quantifiable variation was reported in blood parameters due to management, feeding level, age, sex, health status, method of blood collection, haematological techniques used, diurnal and seasonal variations, ambient temperature and physiological status (excrement, muscular exercise, time of sampling, water balance and transportation. (Kausslish and Arora, 1977; Schalm *et al.*, 1975; Ewuola *et al.*, 2004).

Nutrition, breed, sex, age, reproductive status, environmental factors, stress and transportation are known to affect haematological and biochemical indices (Balikei *et al.* 2007) and thought to play major roles in the differences in haematological and biochemical parameters (Opara and Fagbemi, 2009).

These differences have further underlined the need to establish appropriate physiological baseline values for various breeds of livestock in Nigeria, which could help in the realistic evaluation of the management practices, nutrition and diagnosis of their health condition (Opara *et al.* 2010).

JUSTIFICATION

Gmelina arborea and Elephant grass are widely available as forage plants in the Tropics. So many works have been done on the use of these forage plants. However there is paucity information on the effect of ensiling these forage materials on the blood profile of WAD rams.

OBJECTIVES

BROAD OBJECTIVE

This study is an attempt to come up with normal haematological and biochemical reference values in West African Dwarf rams raised under intensive management system as influenced by feed. This study will be undertaken to evaluate the haematological and serum biochemical indices of West African dwarf rams ensiled Elephant grass and *Gmelina arborea* leaves.

SPECIFIC OBJECTIVES:

- To evaluate the blood parameters (Full Blood Count FBC).
- To come up with the normal serum biochemical reference indices.
- To determine the effect of ensiled *Gmelina* leaves and elephant grass diet in the haematological and serum biochemical indices.

MATERIALS AND METHODS

Sixteen West African dwarf (WAD) rams of $1^{1/2}$ years from the rural settlers with an average live weight of 20-30kg. The animals were housed intensively in well-ventilated pens, in an open-sided house with corrugated aluminium roofing sheet and a concrete floor, which was washed, disinfected with Izal and covered with bedding material (wood shavings) before the arrival of the animals. The rams were given prophylactic treatments which consist of intramuscular application of oxytetracycline at the dosage of 1ml/10kg body weight of the animal. Fresh water was supplied ad-libitum.

Before the commencement of the experiment, the animals were left for a week to acclimatize to the new environment; the experimental units were treated against ectoparasites with 0.5ml/10kg body weight of Ivermectin.

Three test ingredients were used which are *Gmelina* leaves, Elephant grass and cassava peels.

The ensiled mixture of Elephant grass, *Gmelina* leaves and Cassava peel was done at the following ratio T1 (80:0:20%), T2 (60:20:20%), T3 (20:60:20%), T4 (0:80:20%)

xperimental units were subjected to the diets using a complete randomized design.

Blood samples collected from the animals via jugular vein puncture using syringes. One set of the blood samples (5ml) were collected into

plastic tubes containing the anti-coagulant Ethylene Diamine Tetra acetic Acid(EDTA) for the determination of hematological parameters i.e. the analysis for packed cell volume(PCV), hemoglobin(Hb), white blood cells(WBC), red blood cells(RBC),lymphocytes, neutrophils and monocytes. The other set of blood samples (5ml) were collected into anti-coagulant free plastic tubes and allowed to coagulate at room temperature for subsequent biochemical analysis: serum protein, serum glucose, serum albumin, creatinine, urea, serum alanine transaminase (ALT) and serum aspartate transaminase (AST).

The blood cholesterol levels were also analyzed including the HDL (High Density Lipoprotein) and the Low Density Lipoprotein (LDL).

Blood samples were taken from the rams before feeding via the jugular vein puncture between 07:00h and 09:30 h local time at the last day of each experimental period for hematological and blood biochemical assays. The blood samples were taken to the laboratory soon after collection in a sample holder placed in an ice chest. Two different test tubes were used to harvest blood from each of the rams. A plain test tube was used to collect blood to obtain serum for the determination of blood glucose, total protein, albumin, urea, creatinine, AST and ALT. The other test tube, which contained Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant, was used to analyze for Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), White Blood Cell (WBC) count, RBC, lymphocyte, neutrophil and monocytes.

The packed cell volume percentages were measured for each blood sample in fresh ethylene diamine tetra acetic acid (EDTA) anticoagulant samples within 24hours of collection using the micro-hematocrit method. Hemoglobin concentration was also measured in fresh EDTA anticoagulant samples using the Sahl's (acid hematin) method (Benjamin 1978). RBC was measured in fresh EDTA with the aid of Neubaur counting chamber (hemocytometer). Blood smears were used for total WBC counts (Tavares-Dias *et. al.* 2008). Differential relative and absolute counts were classified as lymphocytes, neutrophils and monocytes.

Plasma glucose was measured using the enzymatic glucose oxidase method (Bauer *et. al.* 1974). Total serum protein was measured in serum for individual animal using the biuret method. Serum alanine transaminase and serum aspartate transaminase was analyzed spectrophotometrically by using commercially available diagnostic kits (Randovl Test Kits). Serum creatinine was determined using the principle of Jaffe reaction as described by Bousnes and Taussky (1945).

Resulting hematological and biochemical data obtained from the samples was laid out as Completely Randomized Design and analyzed with one-way Analysis of Variance (ANOVA) using procedure of SAS (2002). The
significant means were compared using the least significant different (LSD) method.

RESULTS AND DISCUSSION

The reference ranges of values were reported by Oyeyemi M.O. and Ajani O.S. (2014) for West Africa Dwarf (WAD) rams of 18–24 months, which weighed 20–25kg and stated to be within normal range. The observed hematological values shows that except for PCV, Hb, lymphocyte and monocytes where the mean values between the four diets significantly ($P<0.05$) differs, although the WBC count is not significant, the means on the same rows differs. All the other hematological parameters did not.

Table 1. Haematological parameters of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	Reference values	T1	T2	T3	T4	SEM
RBC (10^6/mm^3)	9-15	9.83	9.62	9.94	10.30	1.47
PCV (%)	27-45	28.91	30.05	31.92^b	32.46^a	3.92***
WBC (10^6/mm^3)	4-12	6.88^b	7.53^a	7.02^a	7.75^a	0.80
Hb (g/dl)	9-15	8.79^c	10.01^b	10.69^b	11.71^a	3.63**
Lymphocytes (%)	40-75	53.63^c	54.05^c	55.86^b	57.01^a	4.90*
Neutrophils (%)	10-50	35.76^c	37.58^b	37.51^b	38.41^a	4.56**
Monocytes (%)	7-9	2.07	2.04	2.02	2.05	0.49

$^{a, b, c}$: Means on the same row with different superscript are significantly ($p<0.05$) different.*$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; SEM: standard error of mean

RBC=Red Blood Cell, PCV=Packed Cell Volume, WBC=White Blood Cell, Hb=Haemoglobin.

Mean PCV is highly significant $P\leq0.001$ and has the highest mean value in T4 and least in T1. However, these values were within the range of 21-35% reported for WAD goats by Daramola *et.al.* (2005). In contrast, Taiwo and Ogunsanmi (2003) reported higher values 35.5% and 36.9% for clinically healthy sheep and goat respectively. The implication of this observed PCV values,

going by the reports of Dargie and Allonby (1975), is that only the rams on T4 diet could probably have the high tendency for a return of PCV to normal value following an infection through compensatory accelerated production. This is in view of the fact that only the rams on this diet had values above the 32% PCV documented to be normal for circulatory system in sheep (Frandson, 1974).

The Hb concentration in the blood of the studied rams showed a similar pattern of variation as with PCV. Nevertheless, the Hb range in this study was similar to the mean value for goats fed *Prosopis juliflora* by Misri *et al.* (2000) and fell within the range of 7-15g/dL reported by Daramola *et al.* (2005). However, higher in T4 than the value of 11.40g/dL reported for Red Sokoto goats (Tambuwal *et al.* 2002) and in cattle fed different levels of extracted rice bran (Singh *et al.* 2002).

With the relatively higher Hb concentration observed in this study, the dietary treatments generally seems to be capable of supporting high oxygen carrying capacity blood in rams.

The values obtained in this study for lymphocytes and neutrophils fell within the broad range of 47-82% and 51.6% reported by Daramola *et al.* (2005) and Tambuwal *et al.* (2002) and 36.4% for lymphocytes and 17-52% neutrophils reported by the same authors respectively. These values are suggestive of a well-developed immune system in the WAD rams with such number of immune cells to profer good health (Daramola *et al.* 2005). The result also implies that an increase in lymphocytes is associated with a decrease in neutrophils and vice versa (Lazzaro 2001).

WBC count obtained in this study at the end of the experiment though not significant increases across the dietary treatment row compared favorably with values within the range of 6.8-20.1*10^6/mm^3 reported by Daramola *et al.* 2005. WAD rams seem to possess a protective system providing a rapid and potent defense against any infectious agent, and this is probably the physiological basis for the adaptation of these species in their ecological zone (Daramola *et al.* 2005).

Table 2. Serum biochemical indices of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameters	T1	T2	T3	T4	SEM
TP (g/l)	6.32[b]	6.38[b]	7.04[a]	7.22[a]	1.52**
AST (UI/l)	77.37[c]	79.48[b]	81.62[b]	83.92[a]	5.30**
ALT (UI/l)	27.94[b]	28.16[b]	29.20[a]	29.42[a]	2.49***
Albumin (g/l)	2.65[b]	2.59b	3.22[a]	3.29[a]	0.11**
Creatinine (mg/dl)	1.24	1.03	1.24	1.24	0.05
Glucose (mg/dl)	59.01[a]	61.36[a]	54.99[b]	52.81[b]	7.92***
Urea (mg/dl)	8.93	9.65	10.54	10.42	2.30

[a, b, c]: Means on the same row with different superscript are significantly ($p<0.05$) different.*$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; SEM: standard error of mean.

TP=Total Protein; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase

According to Otesile *et al.* (1991); serum biochemistry is a generalized medium of assessing the health status of animals.

Variations in the biochemical indices of the WAD rams placed on different dietary treatments are shown in Table 2.

Aside from the values of urea and creatinine differences between the measured biochemical parameters were not significantly ($P\geq0.05$) different between the diets. ALT and glucose values were highly significantly ($P\leq0.001$) different between the dietary treatments.

Aside from the values of urea and creatinine differences between the measured biochemical parameters were not significantly ($P\geq0.05$) different between the diets. ALT and glucose values were highly significantly ($P\leq0.001$) different between the dietary treatments.

Serum proteins are important in osmotic regulation, immunity and transport of several substances in the animal body (Jain, 1986). However, in this experiment, the dietary treatments differ more significantly in terms of their Total Protein levels in the serum of the rams. Besides, the statistical more significant ($P\leq0.01$) difference between the diets may be related to the findings of Tewe and Maner (1980) that serum protein is not related to the amount of calories contained in the diet but to the availability of protein.

Urea and creatinine levels did not differ significantly between the diets in this study. However, compared to values reported for apparently healthy goats (Tanwar *et al.*, 2000). This study reports high serum urea values across the diets. This may probably have been due to persistent hypoglycemia since according to Radostits *et al.,* (1994) catabolic activity is increased for gluconeogenesis, thus resulting in higher serum urea levels.

Enzymes are protein catalysts present mostly in living cells and are constantly and rapidly degraded although, renewed by new synthesis (Coles, 1986). According to Zilva and Pannall (1984), normal enzyme level in serum is a reflection of a balance between synthesis and their release, as a result of the different physiological processes in the body.

Transaminase enzymes are those mostly responsible for the synthesis of non-essential amino acids through the process known as transamination according to Carola *et al,* (1990).

According to Keele and Neil (1971), serum levels of AST are significantly high under and morbid conditions involving injuries to large numbers of metabolically active cells. However, the result of this study suggests a contrary situation in this regard thus indicating the potential of the studied plant leaves in the feeding of rams.

The monitored activities of transaminases enzymes did not vary widely between the diets. The relatively close mean values observed for transaminases could be an indication that the test diets did not differ in their effects on enzyme secretion mechanism.

Table 3. Cholesterol level of WAD rams fed ensiled Elephant grass and *Gmelina arborea* leaves

Parameter	T1	T2	T3	T4	SEM
Cholesterol (mg/dL)	63.06^{b}	64.33^{b}	68.88^{a}	69.93^{a}	4.41***
HDL (mg/dL)	50.58^{c}	54.13^{b}	54.50^{ab}	55.43	2.56***
LDL (mg/dL)	8.13	7.35	7.73	7.04	2.59

$^{a, b, c}$: Means on the same row with different superscript are significantly ($p<0.05$) different.*P≤0.05; **P≤0.01; ***P≤0.001; SEM: standard error of mean

HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein

The resulting cholesterol values shows that the cholesterol and HDL mean values between the dietary treatments significantly differ ($P<0.05$) while the LDL values did not.

Mean HDL was highest in diet 4 and lowest in diet 1, however, the implication of the observed values by reports is that there is evidence for a protective effect for dietary fibre against arthreosclerosis; a disease of the heart through an increase in the low-density lipoprotein and colon cancer probably through an increased rate of passage of feed residues through the gastro-intestinal tract.

Though, the increase in plasma concentration of HDL cholesterol may be due to the effect of polyphenols, which are involved in the regulation of lipid and glucose metabolism. According to some authors (Norata *et al,* 2003; Bursill and Roach, 2007), this activates the PPAR-α receptor, with an increased stimulation effect in the liver of the expression of key proteins involved in the metabolism of HDL. It was reported that cholesterol concentration is influenced by the degree of stress (Shaffer *et al,* 1981).

CONCLUSIONS

All the haematological and biochemical parameters of WAD rams from this study fell within the normal range of values.

It can be concluded that the ensiled Elephant grass and *Gmelina arborea* leaves can be used as dry season feed for WAD rams without any negative effect on the health status of the animals.

More studies should be carried on the ensiled Elephant grass with *Gmelina arborea* using other ruminant animals such as goat and cattle.

ACKNOWLEDGEMENTS

This work was supported by the University of Ibadan, Animal Science Department.

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A COMPARISON OF SOME FERTILITY TRAITS OF TURKISH HAIR GOATS AND GROWTH CHARACTERISTICS OF KIDS RAISED UNDER BREEDER CONDITIONS BETWEEN BURDUR AND MUĞLA PROVINCES

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Abstract

Study was conducted to compare some fertility traits and the growth characteristics of Turkish Hair goats reared in different conditions. The data of the flocks (concerning totally 10.140 kidding does and 10.702 kids) were examined in the year 2020 of within the scope of the "Project of the improvement of Turkish Hair goat in Breeder conditions in Burdur and Muğla provinces". The effects of factors on growth performance were analyzed by using the analysis of variance, generalized linear model (GLM) procedure. In the study, the birth rate, kid rate and litter size values of Turkish Hair does for Burdur and Muğla provinces were detected as 89.51%, 0.96, 1.07 and 85%, 0.88, 1.04, respectively. Survival rates of the kids until the 90th day of age were 97.36% and 93.33%, respectively. Average birth and live weights of kids on 60th and 90th day of age were detected as 3.38 kg, 13.76 kg and 18.86 kg for Burdur province. The same values were 3.24 kg, 11.09 kg and 14.97 kg for Muğla province. Differences between provinces were found statistically significant (P<0.05). While differences between male and female kids were statistically significant (P<0.05), dam age and birth type had a significant effect (P<0.05) on all the examined growth periods, too. The superiority in favor of Turkish Hair kids especially for survival rate and live weight at weaning for Burdur province are remarkable. It can be thought that the altitude, climate and flora can be count in effective factors on adaptive capacity and growth performance of goats. Additionally, this study has revealed that it is needed to be examine fertility traits of goats and growth performances of kids in more detail by compairing different Turkish Hair goat flocks in other provinces within the scope of "National Sheep and Goat Breeding Project of Turkey.

Key words: *Fertility, Growth, Turkish Hair goat, Burdur, Muğla*

STUDY OF SOME WOOL CHARACTERISTICS IN ZANDI SHEEP BREED

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Abstract

The aim was to study some characteristics on Zandi sheeps breed and the effect of various factors on wool quality including sex, year of birth, birth month, type of birth and the year of sampling.Samples were taken from 105 Zandi sheep during two years. Then, in the wool and skin lab tech, a number of traits were evaluated on wool samples. The analysis were carried out with SAS Statistical software. Phenotypic correlation between characteristics and pearson correlation coefficient were calculated for wool characteristics. Furthermore the regression between compression and resilience, as new traits of Zandi sheep, were examined for wool characteristics and linear pattern was obtained for compression. The mean and standard deviation for staple length (SL), mean fiber diameter (MFD), standard deviation of fiber diameter (SDF), coefficient of variation of fibre diameter (FDCV), sampling variance (SV), kemp fiber (KF), hair fiber (HF), colourful fiber (CF), fiber less than or equal to 30 microns, compression, resilience and medulation fiber (MF) were equal to 6.32 ± 1.28 cm, 31.36 ± 2.76 µ, 10.43 ± 2.02, 32.95 ± 6.05 %, 113.05 ± 43.3, 0.68 ± 0.96 %, 1.63 ± 2.71 %, 45.24 ± 26.68 %, 49.04 ± 12.73 %, 37.56 ± 7.05%, 92.81 ± 3.77 %, 47.34 ± 26.51 %, respectively. The variance analysis was carried out for year of birth, birth month, sex, type of birth and the year of sampling on above mantioned wool characteristics and between them, The effect year of sampling on compression and the effect of sex on SL was significant. The type of birth had no significant effect on any of the wool characteristics. The correlation coefficient between fiber less than or equal to 30 microns with fiber diameter was -0.86. The correlation coefficient between staple length with fiber diameter, variance, standard deviation and fiber less than or equal to 30 microns and was calculated as 0.35, 0.24, 0.26 and -26.0 respectively. Correlation coefficient between compression with fiber diameter and coefficient of variation of fibre diameter were calculated as -0.22 and 0.26, respectively. Regression between compressions with fiber diameter was 0.01. The results indicated that Zandi sheep wool has some useful characteristics to use in sheep breeding programs in comparison with other native sheep breeds.

Key words: *Sheep, Wool, Diameter, Carpet wool, Local breeds*

EFFECT OF HEAT STRESS ON HEIFERS

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Abstract

The optimum ambient temperature for dairy cattle is between 13 and 18 °C and the relative humidity is between 60 and 70%. If the ambient temperature rises above these levels and is accompanied by high humidity, it limits the heat dissipation and the body temperature starts to increase and the cattle get stressed. Heat stress (HS) is a big welfare problem due to its negative effects on livestock's metabolic and reproduction. Heat stress occurs when the animal body temperature increases and cannot dissipate. When animals faced the problems some mechanisms activate and animals try to combat regulate body temperature. Although heifers are less body weight comparatively heat resistant due to less production of metabolic heat and more heat dissipation efficiency, they still suffer from heat stress to some degree. During HS some metabolik and physiologic changes occur in the animal body and heifers decrease dry matter intake and growth ratio, increase respiration, panting, loss of CO_2, respiratory alkalosis, alter blood acid-base balance, elevation of insulin in blood and protein catabolism. On the other hand, decrease in the rumen microbiota and alteration in rumen motility, feed digestibility and rumen fermentation. Also, HS may cause negative effects on luteinizing, estradiol and gonadotrophins hormones' secretion, so, its follow bad estrus cycle, depress follicular development and decrease conception rate. Due to HS yearly economic loses in the USA between $1.69 and $2.36 billion. In order to prevent the losses caused by HS, it is necessary to regulate ventilation, shadow, spray water and well air movement in barn, and feed additives use in the ration to reduce the effect of it.

Key words: *heifer, heat stress, sustainability, reproduction, economic loses*

STUDY ON APICULTURE DEVELOPMENT IN SOUTHERN ETHIOPIA

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Abstract

In the districts, despite the presence of different constraints and challenges, there are high potential and opportunities to maximize the output of resources to improve the livelihoods of the communities in a sustainable way. The study indicated the existence of a lot of indigenous knowledge about practicing beekeeping, which might differ from a beekeeper to a beekeeper and also from one location to another location, mostly depending on the beekeepers' experience. The majority (82.86%) of the beekeepers started beekeeping by catching bee swarms, while the remaining by getting bee colonies as a gift from parents and both catching swarms and gifts from parents (17.14%). It has been necessary intervening to change the very old and unimportant traditional beekeeping practices through adopting improved technologies and management practices and practical skill trainings.

Key words: *apiculture, honey production, development, Southern Ethiopia*

INTRODUCTION

Beekeeping is an important sustainable and alternative source of income in rural areas, benefiting communities living in and around forests. Beekeeping can also be a practical tool for raising the awareness of these communities for better management of their forests and for stimulating their conservation, thereby improving their biodiversity.

Ethiopia has a huge natural resource base for honey production and other hive products, and beekeeping is traditionally a well-established household activity in all parts of the country.

Beekeeping is an environmentally sustainable activity that can be integrated with agricultural practices like crop production, animal husbandry, horticultural crops and conservation of natural resources. Thus, it would be one of the most important intervention areas for sustainable development of poor countries, like Ethiopia (Gibbon, 2001). The contributions of beekeeping in poverty reduction, sustainable development and conservation of natural resources have been well recognized and emphasized by the government of Ethiopia and non-governmental organizations (NGOs).

MATERIALS AND METHODS

Description of study area

The study was conducted in Ana Sora District of Oromia Regional state, the southern part of Ethiopia. The areas have a highland climate with an altitudinal range of 1500 - 2600 meters above sea level. The main rainy season extends from the end of March to the beginning of November. The dry season covers from the end of November to the beginning of March.

Sampling techniques, data collection and statistical analysis

Five representative Peasant Associations (PA) were selected using random sampling techniques. Then, 105 beekeepers were randomly selected based on the proportion of the number of possessed beehives. Besides, questions were prepared and key informants were interviewed. The primary data was collected from sampled respondents through an open questionnaire method and formal discussion. A single-visit-multiple-subject formal survey method was employed to collect data on various aspects of beekeeping production and opportunities and constraints.

In this study, relevant statistical methods, which include tables and descriptive techniques, were used to present the data. The collected data was coded and grouped for analysis. Descriptive statistics were computed using the Statistical Packages for Social Sciences (SPSS) 21.

RESULTS AND DISCUSSION

The survey result indicated that 96.2% of the beekeepers started beekeeping by catching bee swarms, while the remaining by getting bee colonies through gifts from parents and both catching swarms and gifts from parents 3.8% . This finding agrees with Tessega (2009) and Chala (2010) reports that the majority of

beekeepers initiated beekeeping through swarm catching in Burie district of Amhara region and Gomma district of Oromia Region, respectively.

Ana Sora is endowed with a diversity of plant habitat, climate, altitude and rainfall. As a result, quite a large number of bee colonies exist in the district. Because of the diversity of plant habitat and environmental conditions, distribution and flowering times vary.

Table 1. Source of bee colonies

Variables	Frequency	Percent
gift from parent	18	17.14
catching swarm	87	82.86
Total	105	100.0

Table 2. Experiences of beekeepers

Variables	Frequency	Percent (%)
1-10 years	13	13.3
10-15 years	15	15.3
15-20 years	24	24.5
20-25 years	28	28.6
>25 years	25	25.04
Total	105	100.0

HONEY PRODUCTION TRENDS

The frequency and amount of honey harvested varies depending on the flowering condition of major bee forage, colony management practices and number of beehives, and there are two honey harvesting periods; March to April and July to August, which depends on the nature of the yearly rainfall and the floral calendar. During honey harvesting from traditional hives, beekeepers cut and pull the fixed combs one by one. In the case of movable frame hives, beekeepers indicate that during honey harvesting, frames are removed from the boxes and uncapped with the honey fork, then placed in an extractor with the help of a development agent and spun so the honey can drip to the bottom of the tank, pass through a sieve and then collected into a storage tank. (Workneh Abebe, 2011).

Table 3. Honey harvesting frequencies

Frequence of honey harvesting	Frequency	Percent
one per year	23	21.9
two times	82	78.1
Total	105	100.0

AVERAGE OF HONEY PRODUCTION/HIVE

According to the survey results, the types of hives found in the study area were traditional, transitional and modern hives and the amount of honey produced from one traditional, transitional and modern hives vary between 5-8 kg, 8-13kg and 15-22kg, respectively, and about 98.09% of respondents, about 5-6 kg of honey is harvested per traditional hive. This result agrees with the report of BoA (2004), 5-6 kg and Nuru (2007), 5-8 kg, respectively, from local hive per colony per annum. In the case of yields (level), about 26.66% of beekeepers replied that honey yield in the district of all 3 types of beehives is increasing (becoming high) over the years, 20.31 % of respondents replied that honey yield in the district varies the all 3 types of beehives (medium) from time to time and the rest 16.19% respond low all 3 types of beehives. In the study area, honey harvesting takes place at night to avoid the aggressiveness of the honeybee during daylight. As much as possible, the respondents used materials during honey harvesting for modern and transitional hives. But in the case of traditional hives, they did not use any materials, rather than smokers and local honey containers.

Inspection of (hives) honeybee colonies

As the result indicates that PA, more or less, undertake external hive inspection. This indicates that most beekeepers don't visit monthly and inspect their beehives externally, but, they do not inspect internally seasonally unless to check either the hive was filled with honey or not. However, internal hive inspection was limited to those honeybee colonies placed in the backyard due to the use of traditional hives. This result agrees with different previous researchers (Kerealem, 2005; Tesfaye and Tesfaye, 2007; Chala, 2010) who reported that farmers in Ethiopia do not commonly practice internal hive inspection due to the difficulty of the traditional hives for internal inspection, i.e., fixed combs attached to the body of traditional beehive.

Types of honey produced from the study area

Honey is the only most common output that is produced in apiculture farming by small scale farmers in districts of Ana Sora. The farmers in the study area produce and provide honey for the market for their daily income gain. The most available honey colors produced and marketed in the study area were yellow/red, white and somewhat black. Among the colors of honey, the most available colors were yellow/red and the second one was white for small-scale farmers. As most potential production seasons are two, the color of honey produced is also two. What makes the color of honey produced different was the available honey bee flora, season of harvesting and duration of storage in the homes of farmers. The white colored honey is collected during the tree flowering seasons of Schefflera abyssinica from the end of March to the beginning of May (the also called mono flora honey which is very preferred by consumers). The red/yellow colored

honey is collected during the flowering season of vast weeds and tree species like Syzygium, Croton macrostachyus, Eucalyptus tree etc. The black colored honey is especially harvested from Vernonia tree species. The other type of honey which exists in the district is honey which is prepared from stingless honey bees is called locally "Tazma Mar" (honeydew honey).

Opportunities and Constraints of the honey production system in the study area

Opportunities

1. Availability of feeds and water

The District is very special for its diversified shrubs spp. and there are also different kinds of forage trees which flower at different times of the year, which assures a constant supply of feed for bees and enough water supplies in the district.

2. Availability of strong colonies and good yield

During the survey, it was noted that there are a lot of beekeepers in the district with good numbers of traditional beehives (30-140 per household full of strong bee families and modern hives (1-6/household) with some of them being full of colonies, indicating that the area is very suitable for bee business development with poor awareness and adoption of modern technology.

3. Diversity and seasonal availability of bee forages

There are also areas allocated for forest development and soil conservation. In addition, many cultivated crops and coffee production in the area also serve as pollen, nectar, or both pollen and nectar sources. Thus, integration of Apiculture development in the agricultural production system has a huge advantage into pollination, agricultural production and the beekeeping system.

4. Traditional knowledge and Experience

In the study area, beekeeping practice has a long history. As a fact, the beekeepers have developed indigenous knowledge which was passed from generation to generation. The main areas of indigenous beekeeping knowledge are hive construction from locally available materials, especially log hives, swarm catching; hive fumigation, honey and swarming season identification, different medicinal values of honey, identification of important honeybee floras and identification of adulterated honey. This familiarity and pride with beekeeping can support rapid uptake among additional beneficiaries.

This opportunity will give a chance to get support to alleviate major constraints hindering apiculture development in the area.

CONCLUSIONS

Generally, the area has great potential for beekeeping and the majority of households keep bees. Beekeeping contributes to more than 25% of the total household income of the majority of the rural communities in the area. In the districts, despite the presence of different constraints and challenges, there are high potentials and opportunities to maximize the potential of the resource to improve the livelihoods of the communities in a sustainable way. Different tribes have different well developed indigenous knowledge on how to harvest honey successfully without discarding colonies. Some also know how to protect their bees from ants' attack using biological control.

Therefore, it requires intervening to change the very old and unimportant traditional beekeeping practices through adopting improved technologies and management practices and practical skill training.

ACKNOWLEDGEMENTS

Special thanks and acknowledgment of gratitude and appreciation is directed to Committee of International Congress on Domestic Animal Breeding, Genetics and Husbandry - 2022 (ICABGEH-22)

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AN EMERGING PATHOGEN IN THE CANINE INFECTIOUS RESPIRATORY DISEASE (CIRD): CANINE RESPIRATORY CORONAVIRUS (CRCOV)

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Abstract

The betacoronaviruses are currently divided into four lineages (that is, A–D). Lineage A includes human coronavirus (HCoV-HKU1, -OC43), equine coronavirus (ECoV), murine hepatit virus (MHV), porcine hemagglutinating encephalomyelitis virus (PHEV), bovine coronavirus (BCoV), and canine respiratory coronavirus (CRCoV). This lineage has a unique gene that encodes a surface hemagglutinin-esterase (HE) protein that is not present in other coronaviruses. An important secondary receptor for infection, the betacoronavirus lineage A surface HE protein contributes to novel genotypes of the virus with varying host specificity and tissue tropism. Twenty nasal swabs submitted to our laboratory for testing between 2019 and 2021, from private veterinary clinics and animal hospitals were used in this study. The HE gene was targeted using specific primers in dogs with respiratory problems. RT-PCR was used to determine the CRCoV surface HE protein gene (497 bp). 3/20 (15%) of the nasal swab were found positive for the targeted HE gene of CRCoV. Two new findings emerged from our study. In Turkey, CRCoV infection was discovered in nasal swabs of sampling dogs. Though it was reported in 2007 by Erles et al. that CRCoV alone induced moderate respiratory symptoms, in our study, anamnesis revealed three prevalent symptoms: runny noses, coughing, and wheezing in the dogs. For more detail of CRCoV infection should be detected to be done further studies supported by molecular characterizations.

Key words: *betacoronaviruses, canine, hemagglutinin-esterase, RT-PCR, CIRD*

A SURVEY ON HARD TICK FAUNA AND SEASONAL CHANGES IN THEIR POPULATION IN GOATS FROM KHOY, IRAN

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Abstract

External parasites, especially ticks, can lead to stress, anemia and paralysis in livestock. The goal of this study is assessment of goat contaminations development to difference tick family and also determination of found tick species diversity in Khoy city and villages around it. In this study, 462 goats from Khoy city and four regions of north, south, east and west were studied and and the samples were analyzed in the parasitology laboratory of Islamic Azad university branch urmia. A total of 58 ticks were identified and collected during the study. In this survey, the infection rate in goat is 2/59% (P<0.05). Rhipicephalus bursa with 31 numbers (53/45%) determined as maximum contamination and Haemaphysalis punctate with 3 numbers (5/17%) determined as minimum contamination. The highest level of infection was recorded in June with 33/33% and the lowest level of infection was recorded in December with 0% (P<0.05). The highest level of infection was observed on the breast of livestock and the lowest level of infection was observed on the testicles of livestock. The population of ticks increases in hot conditions as well as sufficient humidity, but in general, as in this study, in line with previous studies, in recent years, due to greater awareness of farmers and the effective use of poisons, the rate of tick infection is declining in Khoy city.

Key words: *Hard Tick, Goat, Khoy, Iran.*

INVESTIGATE THE PREVALENCE OF SARCOCYSTIS IN SLAUGHTERED GOATS BY MICROSCOPIC METHOD IN KHOY، IRAN

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Abstract

Sarcocystis is an obligate intracellular parasitic protozoa that can cause digestive disorders in goats and cause high financial losses in the livestock industry. The aim of this study is to determine the prevalence of sarcocystis infection in slaughtered goats in slaughterhouses of Khoy, Iran using microscopic method and comparison with macroscopic method. In this study, carcasses of 202 slaughtered goats were examined for macrocyst with ocular observation and then microscopically. According to the results, 1.96% of the goats were macrocyst-infected. Data analysis showed a statistically significant difference between the rate of infection in different age groups and the rate of infection increased with age ($p<0.05$). However, the rate of contamination was independent of sex and there was no significant difference between the rate of contamination of different sexes. Also, there was a significant difference between the rate of infection in different muscles ($p<0.05$) and the highest microcyst in skeletal muscles with 84.78% and the lowest microcyst in cardiac muscles with 28.26%. This study showed that the microscopic method is preferred in determining the rate of sarcocyst contamination compared to the ocular method, so it is necessary to be careful about determining sarcocytic contamination in carcasses of slaughtered goats.

Key words: *Sarcocystis, the microscopic method, macrocyst, goat.*

INVESTIGATION OF MALE GENITAL SYSTEM ANATOMY IN THE NEW ZEALAND RABBIT (*Oryctolagus cuniculus L.*)

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Abstract

Rabbits are frequently used in research because of their human-like biological structure. In the present study, 10 male rabbits were prefered. Arteries vascularization of the genital system organs were dissected. Morphometric and macroanatomical results of these organs were taken and some organs were examined by magnetic resonance imaging (MRI). In this study right testis length was measured 67.24±5.36 mm and left testis length was 62.25±6.55 mm in New Zealand rabbits. Testicular weight was 10.21±1.17 g on the right and 9.90±1.15 g on the left. Right caput epididymidis 0.68±0.08 gr, left caput epididymidis 0.80±0.13 gr; right corpus epididymidis 0.14±0.03 left corpus epididymidis 0.15±0.03 g; right cauda epididymidis 1.87±0.26 left cauda epididymidis 1.90±0.31 gr. In the present study, right ductus deferens weight was 0.30±0.29 g, left ductus deferens weight was 0.25±0.03 g; The ampulla ductus deferentis weight was 0.35±0.16 g on the right side and 0.37±0.16 g on the left side. The length of the glandula vesicularis was calculated 18.54±0.35 mm on the right and 17.55±0.59 mm on the left. The width of the glandula vesicularis was measured as 16.54±0.28 mm on the right and 16.70±0.45 mm on the left. Glandula vesicularis weight was 0.91 ± 0.07 g on the right side and 0.96 ± 0.09 g on the left side. Prostate length was recorded as 11.68±2.01 mm and width as 13.02±1.38 mm. The length of the glandula bulbourethralis was 13.36±2.00 mm on the right side and 12.39±1.21 mm on the left. Its width was recorded as 6.73±0.98 mm on the right and 5.80±0.90 mm on the left. Respectively, it weighed 0.40±0.20 g on the right and 0.44±0.24 g on the left. The prostate consisted of three parts in rabbits: the prostate, the corpus prostate, and the paraprostate. Glandula bulbourethralis was a green lentil-sized gland found in pairs. It is thought that the obtained results will contribute to the scientific researches on male genital system in laboratory animals and other animal species, artificial insemination studies, reproductive system diseases and operations in rabbits

Key words: *anatomy, male genital system, MRI, New Zealand rabbit*

INTRODUCTION

Rabbit, which is in the Leporidae family, is important in terms of easy availability as a laboratory animal and the benefit it provides in research. Meat is also used. Rabbit breeding is widely practiced for reasons such as giving a large number of offspring in a short time, ease of care and use as food (Anonymous, 1). Male genital system is responsible for the production, maturation, storage of spermatozoa and the transfer of semen to the female vagina by ejaculation. This system also helps the urinary system as the urethra carries both semen and urine (Dyce et al., 2010). Male genital organs are divided into two parts as male internal genitalia (partes genitales masculina internae) and male external genitalia (partes genitales masculinae externae). Partes genitales masculinae internae consists of the testis producing spermatozoa, the male gamete cell, the epididymis where the produced spermatozoa mature and stored, the ductus deferens, which carries spermatozoa to the urethra, and the male attachment glands that produce semen for the formation of sperm. Partes genitales masculinae externae is consists of the penis, which is the mating organ, the urethra masculina, which is responsible for the transport of semen in the penis, and the preputium that covers the penis (König and Liebich, 2015). There are studies on male genital tract organs in different animal species (Alsafy et al., 2021; Baygeldi et al., 2021; Erdoğan, 2011; Harem et al., 2019; Kırbaş Doğan et al., 2021; Pe´rez et al., 2013). There is also literature on the subject studied. However, this study was designed due to the lack of information in the literature and the unidirectional nature of the studies.

In this study, it was aimed to examine the male genital system anatomy in detail in the New Zealand rabbit. The arteriel supplying the male genital tract organs were dissected. Morphometric and macroanatomical findings of these organs were taken and some organs were examined by magnetic resonance imaging (MRI).

MATERIALS AND METHODS

The permission of this study was applied to Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK). Our study was approved with the code of KAU-HADYEK/2021-068. The materials (Figure 1) obtained from Atatürk University Medical Experimental Application and Research Center, which has an official certificate on producing and selling experimental animals, were brought to the Anatomy Department laboratory of Kafkas University Veterinary Faculty and the study was carried out here. The study was carried out on 10 male rabbits, 7 months old, weighing 2862.10 ±73.90 g on average. Two of the rabbits were injected with latex to identify the arteries. After anesthesia, two rabbits were examined with an MRI device. Eight rabbits were used for morphological and macroanatomical results.

Figure 1: Research material

Animal materials were anesthetized intramuscularly (i.m.) with a combination of 5mg Xylazine HCL (rompun) and 35 mg Ketamine (Ketalar) (Holmes 1988). The materials were transferred to the Laboratory of the Anatomy Department of the Faculty of Veterinary Medicine of Kafkas University and the procedures to be applied were carried out here. Before starting the dissection process, some biometric data (body weight) of all animals were determined. The male genital system of the euthanized animals was examined by radiological imaging (MRI). Magnetic resonance imaging was performed with Siemens Essenza brand 1.5 tesla magnetic resonance device. Rabbits were examined in 4 mm thickness in T2W weighted sagittal (TR:4000 TE:87), T2W weighted coronal (TR:6230 TE:87 and T1W sagittal (TR:590 TE:11) planes. Under anesthesia, the arteria carotis communis was cut and blood was drained. The rabbits were euthanized, and the dissection was initiated considering the practices in Mukhopadhyay and Wagner (2020) and Perpiñán (2019). After the dissected organs were photographed, the length, width and thickness of each organ were measured with a digital caliper. The last organ sections were separated and each weighed on a precision balance (min 0.0001 g, max 220 g, precisa code XB220A). The measured morphometric values were evaluated in the SPSS 20 program. Latex was applied to reveal the arteries that vascularize the male genital tract organs on two rabbits whose vessels were washed with saline. Latex (ZPK-580-S; Gerard Biological Center, Preston UK) colored with red fabric dye (Artdeco) was injected from arteria carotis communis dextra and arteria carotis communis sinistra dissected from the neck region, and all arteries were filled with this mixture (Bugge, 1963; Erençin et al., 1967; Aycan and Bilge, 1984). After the arteria carotis communis dextra and arteria carotis communis sinistra were ligated, they were kept at room temperature for 24 hours to freeze the latex in the materials. After it was found that the latex had frozen, the materials were stored in a 10% formaldehyde solution for one week. After it was determined that all formations took formaldehyde, the materials were dissected (Çalışlar, 1986). The other eight rabbits were fixed in formaldehyde after euthanasia. The weights of the cascade organs were weighed, their lengths were measured, and macroanatomical findings were obtained. While abbreviating Latin scientific terms in the text, Aslan (2017)'s Veterinary Terminology dictionary was used. Nomina Anatomica Veterinaria (N.A.V. 2017) was used to name the anatomical terms.

The data of all measurements taken on male genital system organs in New Zealand rabbits were evaluated in the SPSS (20.0 version) package program. Descriptive statistics for numerical parameters; expressed as mean, standard error, median, minimum-maximum values. Since the number of animals evaluated was less than 30, non-parametric test procedures were used without performing normality analysis. The results were evaluated within the 95% confidence interval, and the $p<0.05$ value was considered significant. The differences between the right and left organs in pairs were determined by the 'independent sample t test'.

RESULTS AND DISCUSSION

The testis had two ends, extremitas capitata and extremitas caudata. Extremitas capitata was in association with caput epididymidis, and extremitas caudata was in cauda epididymidis. In the extremitas caudata of testis, ligamentum testis proprium provided the connection between epididymis and testis. The free edge of the testis, margo liber, and the margin in contact with the epididymis, margo epididymalis, were seen macroanatomically. It was determined that the testicles were located in the scrotum between the hind legs in New Zealand rabbits.

The left testis was lower and behind the right testis. When the scrotum was removed, it was observed that the extremitas caudata of the testis curved caudolaterally and the penis extended between the two testicles. When the tunica vaginalis was opened, it was found that the cauda epididymidis was larger than the caput epididymidis, and the corpus epididymidis was located on the ventrolateral of the testis. Caput epididymidis was seen to cover extremitas capitata. Bursa testicularis was not seen clearly. Funiculus spermaticus was found to be long in the rabbit. It was determined that the ductus deferens coursed in the dorsal part of the corpus epididymidis. It was determined that the long axis of the testis was not parallel to the long axis of the body, it was located perpendicularly (craniomedial/caudolateral) and the testis was long, flat and oval shaped. It was noted that although the rabbits were adults, the testicles did not completely descend into the scrotum in all animals. In other words, cryptorchidism was determined in these animals (Figure 2). In the presented study, some parameters of testis are shown in Table 1. While the right testis length was 67.24 ± 5.36 mm and the left testis length was 62.25 ± 6.55 mm in manual measurements in New Zealand rabbits, it was 20.9 mm in MR measurement (Figure 3). It is thought that this is due to the fact that the testicles cannot be measured completely because they are cryptorchid. In manual measurements, the width of the right testis was 15.14 ± 3.67 mm, and the width of the left testis was 14.42 ± 2.90 mm. MR measurements were found to be 12.4 mm (Figure 3). Since all of the P values obtained using the SPSS 20 program for all parameters of the testis were P>0.005, there was no significant difference between them. It was observed that arteria testicularis were separated from the aorta abdominalis at the L2-L3 level. Arteria testicularis was 0.7 mm thick in diameter. Arteria testicularis dextra was separated from arteria testicularis sinistra 6 cm cranially. In other words, the first branch of the aorta abdominalis after giving the arteria renalis was arteria testicularis dextra. Arteria iliaca externa dexter and sinister were separating from the aorta abdominalis at the level of the fifth lumbar vertebra. Arteria iliaca externa and arteria iliaca interna originated from a common root. At this point of separation, a much thinner branch, the truncus umbilicogenitalis, was also starting. Truncus umbilicogenitalis was initially seen to be divided into two branch. It was determined that arteria umbilicalis, which was the branch that split before the branches that split into two, was divided into three among itself. These three branches provided the vascularization of the apex, corpus and cervix parts of vesica urinaria, respectively. The other branch of truncus umbilicogenitalis at the point of separation was also divided into two part. While one of these branches was running in the epididymis, the other branch was feeding the ureter and ductus deferens (Figure 8).

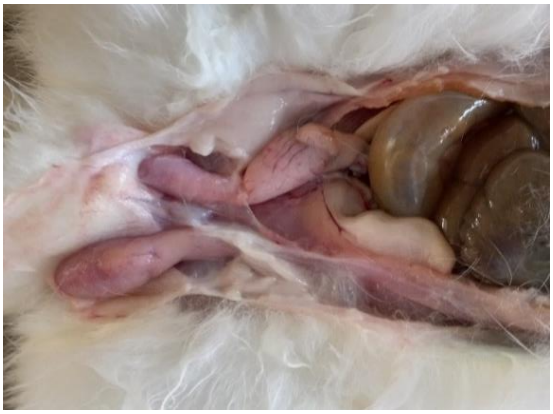

Figure 2: Cryptorchid testicles

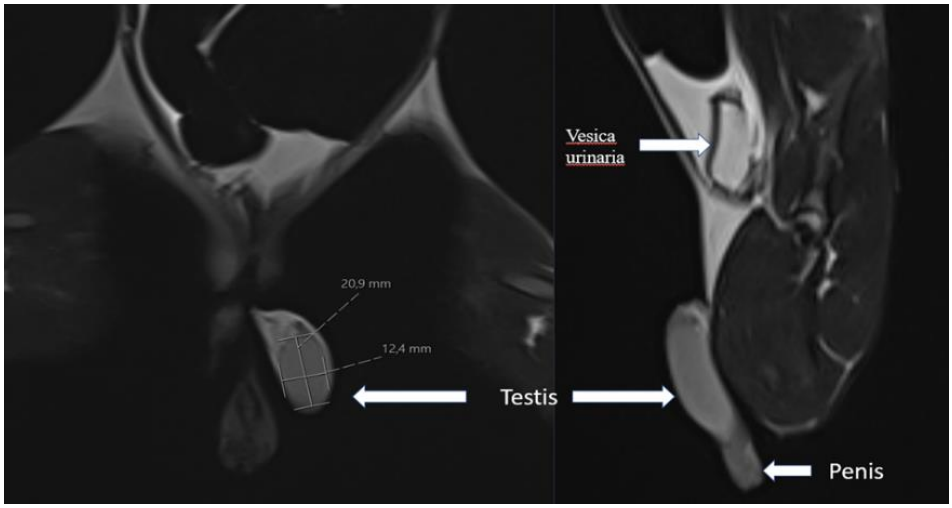

Figure 3: MR image of testis and penis

Table 1: Some parameters of testis

Parameters	Right testis	Left testis	P value
Length of testis	67.24±5.36	62.25±6.55	0.11
Width of testis	15.14±3.67	14.42±2.90	0.63
Thickness of testis	11.30±4.11	10.95±3.85	0.86
Weight of testis	10.21±1.17	9.90±1.15	0.60

Tunica testis consisted of all the membranes surrounding the testis, epididymis and funiculus spermaticus. When the extensions of these membranes of the tunica testis were followed during the dissection procedure, it was observed that there were extensions of some structures forming the lateral wall of the abdomen. When we examine the tunica testis from the outside to the inside, the outermost layer consisted of the scrotum, the second layer of tunica dartos, the third layer of fascia spermatica externa, the fourth layer of fascia cremasterica and musculus cremaster, the fifth layer of fascia spermatica interna, the sixth layer of tunica vaginalis, and the seventh layer of tunica albuginea membranes (Figure 4). The scrotum was an incision formed by the outward invagination of the skin that included both testicles. There was a partition called the septum scroti between the two testicles. There was a border called raphe scroti in the symmetry of the septum scroti towards the external environment. The scrotum was located in the regio perinealis. The tunica dartos, which is a hard and fibrous structure, was inseparably attached to the scrotum. It was observed that the fascia spermatica externa was formed by the continuation of the aponeurosis of the musculus obliquus externus abdominis, the fascia cremasterica and the musculus cremaster from the fascia of the musculus obliquus internus abdominis. It was determined that the fascia spermatica interna was a continuation of the fascia transversalis, and the tunica vaginalis was an extension of the peritoneum. Tunica vaginalis consisted of two leaves as lamina parietalis (periorchium) and lamina visceralis (epiorchium). The tunica albuginea was the innermost layer that formed the parenchyma of the testis tissue, giving it integrity and forming its shape. The tunica albuginea shaped the lobuli testicles by sending multiple divisions called septula testis into the testicular parenchyma. The channels in the lobuli testicles continued and pierced the fibrous capsule and opened into the ductuli efferentes testis, which entered the caput epididymidis.

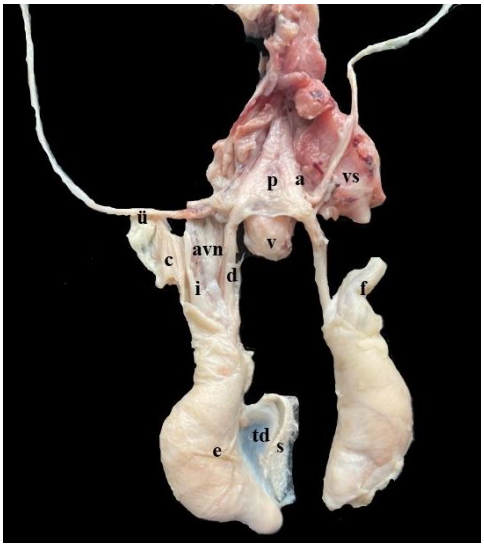

Figure 4: Testis and layers in New Zealand rabbits (s: scrotum, td: tunica dartos, e: fascia spermatica externa, c: fascia cremasterica and musculus cremaster, i: fascia spermatica interna, f: funiculus spermaticus, d: ductus deferens, a: ampulla ductus deferens, v: vesica urinaria, p: plica genitalis, ü: ureter, vs: vesicular gland (vesicula seminalis), avn: arteria testicularis, vena testicularis, plexus testicularis)

The epididymis was an organ consisting of a tubular canal in contact with the margo epididymalis of the testicles. The ductus epididymidis, the canal inside this organ, was seen macroanatomically. It was observed that the epididymis consisted of three parts: caput, corpus and cauda. Caput epididymidis was the head of the epididymis formed by the ductuli efferentes testis leaving the testis. Some parameters of caput epididymidis were as in Table 2. The corpus epididymidis was shaped by the ductus epididymidis, which was formed by the fusion of the ductuli efferentes testicles. Some parameters of the corpus epididymidis were as shown in Table 3. The cauda epididymidis was attached to the testis via the ligamentum testis proprium. The cauda epididymidis was joined by the ligamentum caudae epididymidis through the processus vaginalis. The canal called ductus epididymidis, which came from the corpus epididymidis to the cauda epididymidis, was separated from the epididymis with the name ductus deferens. Some parameters of cauda epididymidis are shown in Table 4. The P values obtained using the SPSS 20

program for the parameters of all parts of the epididymis were P<0.005, in other words, significant for the lengths of the corpus and cauda epiddymis. But since all other parameters were P>0.005, there was no significant difference between them.

Table 2: Some parameters of caput epididymidis

Parameters	**Caput epididymidis**		**P value**
Direction	**Right**	**left**	
Length	19.90±2.71	17.64±0.75	0.04
Width	6.85±0.54	6.78±0.66	0.81
Thickness	4.33±0.64	5.15±0.58	0.02
Weight	0.68±0.08	0.80±0.13	0.05

Table 3: Some parameters of corpus epididymidis

Parameters	**Corpus epididymidis**		**P value**
Direction	**Right**	**Left**	
Length	17.16±1.28	18.95±0.97	0.01
Width	2.95±0.35	3.24±0.52	0.20
Thickness	1.57±0.07	1.53±0.06	0.3
Weight	0.14±0.03	0.15±0.03	0.41

Table 4: Some parameters of cauda epididymidis

Parameters	**Cauda epididymidis**		**P value**
Direction	**Right**	**Left**	
Length	26.19±0.54	25.54±3.61	0.67
Width	12.95±1.14	12.91±1.35	0.95
Thickness	8.15±0.72	8.05±0.46	0.74
Weight	1.87±0.26	1.90±0.31	0.84

Ductus deferens is the sperm canal that takes the semen from the cauda epididymidis and delivers it to the pars pelvina of the urethra. The ductus deferens was reaching the plica genitalis by passing through the canalis inguinalis, after it was entering the cavum abdominis. It was seen that this canal was pass through the ventral of the ureters and open to the urethra, after coming to the facies dorsalis of vesica urinaria (Figure 5). The enlargement of the ductus deferens, called the ampulla ductus deferentis, before opening to the urethra was clearly detected. It was determined that the ductus deferens merged and opened into the urethra at the colliculus seminalis level (Figure 6). Some parameters of the ampulla ductus deferentis and ductus deferens were tabulated as shown in Table 5. Since all P values obtained by using SPSS 20 program for all parameters of ampulla ductus deferentis were P>0.005, no significant difference was found between them. However, the results obtained were significant since all P values obtained for all parameters of the ductus deferentis were P <0.005.

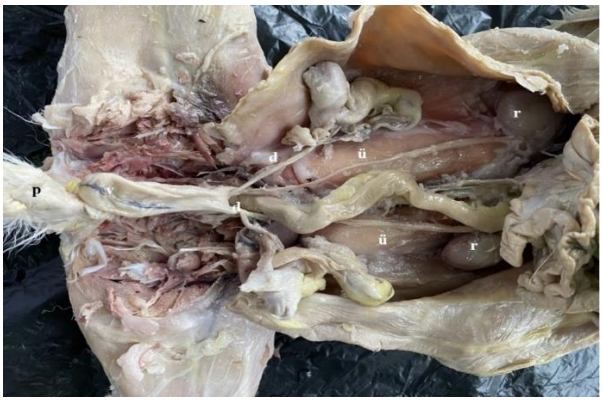

Figure 5: Ductus deferens and ureters (**ü:** ureter, **d:** ductus deferens, **v:** vesica urinaria, **p:** penis, **r:** ren)

Parameters	**Ampulla ductus deferentis**		**P value**	**Ductus deferens**		**P value**
Direction	**Right**	**Left**		**Right**	**Left**	
Length	27.16±3.16	27.44±3.55	0.86	44.18±4.98	37.85±4.69	0.02
Width	3.85 ±0.23	3.88± 0.25	0.8	2.75±0.24	2.39±0.23	0.008
Thickness	1.81±0.43	1.94±0.33	0.52	1.38±0.02	1.33±0.04	0.002
Weight	0.35±0.16	0.37±0.16	0.12	0.30±0.29	0.25±0.03	0.002

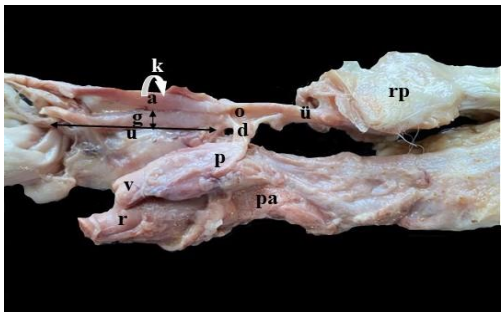

Figure 6: Male genital glands, ampulla ductus deferentis and urethra in New Zealand rabbit (**a:** ampulla ductus deferentis, **u:** Length of ampulla ductus deferentis, **g:** Width of ampulla ductus deferentis, **k:** Thickness of ampulla ductus deferentis, **v:** vesicular gland, **ü:** urethra, **d:** ductus excretorius, **p:** prostata, **r:** rectum, **pa:** paraprostat, **o:** ostium urethra internum, **rp:** radix penis)

It was observed that funiculus spermaticus consisted of arteria testicularis, vena testicularis, plexus testicularis, lymphatic vessels, ductus deferens, musculus cremaster, fascia spermatica externa, fascia cremasterica, fascia spermatica interna structures (Figure 4). The glands forming the glandula genitales accessoriae were located dorsal to the pars pelvina of the urethra from cranial to caudal. These glands were vesicular gland, prostate and bulbourethral gland from cranial to caudal (Figure 7).

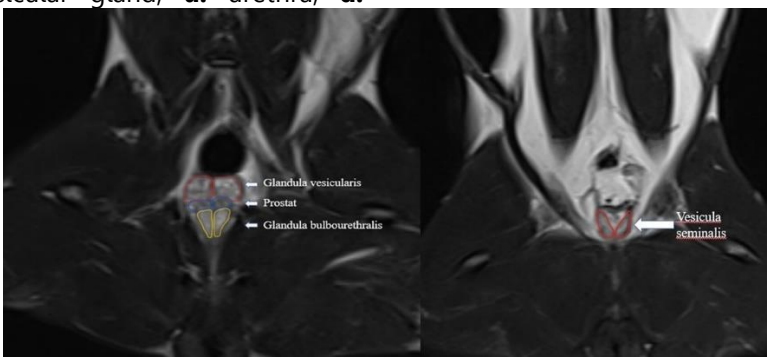

Figure 7: MR image of male accessory reproductive glands (vesicular gland, paraprostate part of prostate, bulbourethral gland)

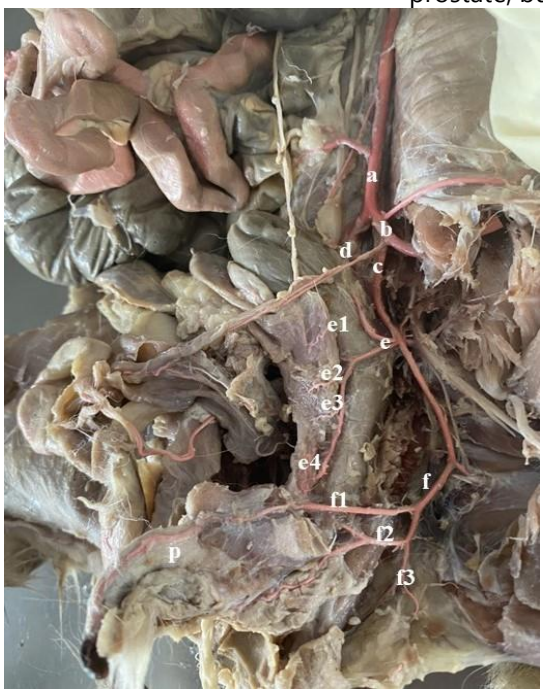

Figure 8: Arterial vascularization of male genital tract organs in the New Zealand rabbit (a: aorta abdominalis, b: arteria iliaca externa, c: arteria iliaca interna, d: truncus umbilicogenitalis, e: arteria prostatica, f: arteria penis, f1: arteria dorsalis penis, f2: arteria profunda penis, f3: arteria bulbi penis, p: penis)

The vesicular gland was a pair of glands located ventral to the ampulla ductus deferens on the dorsol of the rectum (Figure 9-10). Compared to other glands, it was the largest gland macroanatomically. The ductus excretorius, the draining canal of the gland, opened directly into the urethra. It was determined that this gland was located at the L7 level. Arterial vascularization was provided by arteria prostatica originating from the arteria iliaca interna (Figure 8). Some parameters of vesicular gland are shown in Table 6. According to the analysis results obtained by using SPSS 20 program for all parameters of the vesicular gland, the P values were significant because $P<0.005$ in terms of length, while there was no significant difference between them as all other parameters were $P>0.005$.

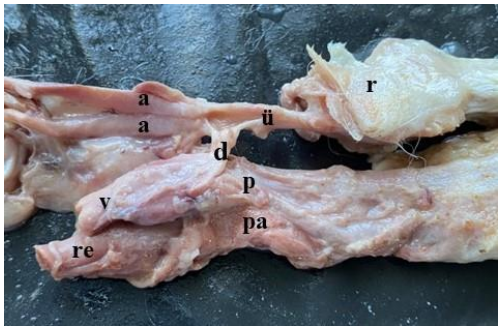

Figure 9: The ductus excretorius of the vesicular gland and its surrounding anatomical structures (**a:** ampulla ductus deferentis, **ü:** urethra, **p:** prostat, **pa:** paraprostat, **v:** vesicular gland, **r:** radix penis, **re:** rectum, **d:** ductus excretorius)

Table 6: Parameters of vesicular gland

Parameters	Vesicular gland		P value
Direction	Right	Left	
Length	18.54±0.35	17.55±0.59	0.02
Width	16.54±0.28	16.7±0.45	0.42
Thickness	4.51±4.77	7.72±4.72	0.93
Weight	0.91±0.07	0.96±0.09	0.25

The prostate gland consisted of three parts in rabbits: the prostate, the corpus prostate, and the paraprostate. The prostate was in the corpus prostatae situation. It was transferring the secretion it produced to the prostate to the pars pelvina of the urethra, right next to it, through channels called ductuli prostatici (Figure 10). In the New Zealand rabbit, the prostate was located at the junction of the ampulla ductus deferens, that is, at the beginning of the urethra, dorsal to the rectum, caudal to the vesicular gland. At the most cranial part of the prostate complex is the prostate. Measurements could not be taken because the prostate was in a size that could not be measured macroanatomically. It was determined that the prostate gland complex was located at the S1-S2 level. Prostate and paraprostate parameters are listed in Table 7. While the P values obtained by using the SPSS 20 program for all parameters of the paraprostate were significant for P<0.005 for length, there was no significant difference between them as all other parameters were P>0.005.

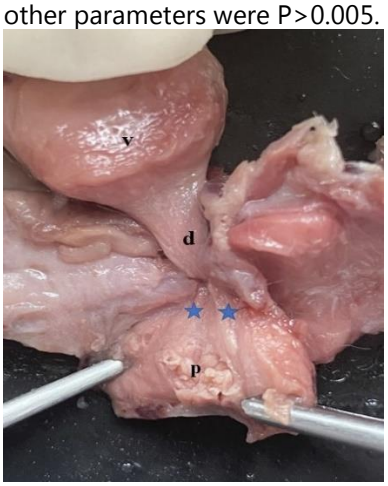

Figure 10: Prostate gland and its drainage canals, ductuli prostatici (**p:** prostat, **v:** vesicular gland, **d:** ductus excretorius, **star signs:** ductuli prostatici)

Table 7: Parameters of prostate and paraprostate

Parameters	Prostate	Paraprostate		P value
Direction		Right	Left	
Length	11.68±2.01	19.56±3.04	14.29±3.34	0.005
Width	13.02±1.38	12.13±2.17	7.47±1.73	0
Thickness	5.08±1.11	3.43±0.99	4.00±0.5	0.172
Weight	0.98±0.09	0.59±0.16	0.70±0.21	0.236

Bulbourethral gland was a green lentil-sized gland found in pairs (Figure 11). It was observed that this gland was located in the cranial of the arcus ischiadicus, in the ventral of the radix penis, and in the dorsal of the caudal part of the rectum. It was determined that the secretion produced by bulbourethral gland directly transferred to the pars spongiosa of the urethra. It was located caudal to the prostate complex in the New Zealand rabbit. The statistical values obtained as a result of the data of this gland are shown in Table 8. Since all P values obtained by using SPSS 20 program for all parameters of bulbourethral gland were P>0.005, there was no significant difference between them.

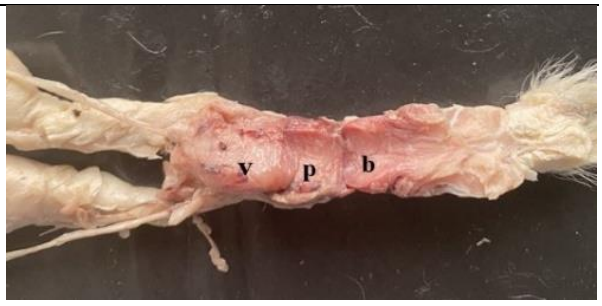

Figure 11: Ventral view of glands containing glandulae genitales accessoria (**v:** vesicular gland, **p:** prostata, **b:** bulbourethral gland)

Tablo 8: Parameters of bulbourethral gland

Parameters	Bulbourethral gland		P value
Direction	Right	Left	
Length	13.36±2.00	12.39±1.21	0.26
Width	6.73±0.98	5.80±0.90	0.07
Thickness	2.88±0.38	2.98±0.21	0.51
Weight	0.40±0.20	0.44±0.24	0.77

It was determined that the urethra masculina is a tubular organ that starts from the vesica urinaria and extends to the external environment with an opening called ostium urethrae externum after traveling inside the penis (Figure 12). The MR image of urethra was as in Figure 13. Urethra was divided into two parts, pars pelvina and pars penina (spongiosa) according to location. It was determined that the ductus deferens, ductus excretorius and ductuli prostatici were opened to the pars pelvina of the urethra masculina. Arteria urethralis, which provides vascularization of urethra masculina in New Zealand rabbits, started from arteria prostatica.

Figure 12: Urethra masculina (**ü:** urethra, **d:** the area where the ampulla ductus deferens unites and opens into the urethra, **v:** vesica ürinaria (bladder))

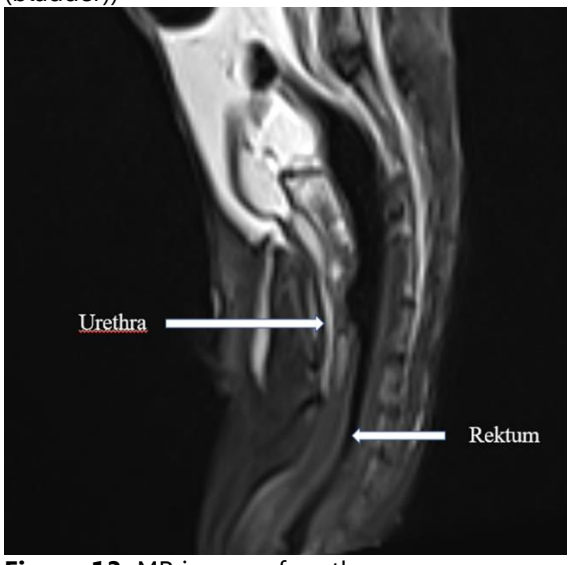

Figure 13: MR image of urethra

It was seen that the penis was placed between the hind legs very close to the scrotum and anus. The direction of the free end of the penis was towards the caudal. The penis began with a double crus penis originating from the arcus ischiadicus. It was seen that these structures combined to form the radix penis, which is the fixed part of the penis. The part that came after the radix penis was the body of the penis and the free corpus penis (pars libera penis). There was no curve in the corpus penis, similar to the flexura sigmoidea penis, which was prominently "S" shaped as in the bull. However, when we looked at the whole penis, a curl was seen on the radix penis. The free end of the penis, the glans penis, was located superficially. In the transversal section of the penis, two sections were distinguished, namely the corpus spongiosum penis and the corpus cavernosum penis. In New Zealand rabbits, the arteria penis feeding the penis was located caudal after the arteria prostatica. Arteria penis was divided into three branches as arteria dorsalis penis, arteria profunda penis, and arteria bulbi penis (Figure 14). It was observed that the arteria dorsalis penis, which was first separated from the arteria penis, provided vascularization of the dorsal part of the penis. The arteria profunda penis, which is separated from the arteria penis, is the branch that provides the vascularization of the corpus penis. The third and most extreme branch of the arteria penis was the arteria bulbi penis. The right and left glans extended up to the penis. It was observed that very thin branches separated from arteria dorsal penis and arteria bulbi penis vascularized the preputium. No osseous formation such as os penis was observed on the glans penis in New Zealand rabbits. But there was a cartilage structure (Figure 15). Some morphometric parameters of the penis are shown in Table 9.

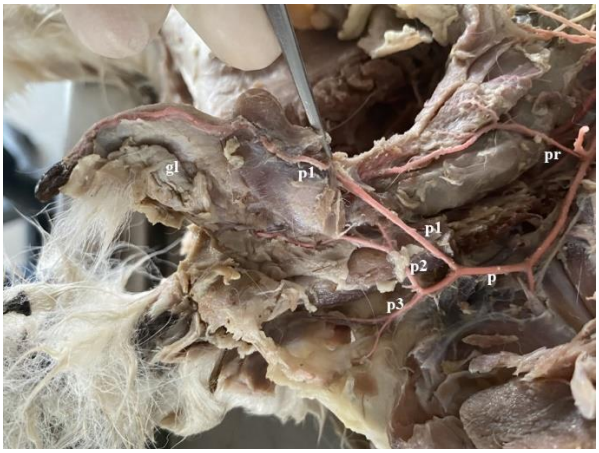

Figure 14: Arterial vascularization of the penis (**pr:** arteria prostatica, **p:** arteria penis, **p1:** arteria dorsalis penis, **p2:** arteria profunda penis, **p3:** arteria bulbi penis, **gl:** glandulae preputiales)

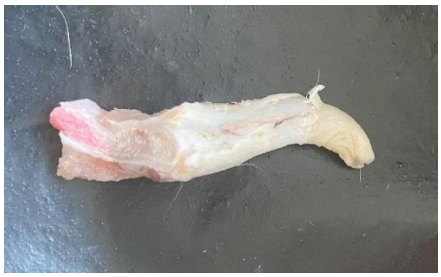

Figure 15: Penis and cartilage structure

Table 9: Parameters of penis

Parameters	Penis
Length	46.15 ± 5.27
Width	12.32 ± 0.79
Thickness	11.29 ± 0.64
Weight	10.56 ± 0.74

New Zealand rabbits are more preferred laboratory animals in scientific studies because they are close to humans in terms of biological structure. Rabbits are mostly preferred as laboratory animals in scientific studies. This study was carried out due to the fact that rabbits are more preferred in the studies carried out and the knowledge of the male genital system organs is not sufficient.

The testicles are an elliptical organ that varies in volume, location and position according to the animal species. This organ is relatively parallel to the long axis of the body in equidae, swine, and carnivora, while in equidae it is in an upright position. In the literature, the shape of the testicles was reported as oval in mice, rats, rabbits and guinea pigs, while in another source it was stated that they were flattened from the sides in rabbits (Mc Laughlin and Chiasson, 1979), plump in guinea pigs, and rounded in mice and rats (Greene, 1963; Cooper and Schiller, 1975). In the presented study, the testicles were perpendicular to the long axis of the body, similar to the ruminant. The testicles are located in the cavum abdominis at birth in adult mice and rats. It descends into the scrotal canal between 4 and 6 weeks after birth. The location of the scrotum is usually between the preputium and the anus. Since the canalis inguinalis is open throughout life in mice and rats, the testicles are mostly retracted towards the body cavity, they are located behind the body cavity and lateral to the vesica urinaria (Türkmenoğlu and Abacıoğlu, 2021). The left testis was lower and behind the right testis. Right testis length was 67.24±5.36 mm and left testis length was 62.25±6.55 mm in New Zealand rabbits. The mean testicular weight in rabbits has been reported as 2.035 ± 0.529 g (Holtz and Foote, 1978). In another study, while the right testis weight was 7.2 g, the left testicular weight was 7.8 g in rabbits (Çakır et al., 2001). In this study, the weight of the right testis was 10.21 ± 1.17 g, while the weight of the left testis was 9.90 ± 1.15 g. In other words, the right testis was heavier than the left testis. When compared with other sources in the literature, it is seen that testicular weight is higher in this study. Research findings Çakır et al. (2001)'s findings were the opposite. Arteria testicularis has been reported in carnivores, at the level of the middle of the distance between arteria renalis and arteria mesenterica caudalis, at the level of L4-L5 (Evans and de Lahunta, 2013). In this study, it was separated between the arteria renalis and arteria mesenerica caudalis at the L2-L3 level, similar to the other studies. Flešárová and Maženský (2017) reported that arteria renalis dextra and arteria renalis sinistra appeared before L2-L3 level in rabbits. In rabbits, arteria testicularis are asymmetrically separated from the aorta abdominalis (Nowicki et al., 2010). Nowicki et al. (2010) reported that arteria testicularis were asymmetrical. It was determined that arteria testicularis dextra 60 mm after the exit of the arteria renalis sinistra, separated from the right ventrolateral wall of the aorta in front of the arteria mesenterica caudalis and at the L5 level in rabbits. In one case, it was observed to separate from the ventral wall. In the same study, in another case, it was determined that both arteria testicularis originated from the back of the arteria mesenterica caudalis. In rabbits and cats, arteria testicularis dextra was observed to be separated from the sinistra 3-4 mm from the cranial (Çakır, 1991). Similarly, in the current study, it was seen that the right testicular artery was located more cranially. In a different study conducted in rabbits, the vessels were typed according to the exit region and ten different typings were mentioned. In the most common type 1 (12 materials), arteria celiaca of the first branch leaving from the aorta abdominalis, arteria mesenterica cranialis of the second branch, arteria renalis dextra of the third branch, arteria renalis sinistra of the fourth branch, arteria testicularis dextra of the fifth branch, arteria testicularis of the sixth branch sinistra and the last seventh branch were arteria mesenterica caudalis (Arredondo et al., 2017). In most of the typings in a different study, it was found that the arteria testicularis dextra separated from the aorta abdominalis before the sinistra (Arizaga, 2018). The diameter of the arteria testicularis is 0.5 mm in the rabbit (Çakır, 1991). In the present study, the diameter of arteria testicularis was measured 0.7 mm. In the rabbit, the arteria testicularis dextra gives out the arteria ureterica media dextra approximately 8-9 mm after its origin. This vessel also divides into two branches that go to the ureter after watching for a while (Çakır, 1991). Arteria ureterica media was not

seen in the current study. Arteria testicularis dextra gives two rami epididymales in funiculus spermaticus. One of these vessels divides into two again. While one branch continue ventrally with the epididymis, the other branch is called rami ductus deferentis that proceeds proximal direction along the ductus deferens. Arteria testicularis dextra alternates between caput epididymidis and cauda epididymidis twice more than arteria testicularis sinistra. At the beginning of the arteria testicularis sinistra, progresses caudal with a slight bend. It gives off the arteria ureterica media sinistra near origin. When it passes through the canalis inguinalis and proceed distal direction along the funiculus spermaticus, it shows a lot of folding. The rami ductus deferentis, which runs dorsal along the ramie epididymales and ductus deferens, leaves the vessel in two or three branches. The continuation of the vessel goes straight to the cauda epididymidis in the margo epididymalis. Turning back again, it moves along the margo liber and reaches the caput epididymidis. Here, it proceeds ventrally within the parenchyma tissue without entering the caput epididymidis and terminates in extremitas caudata (Çakır, 1991). The mean weight of epididymis parts in rabbits was reported as 0.264 ± 0.087 g in caput epididymidis, 0.046 ± 0.019 g in corpus epididymidis, and 0.398 ± 0.123 g in cauda epididymidis (Holtz and Foote, 1978). In this study, right caput epididymidis 0.68 ± 0.08 gr, left caput epididymidis 0.80 ± 0.13 gr; right corpus epididymidis 0.14 ± 0.03, left corpus epididymidis 0.15 ± 0.03 g; right cauda epididymidis 1.87 ± 0.26 gr, left cauda epididymidis 1.90 ± 0.31 gr. When we compare the findings of Holtz and Foote (1978), it is seen that the weights of the epididymis parts in the study are higher.

Ampula ductus deferentis, which is the expansion of the ductus deferens before termination is prominent in equide, ruminant, and canis; however absent in pigs and cats. It was quite evident in New Zealand rabbits such as equide, ruminant and canis. This canal unites with the ductus excretorius to form the ductus ejaculatorius before opening to the urethra in equide and ruminants. But it differed in the presented study in that it opened directly into the urethra without merging with the other glands canals. The initial portion of the ductus deferens in rabbits was 0.098 ± 0.026 g, while the ampulla ductus deferens was reported to be 0.177 ± 0.069 g (Holtz and Foote, 1978). In the presented study, right ductus deferens weight was 0.30 ± 0.29 g, left ductus deferens weight was 0.25 ± 0.03 g; the weight of the ampulla ductus deferentis was 0.35 ± 0.16 gr on the right side and 0.37 ± 0.16 gr on the left side. When we compare the results of Holtz and Foote (1978), it is seen that the weights of New Zealand rabbits are higher. Arteria ductus deferentis arises from the truncus umbilicogenitalis in male rabbits (Takcı, 1992). Kigata and Shibata (2020) stated that arteria ductus deferens originates from arteria umbilicalis. It progresses towards the beginning of the ductus deferens. In rabbits, the truncus umbilicogenitalis originates from arteria iliaca interna, arteria iliaca externa or arteria iliaca communis. Truncus umbilicogenitalis with an average length of 1 cm. It gives branches of the arteria umbilicalis, ramus uretericus, and arteria ductus deferentis. Arteria umbilicalis vascularizes vesica urinaria (Takcı, 1992). Arteria ductus deferens divides into dorsal and ventral branches at the ductus deferens and ureteral passage. The dorsal branch vascularizes the distal ductus deferens. It was observed that the dorsal branch anastomosed with the arteria prostatica at the end of the ductus deferens. The ventral branch passes through the canalis inguinalis and vascularizes the proximal part of the ductus deferens (Kigata and Shibata, 2020).

Generally, male accessory glands are three in domestic mammals: glandula bulbourethralis, prostate and vesicular gland (Demiraslan and Dayan, 2021). However, they do not show the same number and the same characteristics in all domestic mammal species. In domestic mammals, the ampulla ductus deferentis has been recognized as a fourth gland in recent years. While it was reported that the vesicular gland and prostate gland were present in rabbits (Cheeke et al., 1982), the presence of mole and prostate and bulbourethral gland were determined in rats (Gottreich et al., 2001). Four types of glands have been reported in male agouti: prostate, bulbourethral gland, vesicular gland, and coagulate glands (Mollineau et al., 2009). While it was reported that the vesicular gland and prostate gland were present in rabbits (Cheeke et al., 1982), the presence of and prostate and bulbourethral gland were determined in rats and mole (Gottreich et al., 2001). In rabbits, vesicular gland, prostate and prostate gland are found singly, while paraprostate and bulbourethral gland are found in pairs. Arteria prostatica originates from the arteria iliaca interna. It is distributed in vesicular gland, prostate, paraprostate and bulbourethral gland (Barone et al., 1973). In the present study, the vesicular gland, paraprostate, and bulbourethral gland were double, while the prostate gland was single. The prostate could not be determined macroanatomically.

Skonieczna et al. (2019) reported that the vesicular gland is single. While ductus

excretorius, which is the draining canal of the gland, opens directly into the urethra in pigs, it participates in the formation of the ductus ejaculatorius in equide and ruminants (König and Liebich, 2015). It was observed that New Zealand rabbits did not open directly into the urethra as in the pig. In the present study, the vesicular gland was determined to be at L7 level, while Dimitrov et al. (2013) noted that it is at the S1 level. It is approximately 20 mm long and 10 mm wide (Kürtül et al., 2001). In the present study, the vesicular gland length was 18.54 ± 0.35 mm on the right and 17.55 ± 0.59 mm on the left. Kurtul et al. (2001)'s findings were close to the findings of the study but higher. The width of the vesicular gland was measured as 16.54 ± 0.28 mm on the right and 16.70 ± 0.45 mm on the left. The vesicular gland in rabbits has been noted as 0.529 ± 1.169 g (Holtz and Foote, 1978). In the present study, vesicular gland weight was 0.91 ± 0.07 g on the right side and 0.96 ± 0.09 g on the left side. It was greater than the values of Holtz and Foote (1978). It receives arteries from arteria prostatica and arteria rectalis media, which are branches of the arteria pudenda interna. Arteria pudenda interna is the root of the visceral vessel originating from the arteria iliaca interna in carnivores. Arteria prostatica is divided into 2 branches as ramus ductus deferentis and arteria rectalis media (Dyce et al., 2010). In rabbits, the ventral surface of the vesicular gland is in contact with the ampulla ductus deferentis. The cranial of dorsal aspect's is in contact with the colon and rectum. The caudal part of the vesicular gland reaches up to the prostate (Dimitrov et al., 2013).

The prostate is the male accessory gland situated in all domestic mammals (Dyce et al., 2010). This gland is divided into two parts, the pars disseminata prostatae and the corpus prostatae. The pars disseminata prostatae is the internal part scattered in the wall of the pars pelvina of the urethra. Corpus prostatae is the external part surrounding the urethra in the same place (Bahadır and Yıldız, 2014). Pars disseminata prostatae is situated in bos, capra and ovis, and corpus prostatae is situated in bos, equide, carnivor and sus. In the present study, it was similar to bos, equide, carnivor, and sus, due to its corpus prostatae shape. It transfers the secretion produced by the prostate directly to the pars pelvina of the urethra (König and Liebich, 2015). In the New Zealand rabbit, the prostate is located at the beginning of the urethra. While the prostate consisted of a single lobe, the paraprostate was pairs. The prostate complex was in the S1-S2 alignment as reported in the literature (Dimitrov, 2010; Dimitrov, 2021). At the most cranial part of the prostate complex is the prostate. The prostate is approximately 19 mm long by 15 mm wide. In the current study, prostate length was recorded as 11.68 ± 2.01 mm and width as 13.02 ± 1.38 mm. Kurtul et al. (2001) reported the length of the prostate as 18 mm and its width as 15 mm. In the same study, it was reported that the prostate was approximately 19 mm long and 15 mm wide, while the paraprostata could be seen microscopically. In this study, contrary to the literature, while the paraprostate was large enough to be measured, the prostate was not clearly seen. The weights of parts of the prostate gland in rabbits were noted as follows; proprostate 0.633 ± 0.304 g, prostate 0.411 ± 0.181 g, paraprostate 0.040 ± 0.019 g (Holtz and Foote 1978). In the study, paraprostate weight was 0.59 ± 0.16 g on the right side and 0.70±0.21 g on the left side. It was higher than the findings of Holtz and Foote (1978). It was also inconsistent in the presented study in terms of the width of the prostate being greater than its length. Arteria prostatica provides arterial vascularization of most of the male genital glands. It starts from the arteria iliaca interna (Kürtül et al., 2001). Arteria prostatica emerges from the medial aspect of the arteria glutea caudalis and descends towards the beginning of the urethra. In the caudal of the vesicular gland, it divides into two branches, cranial and caudal. The branch that goes to the cranial artery is the arteria rectalis media to the urethralis caudal (Takcı, 1992). Kurtul et al. (2001) reported that arteria umbilicalis provides vesicular gland and prostate complex, while arteria prostatica provides vascularization with several branches going to each gland. The findings obtained in the study were in agreement with Takcı (1992). Arteria urethralis vascularizes the male accessory glands as well as urethra in male rabbits (Takcı, 1992). In the presented study, it was observed that arteria urethralis did not vascularize the male genital glands. In the study conducted by Takcı (1992), it was reported that arteria prostatica proceeded from the arteria penis, not from the arteria glutea caudalis, to the cranioventral over the pars pelvina of the urethra in a male rabbit, and there was no arteria rectalis media. (Takcı, 1992). No such case was found in this study.

Bulbourethral gland is the male accessory gland located in pairs. This gland is situated anterior to the arcus ischiadicus, at the level of the aperture pelvis caudalis, and dorsal to the pars pelvina of the urethra. As reported in the literature (König and Liebich, 2015), bulbourethral gland was transfered the secretion it produced directly to the pars spongiosa of the urethra. It is localized caudal to the prostate complex in the New

Zealand rabbit. It has two lobes and resembles a prostate. Each lobe long is 25 mm and wide 5 mm (Kürtül et al., 2001). In the present study, the bulbourethral gland length was analyzed as 13.36 ± 2.00 mm on the right side and 12.39 ± 1.21 mm on the left side. The width was recorded as 6.73 ± 0.98 mm on the right and 5.80 ± 0.90 mm on the left. The values found in the length of Kurtül et al. (2001), it was observed that it was lower than the value stated and higher in width. Holtz and Foote (1978) reported the weight of bulbourethral gland in rabbits as 0.390 ± 0.133 g. In the presented study, the bulbourethral gland weighed 0.40 ± 0.20 g on the right and 0.44 ± 0.24 g on the left. When the data of our study findings were compared with Holtz and Foote (1978), the values were seen to be similar. Arteria urethralis, which provides vascularization of urethra masculina, started from arteria prostatica in New Zealand rabbits.

The arteria penis is the ventral one of the last two branches of the arteria pudenda interna. Ozgel et al. (2003) reported that arteria penis is divided into two branches as arteria dorsalis penis and arteria profunda penis in New Zealand rabbit. In the present study, arteria penis were in three branches; arteria dorsalis penis, arteria profunda penis and arteria bulbi penis. It was observed that thin branches separated from arteria dorsalis penis and arteria bulbi penis vascularized the preputium. Takcı (1992) noted that arteria penis and branches provide vascularization of penis and preputium in male rabbits. Ozgel et al. (2003) reported that arteria dorsalis penis also gives branches to the preputium. At the beginning of the corpus penis, after giving the arteria dorsalis penis, it enters the corpus cavernosum penis with the name of arteria profunda penis. In one rabbit, it was observed that the arteria penis gave the arteria prostatica towards the cranioventral 4 mm after its origin. It has been reported that the arteria profunda penis and very small branches departing from the arteria penis join the last part of the bulbourethral gland (Kürtül et al., 2001).

CONCLUSIONS

Rabbits are frequently used in research because of their human-like structure. A limited number of literature was identified on the male genital tract of rabbits. It will contribute to the elimination of the lack of knowledge in this field by considering the current and deficiencies. The findings of this study were carried out in scientific studies on the male genital system in laboratory animals and other animal species (Castellini et al., 2022; Emmanuel et al., 2019), artificial insemination studies (Ata et al., 2018; Dimitrova et al., 2009; Lukac et al., 2009) is thought to contribute to reproductive system diseases and operations in rabbits (Harcourt-Brown, 2017; Reineking et al., 2019, Zhu et al., 2018; Zhu et al., 2021).

ACKNOWLEDGEMENTS

This work was supported by the Kafkas University Scientific Research Projects Coordination Unit. Project Number: 2021-TS-65

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CATTLE IDENTIFICATION SYSTEMS ON THE EDGE OF NEW ERA

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Abstract

A crucial component of the processes involved in farm management is the identification of cattle. Conventional methods of identifying cattle include marking them with a brand (either hot or cold), a paint brand, a tag, a notch in the ear, a tattoo, radio frequency identification, a print of the nose, and a variety of other biometric approaches. The fact that existing identification techniques are not actually sturdy and dependable, however, makes it an imperative necessity to design new methods. The development and progress of information technology has significantly increased the accuracy of livestock production management. Because of their ability to create accurate, non-invasive, real-time data, image recognition and computer vision applications that are powered by deep learning are becoming increasingly important in livestock production systems. Real-time animal monitoring and image processing technology can be used to identify animals, improve production quality, and guarantee the safety of livestock products. This will become a major catalyst for the modernization of the livestock business. The applications of image processing that are utilized in livestock identification systems will be the focal point of this presentation's primary discussion area.

Key words: *cattle identification, deep learning, image processing, precision livestock farming, smart livestock husbandry, sustainable livestock production*

INTRODUCTION

The identification of each animal is the cornerstone of maintaining precise production data for the herd or flock (Awad 2016; Neary M and Yager A 2022). Individual animal identification lets producers keep track of important information about an animal's parents, birth date, production records, health history, and many other things (Bhole et al., 2019; Neary M and Yager A 2022; WenFu Yang). Moreover, identification of livestock has been an increasingly important factor in recent years, playing a significant part in the understanding of disease transmission, vaccination, and animal traceability (Awad 2016). When a producer maintains accurate records, they have access to sufficient information from which to manage individual animals or the entire herd or flock (Neary M and Yager A 2022). In many different scenarios, the farmer has to be capable of rapidly identifying the animal in question (Awad 2016; Neary M and Yager A 2022). An effective method of identification makes the completion of this work more productive (Neary M and Yager A 2022). Accurate identification is also essential in order to demonstrate ownership of a certain animal or to demonstrate the herd or flock from which the animal originated (Awad 2016; Neary and Yager 2022). As a direct consequence of this, precise biosecurity is supplied (Awad 2016).

The identification process for livestock, especially cattle, has undergone significant change as a result of advances in technology (Bello et al., 2020). Traditionally, cattle might be identified using any one of the following methods: branding, paint branding, tagging, ear notching, tattooing, radio frequency identification, nose printing, or any of the biometrics approaches (Andrew et al., 2016; Awad 2016; Bello et al., 2020). However, their performance is restricted because of things like losses, duplications, fraud, security difficulties, and ear damage; hence, there is an absolute requirement for the approaches to be improved (Johnston and Edwards 1996; Andrew et al., 2016; Awad 2016; Bhole et al., 2019; Bello et al., 2020 Qiao et al., 2021). In this work, an overview of the identification of cattle is presented, discussing both classic methods of identification and emerging trends in cattle identification.

Traditional Cattle Identification Methods

Finding a measurable, collected, distinct, harmless, and time-invariant identifier for each animal is essential for any precise and efficient cattle identification and tracking system (Awad 2016). In addition, a safe cattle identification system must acquire information in an efficient, dependable, and precise manner that prevents

fraud and facilitates data storage and retrieval (Andrew et al., 2016; Awad 2016). There is evidence of a global demand for a secure and effective animal identification system (Andrew et al., 2016; Awad 2016).

Traditional methods of identification have widespread applications, extended periods of time during which they are utilized, and documented research inquiries (Awad 2016). On the other hand, contemporary technologies that rely on biometrics call for additional research before they can be applied on a big scale (Awad 2016). Traditional methods of cattle identification can be broken down into three distinct subgroups: persistent techniques, provisional techniques, and electronic techniques (Awad 2016).

Losses, distortions, and fraud are all issues commonly encountered when using these techniques, and there are also ethical and welfare considerations to think about when using them on animals (Huhtala et al., 2007; Bowling et al., 2008). Each of the three groups is discussed in detail in this section.

Persistent techniques

Notching of the ears: The technique of ear notching involves cutting a piece in the shape of a V from the right and left ears of an animal; the location of the notch provides information about the identity of the animal (Awad 2016; Kumar et al., 2017; Neary M and Yager A 2022). Animals can experience anguish from ear notching, and such severe pain should be avoided or alleviated by various means, regardless of the potential value to the animal or to mankind (Awad 2016; Roughan and Sevenoaks 2019). Because ear notching is not scaled and can only identify a limited number of animals when done manually, it is not suitable for medium-or large-scale farms.

Tattooing of the ear: Tattooing the ear is another common form of traditional livestock identification (Kumar et al., 2017; Awad 2016). Letters, numerals, or a combination of both can be used as ear tattoo designs (Kumar et al., 2017; Awad 2016). This approach eliminates the issue of animal distress, but it is highly subject to modification, duplication, and elimination (Kumar et al., 2017; Awad 2016). Moreover, the scalability of ear tattooing is limited (Ali Ismail Awad 2016). Real-time livestock identification necessitates an arduous, time-consuming, and demanding procedure for examining, reading, and recording tattoos (Kumar et al., 2017; Awad 2016).

Branding with a hot iron: Branding cattle animals with a hot iron uses the farm's mark, letters or numbers, to visibly identify them (Kumar et al., 2017; Awad 2016; Özbeyaz and Yüceer-Özkul, 2020;). The brand containing the identification is heated to an appropriate temperature, applied firmly to the animal's skin, and then removed promptly (Özbeyaz and Yüceer-Özkul, 2020; Kumar et al., 2017; Awad 2016). Special care must be paid to the temperature of the branding instrument when carrying out this procedure (Awad 2016). Branding with a hot iron may appear to be a straightforward means of identification, but it lacks sufficient accuracy and dependability because it is easily reproduced, removed, or altered (Kumar et al., 2017; Awad 2016). Because of concerns about animal welfare, hot iron branding is illegal in the United Kingdom (Awad 2016).

Branding with a frozen iron: The letters or digits of a farm's brand are used to visually identify cattle animals through freeze branding (Özbeyaz and Yüceer-Özkul, 2020). In contrast to hot branding, freeze branding involves eliminating the natural color in the hair of the animal (Kumar et al., 2017; Awad 2016). This procedure causes white hair to develop in the skin area that the iron contacts(Kumar et al., 2017; Awad 2016). Although this approach is straightforward, it cannot be applied to white animals, which is a disadvantage (Kumar et al., 2017; Awad 2016). Furthermore, such marks can be temporarily concealed by altering the white hue of the brand to match the natural color of the animal (Kumar et al., 2017; Awad 2016). Ear tattoos and iron brands are not permanent because they can be changed or eliminated over time (Awad 2016). As a result, these procedures are not unchanged through time and can change as an animal grows (Awad 2016). The iron branding method only shows who owns an animal, not what it is (Kumar et al., 2017; Awad 2016). This makes it difficult to follow and monitor the animal after it has been sold.

Provisional techniques

Placing Tags in the Ears: One of the methods of identifying cattle that is the most extensively used and approved is ear tagging. Conventional methods have a number of drawbacks, including the fact that they can be stressful for animals and provide challenges for human visual inspection, all of which can be circumvented by using this system, which also offers easy and inexpensive identification (Barron et al., 2009). Ear tags could be made out of plastic or metal components, and they can be marked with bar-code, numerals, or

color (Özbeyaz and Yüceer-Özkul, 2020; Kumar et al., 2017; Awad 2016). The design requirements of every ear tag should make the item tamper-resistant and aesthetically legible, and the tag should remain attached to the animal without causing harm **(**Stanford et al., 2001). It has been discovered that ear tags are prone to damage, duplication, loss, illegibility, and fraud (Ali Ismail Awad 2016). In addition, ear tags are not an effective long-term identifying mechanism (Fosgate et al., 2006).

Electronic techniques

Radio waves can be used to identify people or things through the use of RFID technology. It is regarded as an appropriate method in a broad variety of fields and applications, such as farming, security systems, stockpile tracking, vehicle parking and monitoring, libraries monitoring, and smart shopping systems, amongst others (Awad 2016). An RFID reader, RFID tags, and a control host or server are the primary components that make up a standard RFID system(Awad 2016). RFID tags can be categorized according to the uses for which they are put to use, the operating frequency, and the technology. When considering their applications, RFID tags can be broken down into three categories: boluses, ear tags, and implantable glass tags (Awad 2016). In terms of their operating frequencies, radio frequency identification tags can be categorized as either low-frequency (LF) devices or high-frequency (HF) devices. The identification of animals has been given responsibility for the LF band (Awad 2016). RFID tags can be classified as either active, which produce radio waves, or passive, which do not. Active tags work at high frequency and provide a reading range of 20–100 meters, while passive RFID tags work at low frequency range and provide a reading range of 0.33–3.30 meters (Awad 2016). RFID modules require time and labor to be configured as an identifying and tracking system, which is the principal disadvantage of adopting RFID-based solutions for animal identification. Consider the cost of purchasing RFID tags and updating them, along with the cost of operating the identifying system.

Comparing RFID to conventional identifying techniques has revealed a number of RFID's benefits. RFID tags may store a substantial amount of information and provide a special identifier for each tag. Thus, an animal may be tracked from birth through slaughter with a single tag. Due to the possibility of information storage, an RFID tag can also contain additional information about the animal being followed, such as its age, gender, breed, and color. In addition, it can save data regarding the owner, the farm, diseases, and the vaccination status of the animal. RFID systems can collaborate with other data recording systems to improve their dependability and usability. They can also be integrated with mobile computing to facilitate accessibility, scalability, and operation (Awad 2016).

Permanent and temporary techniques share nearly identical properties with regard to deployment and maintenance simplicity. All require specialized instruments for placement and removal, and improper use of these tools may cause harm to the user or the animal, particularly with branding and tattooing. In contrast, ear tags can be uninstalled with minimal discomfort for the animal. Permanent and temporary systems do not permit automatic data recording; therefore, all livestock must be manually recognized, requiring substantial labor (Awad 2016).

Animal Biometrics

The dependence of current cattle identification systems on equipment affixed to the animal, rather than the animal itself, is a typical issue. Biometric identifiers, like as muzzle prints and retinal scans, offer a quick and secure way to provide a foolproof animal identification system that will ensure the traceability of livestock back to their original farm (Qiao et al., 2021; Bowling et al., 2008). On the other hand, biometrics has significant obstacles with regard to the collectability and accuracy of the data, and as a result, it is regarded as a study topic that is still in the process of developing.

Muzzle print images

Similar to how human fingerprints have distinct ridges, troughs, and beaded structures, cow muzzle prints exhibit these same characteristics. These uneven features, which are spread across the skin surface of the snout region of an animal, are characterized by white skin channels and black convex patches that are surrounded by grooves. The grooves are distributed over the surface of the skin (Awad 2016). An animal's muzzle print can be viewed as an accurate and permanent biometric feature, one that is distinct enough just to recognize an animal with the same level of precision as human fingerprints (Baranov et al., 1993). Animal muzzle impressions, sometimes known as nose prints, have already been studied for a century (Awad 2016). With muzzle print images can be accomplished using either inks or a camera (Awad 2016). The first approach involves restraining the head of the cattle animal with a head door or halter and applying a little quantity of ink to the animal's dry nose. By placing an

index card on the nose of a cow, the ink is copied to a card that is supported by a block of wood or other hard backing (Awad 2016). However, an accumulation of moisture on the animal's snout and an inability to hold the animal still can result in blurred, illegible photos. Although this method of identification has been shown to be accurate, the varying amount of time required to get an exact print is a disadvantage (Awad 2016). In addition, the images of the inked muzzle prints are insufficient for electronic use.

Iris Patterns

The iris of animals, like the iris of humans, bears distinguishing characteristics. The researchers identified bovine animals using SIFT (Awad 2016) as a feature representation. Typically, a local image feature is related to a change in an image characteristic, such as intensity, color or structure (Awad 2016). The advantage of employing feature points in iris detection is that they are calculated at several places within the picture; hence, they are insensitive to image size and rotations. In addition, no additional iris image preparation or segmentation processes are required (Awad 2016). Modern approaches to parallel processing can also cut down a lot on the time it takes to get SIFT features (Awad, 2013).

The authors of Lu et al. (2014) devised a technique for identifying cows that combines iris features with the 2D Complex Wavelet Transform (2D CWT). Iris pictures were collected using a contactless hand-held device.

Even though the generated results are deemed to be accurate, their trust is low due to the small size of the database. Notably, iris-based recognition of cattle faces the same obstacles as iris-based identification of people.

Retinal vascular patterns

Retinal Vascular Patterns (RVPs) provide such a high degree of accuracy in identifying cattle; RVPs share characteristics with human retinal scans. In addition to providing a high level of security, RVPs are immutable over time, and the retinal blood-vessel patterns in a normally formed animal eye remain basically unchanged from birth to maturity (Awad 2016). Retinal patterns are present in virtually all species, not just meat animals (Awad 2016; Barron et al., 2008). Consequently, this approach is applicable to a wide range of animals, including goats and sheep. In addition, corneal injuries do not impede the capacity to obtain an accurate image of the retina (Awad 2016). Cattle retinal vascular patterns face the same constraints of acceptance, collection, and processing time as human retinal patterns (Awad, 2016).

Image Analyses

The biometric approaches that have been detailed up until this point are methods that are expensive, time-consuming, and require a large labor force in order to be utilized.

The automated recognition of individual cattle, made possible by recent developments in computer science such as deep learning, machine learning, and computer vision, amongst other things, would open up a variety of new avenues leading to increased productivity and economic advantage for the production of cattle or animals in general. This part reviewed some newly identified approaches to cattle.

Andrew et al. (2016) presented an investigation into the totally automated visual recognition of individual Holstein cattle using dorsal RGB-D images captured in actual farm settings. They offer a dataset and propose a method that can successfully identify animal identities from top-down still images by first depth-segmenting animals in RGB-D frames and then extracting a subset of local ASIFT coat descriptors estimated to be sufficiently distinctive across species. They indicate that learning a species-specific identification model is effective, and we exhibit robustness to poor or complex input image conditions, such as the presence of several cows, improper depth segmentation, etc. The suggested approach achieves identification accuracy of 97% based on testing with roughly 86,000 image pair comparisons encompassing a herd of 40 individuals from the FriesianCattle2015 dataset.

The system divides the detected area into animal regions by fitting a depth model and then extracting ASIFT descriptors. To select and use features for cattle identification recovery, an SVM is used to learn a species-wide predictor of descriptor-individuality.

Qiao et al. (2021) developed a deep learning architecture consisting of a convolutional neural network (CNN) and a bidirectional long-short-term memory (BiLSTM) with a self-attention algorithm. In particular, the Inception-V3 CNN was utilized to extract features from a rear-view cattle video clip captured in a feedlot. A BiLSTM layer was then fed with extracted features to collect spatio-temporal information. For the final step of cow identification, they used self-attention to offer a new focus on the traits collected by BiLSTM. They collected 363 rear-view films of 50 cattle at three distinct dates, with a one-month delay between data collection sessions. Using a 30-frame video duration, the proposed method achieved 93.3% identification accuracy, outperforming current state-of-the-art methods (Inception-V3, MLP, SimpleRNN, LSTM, and BiLSTM). In addition, a comparison was

made between additive and multiplicative attention methods. Compared to the multiplicative attention mechanism, which obtained 90.7% accuracy and 87.0% recall, the additive attention mechanism achieved 93.3% accuracy and 91.0% recall. The duration of the film also affected its precision, with 30-frame video sequences boosting identification performance. So their method can take into account important spatial and temporal variables to improve the accuracy of identifying cattle. This makes it possible to automatically identify cattle for precise livestock farming.

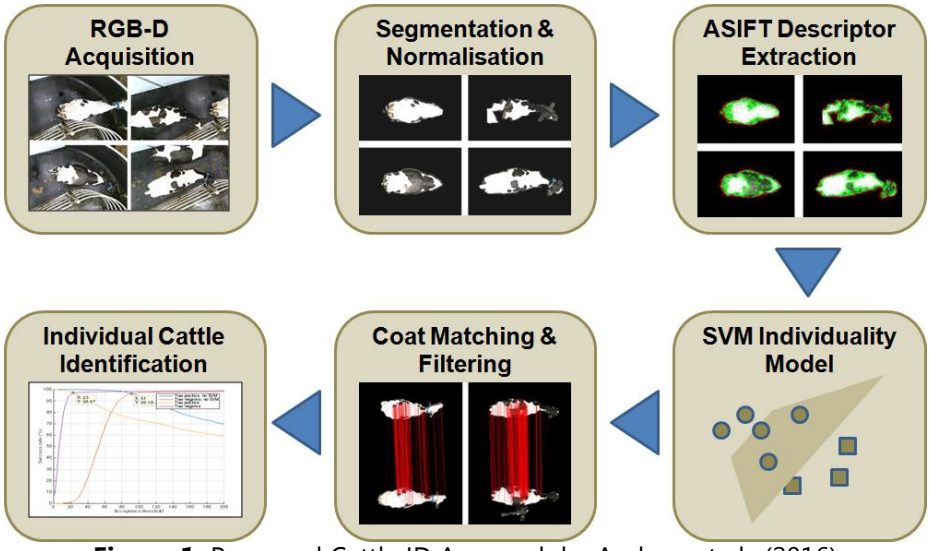

Figure 1. Proposed Cattle ID Approach by Andrew et al., (2016)

Another study conducted by Kumar et al. in 2017 involved animal facial photographs. In the burgeoning study subject of animal biometrics and computer vision, it revealed a current state-of-the-art method for the recognition of individual cattle based on a facial image database. At the initial smoothed level of the Gaussian pyramid of the cow face picture database, the appearance (holistic)-based face recognition approach, independent component analysis (ICA) algorithm, achieved an identification accuracy of 86.95%. The recognition accuracy of the PCA-LiBSVM and ICA-LiBSVM techniques was 95.62 and 95.87%, respectively. Experimental results on a cow face database of 5000 face images (e.g., 500 subjects (cattle) × 10 photographs of each subject) demonstrate the viability of face recognition for cattle.

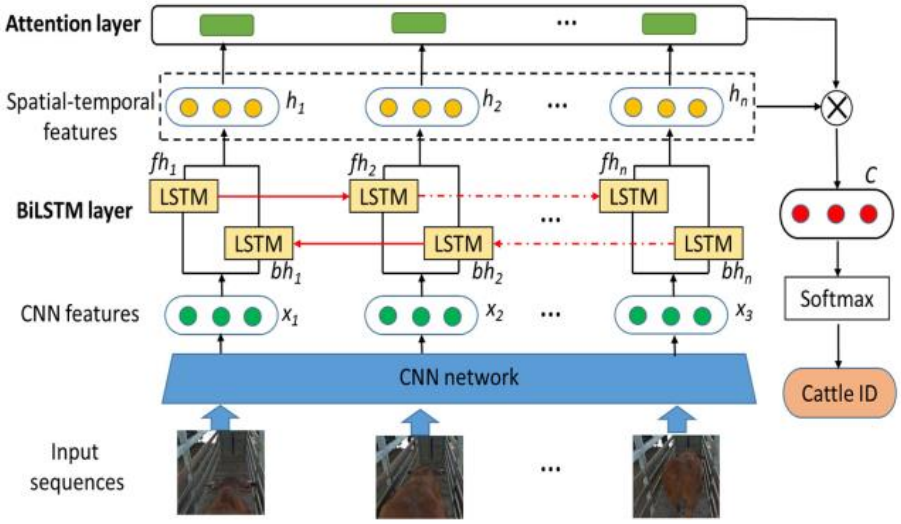

Figure 2. The overall structure of proposed attention-based BiLSTM for cattle identification (Qiao et al.,2021)

As computer technologies like machine learning (ML), the internet of things (IoT), and others get better, animal identification will be able to follow the welfare criteria of animals, vaccines, drugs, and products. This will make animal breeding more economically sustainable.

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BLOOD SAMPLING TECHNIQUES AND PREPARING FOR ANALYSIS IN RAINBOW TROUT (Oncorhynchus mykiss)

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Abstract

In aquaculture, biochemical and hematological analyses are frequently performed for scientific research, health screening and disease diagnosis. In fish, as in other vertebrates, biochemical and hematological parameters vary due to diet, water quality, pathogens and various environmental factors that may cause stress. Blood sampling via caudal venipuncture is a non-invasive method widely used to investigate fish health, biochemistry and physiology. This method is performed under the influence of a properly selected anesthetic agent, with minimal impact on animal welfare and therefore minimal stress, and avoids serious changes in biochemical parameters. This review summarizes the appropriate sampling and selection of the correct anesthetic agent for the analysis to be performed.

Key words: *rainbow trout, blood sampling, biochemistry, haematology*

ANTIMICROBIAL ACTIVITY OF THYME (THYMUS VULGARIS L.) EXTRACTS WITH DIFFERENT SOLVENTS ON THE SIX DIFFERENT MULTIDRUG-RESISTANT ESCHERICHIA COLI STRAINS

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Abstract

This study investigated the antimicrobial activity of methanol, ethanol, acetone, and water extracts of thyme (Thymus vulgaris L.) against multidrug-resistant Escherichia coli strains. The thyme extracts were prepared with methanol, ethanol, acetone, and water as solvents. The disc diffusion method was used to evaluate the bacterial inhibition of thyme extracts against E. coli strains (EC1-6) isolated from the cecum of broiler chicken. Methanol and water extracts had higher (P<0.05) extraction yields than ethanol and acetone extracts. Ethanol, methanol, and acetone extracts showed higher (P<0.01) antibacterial activity than water extract against EC1, EC3, EC4, and EC6, while water extracts had no inhibition zone against EC1-4. Methanol extract also showed the highest (P<0.001) inhibition zone against EC2 compared to other extracts. Similarly, methanol and ethanol extracts had the highest (P<0.001) antimicrobial activity on EC5 compared to other extracts. Water extracts had lower (P<0.05) inhibition zones to EC5 and EC6 strains than the other extracts. The results showed that methanol, ethanol, and acetone extracts of thyme have a high potential for bacterial inhibition against multidrug-resistant E. coli strains.

Key words: *thyme, antimicrobial activity, methanol, Escherichia coli, Thymus vulgaris L.*

OKSIDATIVE STRESS INDEX (OSI) OF SEMINAL PLASMA IS ASSOCIATED WITH POST-THAW SPERM QUALITY IN LOCALLY ADAPTED ANATOLIAN BLACK BULLS

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Abstract

The objective of this study was to determine the oxidative stress index (OSI) of bovine seminal plasma via analyses of total antioksidant status (TAS) and total oksidant status (TOS) and to evaluate the correlation between OSI of seminal plasma and post-thaw sperm quality of bulls. For this purpose, 72 ejaculates were collected from 6 sexually matured Anatolian Black Bulls and split into two aliquots. First one was santrifuged for seperating seminal plasma and latter samples with motility ≥80% were cryopreserved using an automated freezing machine. After thawing ejaculates were classified into two groups as good (GFE) and poor freezability (PFE) through cluster analyses based on post-thaw total motility (PTM) and plasma membran and Acrosome Integrity (PMAI) . To calculate OSI in seminal plasma, TAS and TOS analyses were performed using an automatic chemistry analyzer. OSI levels was negatively corelated with post-thaw Total Motility (r:-0,357 ; P<0,01), Progressive Motility (r:-0,255 ; P<0,05) and PMAI (r:-0,376 ; P<0,05). There was no correlation between HMMP and OSI. Furthermore, TAS levels were found higher in GFE (P<0,05), while TOS (P<0,05) and OSI (P<0,05) levels was found higher in PFE. In conclusion, OSI of seminal plasma was negatively related with post-thaw sperm quality and could be used as predictive biomarker for sperm freezability in bulls. It can be also concluded that the measurement of seminal OSI, which is an easy and quick method, would be useful for determining the amount of exogenous antioxidant to be supplemented to semen extenders.

Key words: *Oxidative Stress, Antioxidants, Sperm Quality, Seminal Plasma, TAS, TOS*

SEMEN COLLECTION, CRYOPRESERVATION AND ARTIFICIAL INSEMINATION IN DOGS

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Abstract

The first scientific artificial insemination in history was made by the Italian L. Spallanzani who used fresh semen in bitches whelping two live puppies in the 1780s. Then, American S.W.J. Seager who used frozen semen obtained the first puppy in 1969. Today, the methods of artificial insemination (AI), which have recently become a biotechnological phenomenon, have been used, apart from the scientific studies in dogs, upon the request of clients and especially for those partners that are largely unable to mate because of differences in their body sizes. The AI is possible only by diluting the semen collected in order to protect spermatozoa from adverse environmental conditions, by using some short- and long-term preservation methods and by transferring processed semen into the oestrous females with proper AI techniques. In the present study, brief information is given about the approach to the male during semen collection, semen collection, dilution, short (at +4 oC) or -long-term storage/freezing (in liquid nitrogen, -196 oC) and AI methods in dogs. For semen collection, mainly digital manipulation, conic plastic rubber and hand massage and also electrical stimulations (electro-ejaculator) are used. For semen dilution, Tes, Bes, Hepes, Pipes, Tris, Tes/Tris and commercial extenders are mostly preferred. A ratio of 1:1-1:5 (semen vs. extender) is used as the dilution rate. In the long-term freezing of semen, freezing mainly within ampoules, pellets and straws is preferred to store semen. Finally, the main AI methods used were intravaginal catheter, intrauterine Norwegian catheter, intrauterine endoscopic method and intrauterine surgical techniques. Therefore, in this short review, brief information is given about the collection and storage of semen along with its use for AI in dogs.

Key words: *Canine, Semen, Preservation, Storage, Insemination*

BIOMARKERS IN THE DIAGNOSIS OF FELINE IMMUNE DEFICIENCY VIRUS (FIV) INFECTION

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Abstract

Feline immunodeficiency virus (FIV), which is observed in domestic and wild cat species. There are 7 subtypes (A, B, C, D, E, F and U-NZenv) of the virus, which is widespread worldwide. Many retroviruses reproduce only in rapidly dividing cells. Most mammalian and avian retroviruses are non-cytocidal and do not significantly alter the metabolism of the cells they infect, but this is not the case with lentiviruses. Virions adsorb to specific cell receptors via one of the enveloped glycoproteins. Entry occurs by receptor-mediated endocytosis or direct fusion with the plasma membrane for different retroviruses. DsDNA syntesis occurs br reverse transcriptase and it moves towards the nucleus and few such molecules integrate as proviruses at random sites in the cell DNA. Integration requires viral integrase and involves removing two nucleotides from the ends of the viral DNA and producing a short copy of the cell lines at the integration site and joining the ends of the viral DNA into the cell DNA. Biomarkers Used in Diagnosis and Treatment; Acute phase proteins (AFR) are molecules in protein structure that are formed as a result of the main reactions that occur during FIV infection. In response to immunity, it is a response to complex nonspecific phenomena that occur in the early stages of the disease. It is a result of the production and release of cytokines such as acute phase proteins, IL-1, IL-6 and Tumor Necrosis Factor -a (TNF-a). AGP is believed to act as an immunomodulator and anti-inflammatory protein. Serum amyloid A (SAA) is a minor protein that appears to potentially be a precursor to a major α-amyloid protein A. C-reactive protein (CRP) was the first AFP described and is considered a major protein that can be found in different species. Treatment: Appropriate treatment should be initiated as early as possible after clinical signs of FIV-infected cats have been identified. Antiviral Treatment, Azidothymidine (AZT) treatment, AMD3100,1,1'-(1,4fenilenbis(metilen))bis1,4,8,11tetraazasiklotetradekan oktahidroklorür treatment, Feline interferon – ω treatment, Recombinant feline interferon-ω therapy, Human interferon – α tretment, Griseofulvin and phylogastrim treatment, Eritropoietin (EPO) treatment, Insulin-like growth factor (IGF-1) treatment, Vaccination are used in the treatment.

Key words: *FIV (Feline immunodeficiency virus), biomarker, HIV1*

INTRODUCTION

Feline immunodeficiency virus (FIV), which is observed in domestic and wild cat species worldwide and is in the retrovirus class, was first described in 1986 by Niels Pedersen et al. Feline immunodeficiency Virus has been the subject of many studies due to its antigenic similarity with HIV-1, which causes AIDS in humans. In studies, FIV is accepted as a model for HIV-1. There are 7 subtypes (A, B, C, D, E, F and U-NZenv) of the virus, which is widespread worldwide (Westman et al 2016). It is an enveloped RNA virus with icosahedral symmetry.

VIRAL REPLICATION

Many retroviruses reproduce only in rapidly dividing cells. Most mammalian and avian retroviruses are non-cytocidal and do not significantly alter the metabolism of the cells they infect, but this is not the case with lentiviruses. Virions adsorb to specific cell receptors via one of the enveloped glycoproteins. Entry occurs by receptor-mediated endocytosis or direct fusion with the plasma membrane for different retroviruses. The nucleoprotein complex is releases into the cytoplasm. Reverse transcriptase copies negative polarity cDNA from the viral genome by using genome-associated tRNA as the primer. In parallel, the viral RNA template is digested by a second domain of the reverse transcriptase molecule carrying RNase H enzymatic activity. The oligonucleotides resulting from this hydrolysis serve as primers for the synthesis of positive polarity cDNA

using newly made negative polarity cDNA as template. The resulting linear duplex DNA contains long terminal repeats (LTRs) consisting of sequences amplified from the 3' (U3) and 5' (U5) ends of viral RNA. So that each end of the provirus contains an LTR consisting of a U3, R and U5 region; Within the LTR are regulatory sequences, including an enhancer/promoter region with binding sites for viral and/or cellular regulatory proteins. DsDNA moves towards the nucleus and few such molecules integrate as proviruses at random sites in the cell DNA. Integration requires viral integrase and involves removing two nucleotides from the ends of the viral DNA and producing a short copy of the cell lines at the integration site and joining the ends of the viral DNA into the cell DNA.

Integration is a prerequisite for virus replication. The integrated provirus is transcribed by cellular RNA polymerase 11. The complete RNA transcript is the same as the original genomic RNA and functions as mRNA. The rnRNA is translated to yield the Env precursor, again in a different reading frame, which is transcribed from full-length RNA. In the case of HTLV and HTV, the differently spliced mRNAs encode various regulatory proteins. All mRNA's shares a common sequence at their 5' ends. Viral protease is responsible for post-translational cleavage. Gag polyprotein is involved in obtaining reverse transcriptase / RNase H and integrase to yield matrix, capsid and nucleocapsid proteins, as well as for cleavage of Pol polyprotein. On the other hand, the Env polyprotein is cleaved by a cellular protease to yield two enveloped glycoproteins.

The assembly details of the virion are not fully understood and differs from genus to genus. Two covalently linked molecules of full-length positive-sense genomic RPJA form a core as a result of the interaction of a packaging signal in the leader sequence of RNA with binding sites in the nucleocapsid protein. Capsids are usually assembles on the cell surface. Myristicized and glycosylated envelope proteins penetrates the plasma membrane and form peplomers of the envelope that the virion gains by exocytosis. The final stages of proteolytic cleavage occurs during and even after budding.

BIOMARKERS USED IN DIAGNOSIS AND TREATMENT

Acute phase proteins (AFR) are molecules in protein structure that are formed as a result of the main reactions that occur during FIV infection. In response to immunity, it is a response to complex nonspecific phenomena that occur in the early stages of the disease. It is a result of the production and release of cytokines such as acute phase proteins, IL-1, IL-6 and Tumor Necrosis Factor -a (TNF-a). These mediators cause changes in the body including fever, leukocytosis and protein synthesis. Acute phase proteins that increase during infection are believed to act as immunomodulators. The best described AFPs in cats on this subject are a-1-glycoprotein (AGP) and serum amyloid A (SAA) (Petersen et al. 2004).

AGP is believed to act as an immunomodulator and anti-inflammatory protein. IL-1R antagonists modulate production by reducing platelet aggregation and lymphoid proliferation by macrophages. Studies have shown that FIV positive cats have lower AGP concentrations than healthy ones (Korman et al2012).

Serum amyloid A (SAA) is a minor protein that appears to potentially be a precursor to a major α-amyloid protein A, and has been implicated in a variety of chronic inflammatory diseases (Uhlar and Whitehead 1999). Among the main functions of serum amyloid A is to clear oxidized metabolites and protect tissues from excessive damage caused by fire (He et al. 2006).

C-reactive protein (CRP) was the first AFP described and is considered a major protein that can be found in different species. It has an important role in the immune system of humans and dogs (Ceron et al. 2005). CRP is involved in the activation of the classical complement pathway and increases phagocytosis (Schultz et al 1990). Although CRP is not involved in the acute phase reaction in cats, its functions have not been well studied or fully demonstrated (Ceron et al. 2005). It has been found in many studies in human medicine that it increases in HIV-positive patients (Jahoor et al. 1999). In addition, immunomodulation treatment with exogenous IL-2 is also performed (Barbai et al.2010). Despite the similarity between HIV and FIV, CRP levels in FIV-positive cats are still unknown in patients receiving immunomodulation therapy (Hosie et al.2009).

Recombinant feline interferon-ω (rFeIFN-ω) therapy (Virbagen, Virbac) is an immunomodulating drug that plays an important role in the therapeutic approach for various feline diseases. It is also used in feline retrovirus infections. (Collado et al. 2006). APPs have been used to monitor treatment after immunotherapy. The main purpose of the studies was to determine whether rFeIFN-ω treatment affects AFPs (SAA, AGP and CRP) and

to determine whether these parameters stimulate innate immunity in naturally retroviral infected cats (Mari et al 2004). Studies describing the use of AFPs as a clinical monitoring tool are a weak tool for the immune system in IFN-treated animals (Leal et al 2014).

TREATMENT

Appropriate treatment should be initiated as early as possible after clinical signs of FIV-infected cats have been identified. If FIV-infected cats are sick, prompt and accurate identification of secondary infections is essential for early initiation of therapeutic intervention and successful treatment outcome. Therefore, more intensive diagnostic testing should be performed during illness than is recommended for uninfected cats.

Antiviral Treatment

Most antiviral drugs used in cats are licensed for humans and are specifically designed for the treatment of HIV infection. Some of these can be used to treat FIV infection. However, many of the available drugs are toxic or ineffective for cats (Hosie et al 2009).

Azidothymidine (AZT) treatment

AZT (3'-azido-2',3'-dideoxythymidine) is a nucleoside analog (thymidine derivative) that blocks reverse transcriptase of retroviruses. AZT has been shown to inhibit FIV virus replication in vitro and in vivo. It can reduce the plasma virus load, improve the immunological and clinical condition of cats with FIV, and increase the quality of life. In a placebo-controlled study, it was determined that AZT administration developed stomatitis in naturally infected cats (Hartmann et al. 1995). The recommended dosage is 5-10 mg/kg PO or SC every 12 hours. It should be used with caution in high doses as side effects may occur. The lyophilized product for SC injection should be diluted in isotonic NaCl solution to prevent local response. For PO administration, syrup or gelatin capsules (dose/weight for each cat individually) may be given. During the treatment, hemogram analyzes should be performed regularly (once a week for the first month), because non-regenerative anemia, which is an important side effect, develops, especially in high-dose drug use. If the values are stable after the first month, the monthly check is sufficient. Bone marrow suppressed cats should not be treated with this method. Studies in which cats infected with FIV were treated for two years showed that AZT was well tolerated (Hosie et al 2009). Some cats may develop a slight hematocrit reduction within the first three weeks of treatment initiation. If the hematocrit falls below 20%, it is recommended to discontinue AZT and the anemia usually resolves within a few days after discontinuation of the drug (Hosie et al 2009).

AMD3100,1,1'(1,4fenilenbis(metilen))bis1,4,8,11 tetraazasiklotetradekan oktahidroklorür treatment

AMD3100,1,1'-(1,4phenylenebis(methylene))bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride, JM3100, SID791 belongs to the new class of bicyclamlams that act as selective antagonists of the chemokine receptor. CXCR4 is the main co-receptor for T-cell-stimulated HIV strains. Blocking the CXCR4 receptor leads to inhibition of virus entry. FIV also uses CXCR4 for virus entry (Willett et al 1999). There is a significant degree of similarity between human and feline CXCR4s. AMD3100 is licensed as a stem cell activator for patients undergoing bone marrow transplant rather than an antiviral drug. No adverse effects were observed in cats receiving AMD3100 (Hartman et al 2002).

Feline interferon – ω treatment

Recombinant feline interferon-ω therapy is an immunomodulator used in the treatment of different retroviral diseases, including FIV and FeLV. Although its mechanism of action has not been determined, it has been observed that this drug enhances innate immunity. Feline interferon-ω has recently been licensed for use in veterinary medicine in some European countries and Japan. Because interferons are species-specific, feline interferon-ω can be used for life without inducing antibody development. Although there are not enough studies on this subject in cats, no side effects have been reported (Mari et al 2004).

Human interferon – α tretment

Besides the immune modulatory effects of human interferon-α, it acts as a true antiviral compound that protects the body against virus replications (Tompkins et al 1999). There are two common treatment protocols for the use of human interferon-a in cats, either high-dose subcutaneous injection (104-106 IU/kg every 24 hours) or low-dose oral administration (1 to 50 IU/kg every 24 hours). When given subcutaneously in high doses, interferon-α results in measurable serum levels. However, it becomes ineffective after 3 to 7 weeks due to the development of neutralized antibodies (Zeidner et al 1990).

Griseofulvin and phylogastrim treatment

Many cats with FIV, unlike non-infected cats, require a longer or more aggressive course of

therapy (eg antibiotics) and respond well to appropriate medications. Some clinicians have reported that the use of corticosteroids and other immunosuppressive drugs in cats with FIV with chronic stomatitis is clinically beneficial, but their use is controversial because of their potential side effects. It has been reported that griseofulvin causes bone marrow suppression in cats with FIV and should not be used (Shelton et al 1990). Phlogastrin, a recombinant human product (rHuG-CSF) cytokine, can increase the neutrophil count in FIV lichens with veneutropenia (Phillips et al 2005). The increase in the amount of virus also causes an increase in mononuclear cells and lymphocytes in the peripheral blood (Araive et al 2000).

Eritropoietin (EPO) treatment

Erythropoietin is commercially available as a recombinant human product (rHuEPO) and is effectively used in cats with non-regenerative anemia due to endogenous erythropoietin deficiency in chronic renal failure. A gradual increase in erythrocyte and leukocyte counts was observed in cats with FIV treated with human erythropoietin (100 IU/kg SC every 48 hours) (Aral et al 2000). No increase in viral loads was observed and therefore human erythropoietin was found to be safe to use in FIV-infected cats.

Insulin-like growth factor (IGF-1) treatment

Insulin-like growth factor-1 is commercially available as a recombinant human product (rHuIGF-1). Among other actions, it has the ability to induce thymic growth and stimulate T-cell function. Administration of human IGF-1 to cats experimentally infected with FIV resulted in a significant increase in thymus size and thymic cortical regeneration, which replenishes the peripheral T cell pool (Woo et al 1999). IGF-1 can be considered as supportive therapy in young cats infected with FIV, but there is no study showing the effect of IGF-1 in naturally infected cats (Hosie et al 2009).

Vaccination

There are many subtypes of FIV infection common in cats. A and B subtypes are common in the world. An effective FIV vaccination should include predominantly circulating subtypes A and C. In a study conducted in Australia, it was determined that it was almost impossible to understand when cats were infected with FIV and to ensure that control groups were negative for FIV. Screening of both vaccines and controls with FIV testing at day 0 in such studies will avoid potential bias, but a fairly large sample population is required to account for such a study design.

The mechanisms by which the FIV vaccine confers sterile immunity against certain subtypes are still unclear. In experimental studies, it is thought that protection occurs through cellular and humoral immunity, especially antibody production directed in humoral immunity.

There are many role models for FIV vaccine design. Different protocols of tried and formulated vector-based, Deoxy Ribo Nucleic Acid (DNA)-based adjuvants including IWV, IWC, recombinant gene (eg p24), gene deletion were applied. Vaccination of cats against FIV using an IWC vaccine works better than an IWV vaccine, and this vaccine is generally believed to have VNA induction of both virus neutralizing antibodies to prevent virus binding. The prototype FIV vaccine it contained only IWV (no IWC), surpassing the European FIV vaccine (Hosie et al 2007). The vaccine known as Fel-O-Vax is also the IWV formulation in FIV (Hosie et al 2009).

There is no currently available FIV vaccine commercially in Europe. Experimentally, inactivated cell vaccines and vaccines against canary disease have been reported to protect against FIV infection (Hosie et al. 2007). The most successful of these vaccines to date are inactivated virus vaccines (WIV) preparations. One such vaccine was commercially available to veterinarians in the USA in 2002 and in Australia and New Zealand in 2004. However, the vaccine is no longer available in the US.

In addition, a recent Australian study cast doubts on the effectiveness of Fel-O-Vax as FIV was only 56% effective in field conditions.

Sterilization immunity cannot be provided in FIV and HIV, and as a more realistic goal, a vaccine that reduces viral load should be found. What should be emphasized in future research is to reduce FIV disease rather than prevent infection (Hosie et al 2009).

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FAECAL PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* SPP IN DAIRY CATTLE IN ALGERIA

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Abstract

Salmonellosis is an inoculable and contagious infectious disease caused by ubiquitous enterobacteria of the genus Salmonella. This is one of the main causes of foodborne illness in humans in developed countries. In cattle, many Salmonella enterica serotypes are responsible for the wide variety of clinical manifestations that can cause considerable economic loss. The purpose of this study is to determine the prevalence of Salmonella spp. isolated from cow feces, as well as to serological identification and the study of the susceptibility of these strains to antibiotics. This study was carried out in different cattle farms in the Khenchela region. The prevalence was established on the one hand after bacteriological analysis of 307 samples of fecal material from cows belonging to 39 different farms. Bacteriological results using the reference method NF U 47-100 showed a prevalence of 0.97% (IC95% 00% - 2.08%). Serotyping revealed a prevalence of 0.97% for S. Mbandaka. The in vitro antibiotic susceptibility test indicated that Salmonella Mbandaka, they were 100% resistant to cefazolin, ceffoxitin, kanamycin, gentamicin, tobramicin, amikacin and netilmicin. This report shows that the animal world is a huge reservoir of Salmonella and bovine salmonellosis is only a part of it. Control of bovine salmonellosis requires the simultaneous application of therapeutic, sanitary and hygienic measures. In addition, the acquisition by Salmonella of many antibiotic resistance becomes of concern in both animal health and human health.

Key words: *Cows, Feces, Salmonella* spp*, Prevalence, Antibiotic resistance.*

INTRODUCTION

In cattle, salmonellosis is a zoonotic disease caused by several serovars of Salmonella *enterica* that can cause considerable economic losses. Due to its wide dissemination in the environment, its prevalence in the global food chain, its virulence and its adaptability, *Salmonella enterica* is the germ most frequently encountered in human and animal pathology (Tindall et al., 2005). It has a considerable impact in medicine, public health and the world economy (Miller et al., 2000). Currently, more than 2600 serovars of *Salmonella enterica* are described (Nataro et al., 2007). Most salmonellae are localized in the gastrointestinal tracts of domestic or wild mammals, as well as in reptiles, birds and insects. (Gillespie et al., 2005).

Furthermore, the use of antibiotics outside the legislative framework in veterinary practice has resulted in the emergence of multi-resistant strains that can reach humans; this phenomenon is all the more worrying in that it affects strains that were sensitive until now and extends to antibiotics reserved for human medicine, hence the risk of therapeutic impasse.

The aim of our work concerns the search for *Salmonella* in bovine faeces and the determination of the serovars, followed by the study of the sensitivity to antibiotics of the isolated strains.

MATERIALS AND METHODS

Study area

This study was carried out in Khenchela region. This region is located in the east of Algeria, and it's characterized by *a large number of cattle* (4478 cows in 2018), and a promising milk sector (27 million liters of milk per year). The altitude range is from 1050 to 1710 meters and the daily average temperature ranges from -2°C to 42°C.

Sampling

A total of 39 farms were randomly selected, from which, 307 fecal samples were taken and analyzed. About 25g of individual fecal samples of cows were collected directly from the rectum using disposable gloves, and then stored in sterile pots. Samples were then sent for analysis on the same day.

The minimum number of cattle to be tested on each farm was established as 10 (Cannon and Roe, 1982), the number of cattle to be sampled on each farm was defined on the basis of the total number of cattle in the farm: the farm consisted of less than 10 cattle, in which case all cattle were harvested or the farm contained more than 10 cattle and, in this case, at least 10 individuals were taken.

BACTERIOLOGICAL CULTURE

Isolation of *Salmonella* spp.

The isolation was performed according to the AFNOR standard (NF U: 47–100) (2007). 25g of individual fecal samples were mixed with 225 mL of buffered peptone water (Condalab, Spain) and incubated for 24h at 37°C. Then, 1 mL of the pre-enriched culture was transferred to Müller Kauffmann Tetrathionate- novobiocin broth (Bio-Rad, France) and 0.1 mL of the same pre-enriched culture was transferred to Modified Semisolid Rappaport Vassiliadis Medium (MSRV; Condalab, Madrid, Spain) and incubated at 37°C and 42°C for 24h respectively. A loopful from each culture was streaked into selective xylose-lysine-deoxycholate agar (Condalab, Spain) and Hektoen agar plates (HK; Institut Pasteur Algeria (IPA)), and incubated at 37°C for 24h. The initial biochemical tests were performed on a 24h pure culture using Triple Sugar Iron (TSI; IPA) agar slant, indole urea reagent (IPA), Lysine Decarboxylase (LDC; IPA) reagent and ortho-NitroPhenyl-β-galactoside (ONPG; IPA). Then, the API 20E system (BioMérieux, France)**.**

Serotyping of *Salmonella*

Salmonella serovars were identified serologically by slide agglutination test using diagnostic polyvalent and monovalent O and H *Salmonella* antisera according to Kauffman–White scheme (Grimont and weill, 2007).

Antimicrobial susceptibility testing

The agar disk diffusion method was used to determine the antimicrobial susceptibility patterns of *Salmonella* isolates according to the Clinical and Laboratory Standards Institute guidelines, (CLSI) (2018) Using Mueller- Hinton agar (IPA, Algiers, Algeria). The isolates were tested for the following antibiotics (disk content): ampicillin (10 µg), piperacillin (100 µg), ticarcillin (75 µg), amoxicillin/clavulanate (20 µg/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), imipenem (10 µg), sulfonamides (300 µg), trimethoprim (5 µg), cortrimoxazol (25µg), nalidixic acid (30 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), colistin (10 µg), furans (300 µg), chloramphenicol (30 µg) and tetracycline (30 µg), the results were evaluated after 24h of incubation at 35°C.

RESULTS AND DISCUSSION

Bacterial isolation and serotyping:

Of 307 faecal samples collected from dairy cows, 3/307 (0.97%) bacterial isolates (95% CI: 0 to 2.08) were recovered. The three serotyped *Salmonella* isolates are all from Mbandaka.

Antibiotic sensitivity test

The three Salmonella isolates from cows were tested against a panel of thirty antimicrobials. The antimicrobial susceptibility profile of the isolates indicated that all isolates had high susceptibilities against ampicillin, piperacillin, ticarcillin, amoxicillin, amoxicillin/clavulanate, norfloxacin, ciprofloxacin, colistin, furans, chloramphenicol, tetracycline. On the contrary, they were 100% resistant to cefazolin, cefoxitin, kanamycin, gentamicin, tobramicin, amikacin and netilmicin.

Salmonella infections are a major concern for the various animal productions and for public health. Ruminants, particularly cattle, are victims of salmonellosis with serious symptoms and heavy economic consequences.

In the present study, out of 307 faecal samples collected from dairy cows, 3 (0.97%) *Salmonella* spp. were isolated. Almost similar results had already been reported: 0.9% in Spain (Adesiyun et al., 1996), 0.97% in Egypt (Mohamed et al., 2011), 1.25% in Iran (Halimi et al. , 2014), 1.74% in Turkey. (Hadimli et al., 2017). The prevalence of *Salmonella* in cattle feces can be much higher: 7.6% in Algiers (Hezil et al., 2021), 9.3% in the states of Minnesota, Wisconsin and New York (Warnick et al ., 2003); 9.96% in the United States (Callaway et al., 2005), 10.1% in the United States (Cummings et al., 2010). Callaway et al. Found an isolation rate of 9.96% in a sample of 960 cows. The higher prevalence than that obtained in our study can be explained by the number of samples, which is much higher in the other studies. Region may also influence the frequency of isolation across studies. Indeed, several researchers have confirmed this hypothesis (Eguale et al., 2016; Hezil et al., 2021).

In our study, isolates detected in faecal samples belonged to a serovar (Mbandaka) infrequently reported in cattle. Nevertheless, in some studies conducted in the United States, it was one of the most prevalent serotypes in the slaughterhouse (Wells et al., 2000). *S.* Mbandaka could survive on cattle hides and be transmitted from cattle to the slaughterhouse environment.

The three *Salmonella* isolated in the current study were 100% resistant to cefazolin, cefoxitin, tobramycin, amikacin and netilmicin. This result is consistent with results reported in Ethiopia (Addis et al., 2011) and Turkey (Hadimil et al., 2017).

Nevertheless, not all *Salmonella* isolates from Ethiopia and Nigeria were susceptible to ampicillin and amoxicillin (Addis et al., 2011) unlike our study where all isolates were susceptible to ampicillin and amoxicillin. Studies of antimicrobial use in dairy cow herds in Ethiopia and the United States have shown that beta-lactams are among the most commonly used antimicrobial agents (Sawant et al., 2005). However, few studies on dairy farms have examined the association between antimicrobial treatment at the herd or animal level and the prevalence of antimicrobial resistant *Salmonella.*

Quinolones and chloramphenicol showed antimicrobial activity against cow isolates. This finding is consistent with previous reports by Addis et al. (2011) from Ethiopia (Addis et al., 2011) Egypt (Mohamed et al., 2011). These antimicrobials are not commonly used on most farms for the management of bacterial infections leading to less resistant bacteria.

CONCLUSIONS

In conclusion, This report shows that the animal world is a huge reservoir of salmonella and bovine salmonellosis is only a part of it. The importance of this disease has been steadily increasing for several years.

The control of bovine salmonellosis requires the parallel application of restrictive therapeutic, sanitary and hygienic measures. In addition, the acquisition by *Salmonella* of many antibiotic resistances is becoming a concern in both animal and human health.

ACKNOWLEDGEMENTS

The authors wish to thank the animal and local resource management lab and the national veterinary school in Algiers, for the resources made available by them, and the veterinarians of the Khenchela region for their participation in providing the samples.

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EFFECT OF HEXAFLUMURON ON LIPID CONSTITUENTS IN ADULT WISTAR RATS

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Abstract

Excessive use of insecticides is one of the main reasons for contamination of the material cycle in nature and causes many harmful effects on humans and other living organisms. Hexaflumuron is a systemic pesticide of the benzoyl urea group that kills insects by Inhibition of chitin synthesis. Chitin is the main component of the insects´ exoskeleton. The current study was performed to assess the effect of Hexaflumuron on plasma lipid constituents in adult Wistar rats. twenty rats were divided in five groups of 4 each. Group A presented as normal control. Group B, C, D and E were administered hexaflumuron in the technical form by gavage with different doses(110 or 22 or 16.5 or 11 mg/kg respectively, corresponding to LD50, 20% of LD50, 15% of LD50, 10% of LD50) for 28 days. The plasma levels of total cholesterol(TC) and triglycerides(TG) were measured. The highest amount of triglycerides and total cholesterol was observed in 20% of LD50 group (B) and no significant difference observed between groups.Although pesticides have several adverse effects on lipid concentrations, hexaflumuron in its technical form had no significant effect on lipid concentration levels in adult wistar rats.

Key words: *Hexaflumuron, Triglycerides, Total cholesterol, Rats*

THE EFFECTS OF PROGESTOGEN AND RAM EXPOSURE, WITH OR WITHOUT ECG ON REPRODUCTIVE PERFORMANCE OF FAT TAIL EWES IN NON-BREEDING SEASON

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Abstract

Sheep are considered short-day breeders. Exposure of rams to anestrus ewes results in an increase in LH secretion. The aim of this study was to determine whether progesterone (p4) analogue with or without equine chorionic gonadotropin (eCG) and exposure of rams can effect on reproductive performance such as: pregnancy rate, litter size, sex of lamb, fecundity rate and prolificacy rate of fat tail Iranian Shaal breed ewes in non-breeding season. Totally, 100 ewes out of 138 non-cyclic ewes with <0.5ng/ml P4 were selected for this study. They were aged 2 to >7 years old. They were stratified to two groups as control and treatment. They received 13 days a sponge containing 60 mg medroxyprogesterone acetate (MPA). The treatment group consists of 3 subgroups and each group contains 25 ewes equally. They were included: 1- P4, eCG and Ram exposure. 2- P4 and ram exposure. 3- P4 and eCG. Ewes in control group received only P4. The ewes were located in isolated corrals for 40 days. The ram to ewe ratio was 1 to 5. Twenty healthy rams were housed in an isolated pen from ewes with distance 1500 m during 40 days. Rams exposed three days before sponge withdraw (day 10). They were separated by considerable distance (2 meters) using fence. The related groups received 500 IU eCG on the day of sponge removal (day 13). The rams were released into the ewe flock after removing the sponge in treatment and control groups. Pregnancy diagnosis performed using transabdominal ultrasonography. The obtained data analyzed using SPSS version 16. The conception rate in treatment and control groups were differed significantly (P<0.01). The conception rate in P4+ eCG+ ram exposure, P4+ramexposure and P4+ eCG were 96%(no.24), 80%(no.20) and 88%(no.22), respectively. However, the conception rate in control group was 60% (no. 15). The fecundity rates were 136%, 100% and 124% in treatment subgroups, however, it was 84% in control group, respectively. There were no significant differences among genus and weight of lambs in treatments subgroups and control group (P>0.05).
It is concluded that progestogen, ram exposure with eCG can increase significantly reproductive performance in non-breeding season fat tailed ewes Shaal breed.

Key words: *Reproductive performance, ewe, eCG, progestogen, ram exposure, Shaal*

INTRODUCTION

Sheep are seasonally polyestrous, short-day breeders, cycling in late summer and early autumn, resulting in late winter and spring lambing (Shinomiya et al., 2014). Their breeding season is regulated by the length of the day or, more specifically, by the increased duration of night which is the main environmental factor responsible for the seasonality of reproduction in sexual activity.

Seasonal breeding in sheep is a problem for sheep industry. Although the gestation length of the ewe averages only 148 d, the most ewes are not able to have cycle in spring. Therefore, it is a natural limitation for producer to have only one lamb crop per year.

The stimulus for the onset annual of reproductive activity is the reduction length of daylight. Artificial manipulation of reproduction system using hormonal agents can induce the out of breeding season sexual activity. Exogenous progesterone can be used in anestrous ewe to induce estrus in out of breeding season. It is administered continually over a period of time similar to the normal luteal phase, therefore, it mimics the luteal phase and prime the brain for estrus cycle, and behavioral estrus after withdrawal. After that, the natural

follicular phase process will be stimulated (Catalina Cabrera et al., 2019. Noaks et al., 2019). Progesterone analogues or progestogens(P4) are marketed as sponges, vaginal devices or as implant (Garoussi et al., 2019, Talebkhan Garoussi and Golzar., 2005). The combination of equine chorionic gonadotropin (eCG), formerly pregnant mare serum gonadotropin (PMSG) with progestogens priming is a popular option for small ruminants to enhance follicular growth and ovulation (Joaquim de Sousa Lima et al., 2021. Garoussi et al., 2019. Ozyurtlu et al., 2010. Windorski et al., 2008).

The response of isolated anestrous ewes to the exposure of rams characterized by an increase in luteinizing hormone (LH) pulse frequency and a reduction in the negative feedback of estradiol-17β on the hypothalamic-pituitary axis culminating in an LH surge (approximately 30 h after ram releasing) that is followed by an ovulation (Noaks et al. 2019. Youngquist and Threlfall 2007. Martin et al. 1986 Martin et al., 1980). The resulting corpus luteum (CL) has either a normal or a short life span (Martin et al. 1986). Most ewes with a normal life span CL following their first ovulation display estrus prior to their second ovulation. Exposure of anestrous ewes to rams has also been shown to increase the number and diameter of ovarian follicles (Atkinson and Williamson, 1985). It is shown that ewes which run continually with rams are more fertile than ewes that are kept isolated from rams (Abecia et al., 2015, Nugent et al., 1988).

Ram exposure allows rams to have only visual, olfactory, auditory without any tactile contact with ewes. Ewes must have no contact with rams by either sight, sound, or smell, which means that they must be separated by considerable distance. The great value of the ram exposure is synchronization of estrus activity (Abecia et al., 2015. Hawken et al., 2005).

In out of breeding season anestrous ewes, the exposure of males triggers an increase in the frequency of ovulation (Delgadillo et al., 2009). This increase stimulates follicular development and is followed by a preovulatory surge of LH. Consequently, a high percentage of females exposed to rams ovulate within the first 3-4 days after the stimulus (Martin et al., 1986). However, this ovulation is not accompanied by estrous behavior unless ewes are primed with progestagens (Hunter and Einer-Jensen.,2005). During the non-breeding season, progestagen priming may be used for a period of time (9-14 days), followed by the administration of equine chorionic gonadotrophin (eCG) (Garoussi et al., 2019. Garoussi et al., 2012. Ungerfeld and Rubianes, 1999.) or the introduction of rams (Ungerfeld et al., 2003).

The objective of this study was to determine whether use of progestogen and ram exposure with or without eCG can effect on reproductive performance such as: pregnancy rate, litter size, sex of lamb, fecundity rate and prolificacy rate fertility, fecundity of Shaal breed ewes in non-breeding season.

MATERIALS AND METHODS

The experiment was performed in May-July (the nonbreeding season) under the influence of natural lighting in the suburb of Tehran-Iran (the capital). Shaal breed sheep is a local breed from shaal area in Ghazvin Province-Iran whose characteristics have been described (Akbarinehad et al., 2014). Rams used in this experiment were of the same breed.

In total, 100 out of 138 non-pregnant, non-lactating, fat-tailed Shaal breed ewes almost 2 to 7 years old were selected for out of breeding season program. The weight of the ewes ranged 50-60 Kg. Palpation of the ewes back were used for evaluation of Body Condition Scoring (BCS) (1-5 points) (Kenyon et al. 2014). The selected ewes were located in isolated corrals under strict sanitary programs and dietary measures including hay, corn silage, straw and concentrate with trace elements and minerals.

Twenty healthy rams were selected for breeding. They were housed in an isolated pen from ewes with distance 1500 m during 40 days before the initiation of the experiments not to see, hear the sounds and smell of the ewes.

Three experiments were conducted to examine the effects of the combination of Progestogen (Medroxyprogesterone acetate) (MPA) intravaginal sponge treatment consist of 60 mg MPA and ram exposure with or without eCG on estrus synchronization, reproduction performance, such as: pregnancy rate, litter size, sex of lamb, fecundity rate and prolificacy rate (Youngquist and Threlfall 2007).

They were included 2 groups: treatment and control. All of the ewes in both groups received 13 days of P4-impregnated intravaginal sponges (Gonaser, Hipra, Spain). However, ewes in treatment group divided to 3 subgroups included: 1- P4, eCG and Ram exposure (no.25). 2- P4 and ram exposure (no.25). 3- P4 and eCG (no.25). Ewes in control group received only P4 (no.25).

They were separated by considerable distance (2 meters) using fence. Rams exposed to related ewes 3 days before sponge withdrawal (day 10). Rams removed at sponge withdrawal. At sponge withdrawal ewes in related treatment subgroups received 500 IU eCG. The rams were released into the ewe flock after removing the sponge and eCG injection (Fig 1).

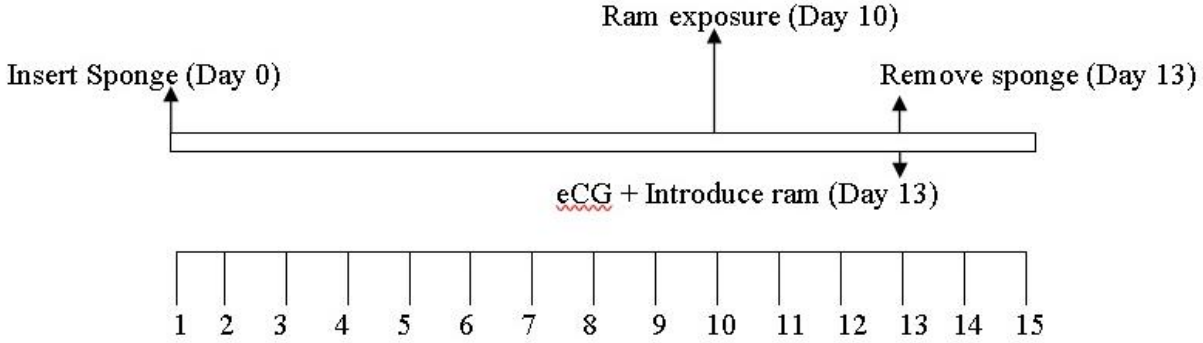

Figure 1: Protocol used for oestrus-synchronization in the anoestrous ewes during the non-breeding season using progestagen (MAP) intravaginal sponge, ram exposure with or without eCG.

Hormonal assays

1. Progesterone assay

In order to make sure that the ewes were in seasonal anestrus (blood P4 < 0/5 ng/ml) (Noaks et al. 2019), approximately 10 ml two blood samples from jugular vein were collected 10 days apart for serum progesterone analysis. The samples were drawn into 10ml sterile collecting tubes and sent to the laboratory for analysis. The collected samples were centrifuged at 3000×g for 20 min. The extracted serum poured into a 2-ml Eppendorf tubes and the number of each ewe was established on the tubes. They were stored at − 21 °C until the P4 analysis. The P4 level was assayed using ELISA kit (DRG Instruments GmbH, Germany). Ewes (no. 38) with the P4 level > 0/5 ng/ml were eliminated from the experiment.

Statistical analysis

Data were analyzed at $P \leq 0.05$ using the SPSS statistical software (Version 25 SPSS Inc, Chicago, Illinois).

RESULTS

Ewes exposed to the rams and used eCG exhibited a higher proportion of pregnancy rate throughout the experiment than the ewes in control group (P<0.05) (Table 1). The pregnancy rate in P4, ram exposure (RE) + eCG and P4+eCG were 96% and 88%, respectively. However, there were no significant differences in treatment ewes without eCG in comparison with control group (P>0.05) (table 1). The exact P-values were 0.002 and 0.02 for the above mentioned groups, respectively.

Table 1. The effect of ram exposure, eCG and Progestogen on conception rate in nonbreeding season in fat tail shaal breed ewes

Groups	Pregnancy rate		Total	P-Value
	+ (%)	-(%)		
Control (P4)	15(60)[a]	10(40)[a]	25	
Treatment				
P4,+RE+eCG	24(96)[b]	1(4)[b]	25	0.002
P4+RE	20 (80)	5(20)	25	0.12
P4 + eCG	22(88)[c]	3(12)[c]	25	0.02
Total	81(81)	19(19)	100	

a,b and c within a panel, categories without a common superscript differed (P<0.05).
RE: Ram Exposure, eCG: Equine Chronic Gonadotropin, P4: Progesterone analogue

Table 2 shows the reproductive performance of the ewes in progestogen and ram exposure programming with or without eCG in non-breeding season in fat tail Shaal ewes. There were no significant differences between single or twin lambing in treatment and control groups (P>0.05). The fecundity rate increased in treatments group in comparison with control group. They were 136%, 100% and 124% in treatment subgroups, however, it was 84% in control group, respectively. The prolificacy rates are shown in table 2.

Table 2. Reproductive performance in ram exposure, P4 analogues, with or without eCG program in non-breeding season fat tail Shaal breed

Groups	Lambing		Total (%)	P-Value	Lambing rate (%)	Fecundity rate (%)	Prolificacy rate (%)
	Single (%)	Twin (%)					
Control (P4)	9 (11.11)	6 (7.4)	15 (18.51)		60	84	1.4
Treatments							
P4+RE+eCG	14 (17.28)	10 (12.34)	24 (29.62)	0.92	96	136	1.42
P4+ RE	15 (18.51)	5 (6.17)	20 (24.69)	0.34	80	100	1.25
P4+ eCG	13 (16.04)	9 (11.11)	22 (27.16)	0.96	88	124	1.4
Total	51 (62.96)	30 (37.03)	81	0.83			

Non-significant differences (P < 0.05).

Table 3 shows the distribution of genus of lambs and birth weight of the lambs in out of breeding season in progestogen and ram exposure programing with or without eCG. There were no significant differences among lamb genus in treatments and control groups (P>0.05). The weight of male lambs averaged greater than female lambs. However, they did not differ significantly in treatments and control groups (P>0.05).

Table 3: Distribution of lamb genus and average birth weight in nonbreeding season program using ram exposure, P4 analogues with or without eCG in fat tail Shaal breed

Groups	Lamb Genus					Total (%)	Weight (Kg)					
	Single		Twin				Single		Twin			
	♂ (%)	♀ (%)	♂♂ (%)	♀♀ (%)	♂♀ (%)		♂	♀	♂♂	♀♀	♂♀	
											♂	♀
Control (P4)	4 3.6	5 4.5	1 0.9	1 0.9	4 3.6	21 18.91	5.02	5.22	4.4	4.25	4.37	3.7
Treatments												
P4+RE+eCG	8 7.2	6 5.4	3 2.7	3 2.7	4 3.6	34 30.63	4.24	5.08	3.97	4.55	4.77	4.57
P4+ RE	4 3.6	11 9.9	1 0.9	2 1.8	2 1.75	25 22.52	5.4	4.9	4.4	3.7	4.55	4.35
P4+ eCG	7 6.3	6 5.4	3 2.7	2 1.8	4 3.6	31 27.92	5.18	4.2	3.9	4.85	4.22	3.95
Total	23 20.72	28 25.22	16 14.41	16 14.41	28 25.22	111						

There were no abortion and pregnancy toxemia during this experiment.

DISCUSSION

There were significant differences in pregnancy rate among the ewes in control and treatment groups (P<0.05) (Table 1). The pregnancy rate was increased (24/25, 96% and 22/25, 88% in treatment subgroups, respectively) in comparison with control group (15/25, 60%) using (MPA) alone. The different treatment subgroups received MPA with variable experiments. The pregnancy rates in ewes using MPA, ram exposure and eCG subgroup were the highest (P=0.002). However, it was higher in MPA and eCG subgroup in comparison with control group (P=0.02), too. But, there were no significant differences in MPA and ram exposure treatment subgroup in comparison with control group (P>0.05) (Table 1). It showed that the genital system of ewes could be influenced by progestogen (MPA), ram exposure and eCG in out of breeding season. In addition, twinning, lambing, fecundity and prolificacy rate increased in this subgroup comparison with control group (Table 2).

Ram exposure results in increased LH production in receptive ewes, followed by ovulation within a few days. Administration of progestogen prior to ram exposure can improve the synchrony of estrus. It is the best that the females be abruptly exposed to the male following isolation from the sight, sound and odor of rams for the 30 to 60 days prior to exposure. The ram need only be exposed for 48 hours to have full effect. For optimum response, ewes must be weaned prior

to exposure and be in good breeding condition (Youngquist and Threlfall., 2007).

It was showed for the first time that the male exposure stimuli to bypass the suppressive effect of progesterone in ewes which were treated using progesterone implants (Martin et al., 1983. Pearce and Oldham., 1983) or progesterone analogue (medroxyprogesterone acetate) (Evans et al., 2004) where exposure of treated ewes to rams increased LH secretion. The effect of ram exposure stimuli on LH secretion is not limited to exogenous sources of progesterone and cyclic ewes at all stages of the oestrous cycle respond to rams with an increase in pulsatile LH secretion (Hawken et al., 2007). It was found that, ram exposure in Suffolk crossbred ewes during the last 3 days of exogenous P4 synchronisation, the LH surge, oestrus and ovulation all began earlier, whereas the duration of oestrus was reduced (Evans et al., 2004).

In non-breeding season ewes, management of the ovine oestrous cycle is mainly based on the use of exogenous hormonal agents to mimic or enhance (progesterone and its analogues) the activity of the corpus luteum, combined with the application of other hormones mimicking the pituitary secretion of gonadotrophins (e.g. eCG). However, in this study, the high pregnancy rate occurred on the day 10^{th} after ram exposure and eCG indicates that ovulation did happen in most ewes (24/25, 96%) (P=0.002). On the other side, the pregnancy rate was lower in P4 + eCG treatment subgroup which did not expose to the rams (22/25, 88%) (P=0.02) (Table 1). Possible explanations for the reduction of pregnancy rate in P4-treated and eCG animals are alterations in final follicle growth (Gonzalez-Bulnes et al., 2005) and ovulation (Vinoles et al., 2001. Killianet al., 1985) as well as impairment of sperm transport and survival in the female genital system, reducing the number of fertilized ova (Allison and Robinson., 1970 Hawk and Conley 1972). MPA alone in control group is not highly efficacious in treating seasonal anoestrus ewes because some individual animals: (1) have not enough follicular activity at the beginning of treatment; (2) may ovulate spontaneously during treatment; and (3) have follicle growth but fail to ovulate after withdrawal of the progesterone.

In this study, lambing rates (80-96%) and fecundity rate (100-136%) were higher than control group (60% and 84%, respectively). The main reason for the high prolificacy achieved even without eCG treatment was probably that this experiment was performed only well-managed and conditioned ewes.

In our study, we found no effect on single, twinning and lamb genus (Table 3). However, twinning rate was higher in treatment subgroups in comparison with control ewes.

CONCLUSION

This study reports that using MPA as a synthetic hormone and exposing Shaal breed ewes to ram for 3 days followed by eCG administration in progestogen programming was highly effective to induce fertile estrous activity during the nonbreeding season. When ram exposure used in out of breeding season with MPA and eCG, appear to be inferior to P4 + eCG or P4 ram exposure in ability to stimulate anovular ewes to cycle in out of breeding season. The male exposure and eCG have great potential for synchronizing oestrus in seasonal Shaal breed during non-breeding season. This leads us to consider new perspectives of research on the issue of isolation of females from males in non-breeding season programming.

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GEOMETRIC MORPHOMETRIC INVESTIGATION OF INCUS IN HORSE (EQUUS FERUS CABALLUS) AND DONKEY (EQUUS ASINUS)

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Abstract

In this study, it was aimed to determine the shape of the incus in horse and donkey by geometric morphometric method and to evaluate the shape differences between horse's and donkey's incus. The left incus bone of 5 adult horses and 5 donkeys were used in the study. Incus were photographed at same lateral direction. Thirteen homologous landmarks were marked from the photographs using TpsUtil (Version 1.79) and TpsDig2 (Version 2.31) software. As a result of the study, the first principal component explained 38,642% of the total shape variation. In the PC1 plot, samples were clearly clustered by group. Acording to canonical varians analysis, in the wire frame warp graphic, the corpus incudis edges (right, left, and bottom) were flatter in donkeys. Angle at the LM13 level was more pronounced on the crus breve. The apex of the crus longum (Landmark 4, 5, and 6) was wider in donkey. In the study, the morphological features of horse's and donkey's incus were determined by geometric morphometric method. This study is important in that it is the first geometric morphometric study on the incus, one of the ossicula auditus. We thinks that the study will contribute to the anatomy of the ossicula auditus in the equide family.

Key words: *Donkey, Geometric morphometry, Horse, Incus.*

INTRODUCTION

The ossicula auditus are located in the pars petrosa of the os temporale, dorsal to the cavum tympani. These are located between the membrana tympanica and the fenestra vestibuli as the malleus, incus, and stapes, respectively (Pazvant and Gündemir, 2021). There is also os lenticulare between the incus and stapes in young animals. This ossicle fuses with the incus in later ages to form the processus (proc.) lenticulare. The ossicula auditus magnify the vibrations from the eardrum by 20 times and transmit them to the inner ear and cause the fluctuation in the endolymph (König and Liebich 2007). At the same time, the ossicula auditus can also reduce sound pressure by separating each other through certain muscles (m. tensor tympani and m. stapedius) (Reece WO, 2012).

In the studies carried out to date, there is information about the anatomy (Özgüden 1962, Hebel and Stromberg 1986, Masuda et al 1986, Huang et al 1996, Kristensen et al 1996, Botti et al 2006, Solntseva 2013) and morphometry (Gürbüz et al, 2016; Kurtül et al. 2003, Mohammadpour 2011, Demiraslan et al. 2015; Gürbüz et al. 2016; Gürbüz et al., 2019; Dalga and Aslan, 2019) of the ossicula auditus in different animal species However, no study was found in which the shape of the ossicula auditus was determined by the geometric morphometric method. For this reason, in this study, it was aimed to reveal the morphological anatomical values of the horse's and donkey's incus belonging to the Equidae family and to evaluate the shape differences between the horse's and donkey's incus.

MATERIALS AND METHODS

Samples

In the study, the incus that one of the left ossicula auditus of 5 adult horses and 5 donkeys, were used. Ethical permission was not required as the materials were obtained from previously dead animals.

Imaging and Digitization

Incus were photographed laterally with a stereo microscope (Leica S6D) focusing on the median line. The distance between the lens and the material was determined as 10 cm. The photos were saved on the computer with the Jpg extension. From the photographs, 13 homologous landmarks were marked using TpsUtil (Version 1.79) (Rohlf, 2019) and TpsDig2 (Version 2.31) (Rohlf, 2018) software (Figure 1 and 2). Thus, the *x* and *y* Cartesian coordinates of homologous anatomical points representing the general shape of the incus from the lateral direction were determined. Before statistical analysis, confirmation test was performed for landmarks in TpsSmall (Version 1.34) (Rohlf, 2017) program. In TPS small analysis, slope and corelation values of landmarks were found as

0.998850 and 1.000000, respectively. These values show that the landmarks are placed correctly.

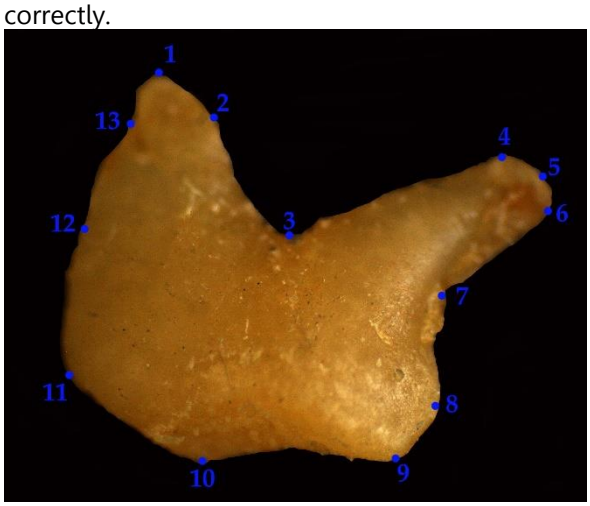

Figure 1. The Landmarks on Horse's incus

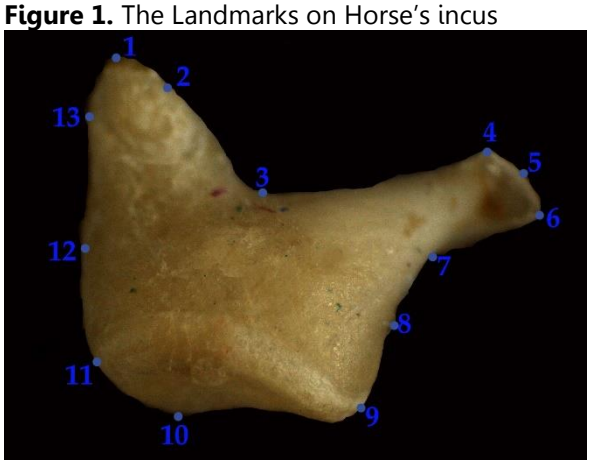

Figure 2. The landmarks on Donkey's incus

1. The highest point of the crus breve, **2.** The rightmost peak of the crus breve**, 3.** The angle of the crus breve with the crus longum, **4.** The leftmost peak of the crus longum**, 5.** The midline of the crus longum of the peak point, **6.** The righmost peak of the crus longum, **7.** The angle of crus longum and corpus incudis, **8.** The most protruding point of corpus incudis on the right edge, **9.** Right corner point of corpus incudis, **10.** The most protruding point of corpus incudis on ventral edge, **11.** Left corner point of corpus incudis, **12,** The most protruding point of corpus incudis on the left edge, **13.** The rightmost peak of the crus breve.

Statistical analysis

The differences in size, position and orientation of Incus' lateral photographs were superimposed by General Procrustes Analysis (superimposition) (Slice, 2007). PAST (Version 4.02) (Hammer et al, 2001) program was used for this analysis. With the same program, principal components analysis was performed on the new coordinates obtained as a result of the Procrustes analysis, and the components between the groups were calculated. In addition, 2-t test was applied to compare the landmark coordinate values (procrustes) according to the groups. The degree of closeness (Classical cluster) of individuals was analyzed in the PAST (version 4.02) program. Using the MorphoJ (Klingenberg, 2011) program, at which landmarks the shape differences were concentrated (PCA) and grouping characteristics (Canonical variance analysis-CVA) were analyzed.

RESULTS

The results of principal component analysis performed with the landmark coordinates are shown in Table 1. Accordingly, the first principal component (PC.1) explained 38.642% of the total shape difference, and the first four principal components (PC1+PC2+PC3+PC4) explained 85.903%. Evident breakpoint among principal components was observed between PC1 and PC2. The distribution of samples according to PC1 was shown in the graph in Figure 3. Accordingly, the samples were clearly clustered according to the groups. It was observed that the donkey samples were collected on the right of the y axis, and 4 of the horse samples were collected on the left of the y axis.

Table 1. Results of the principal component analysis, PC: principal component

PC	Eigenvalue	% variance
1	0,00407434	38,642
2	0,00244738	23,211
3	0,00151831	14,4
4	0,00101748	9,65
5	0,000636602	6,0377
6	0,000360864	3,4225
7	0,000296456	2,8116
8	0,00015774	1,496
9	3,46808E-05	0,32892

In the study, the graph obtained as a result of the test performed to determine the proximity of the samples is given in Figure 4. Accordingly, the samples were largely grouped according to the race factor.

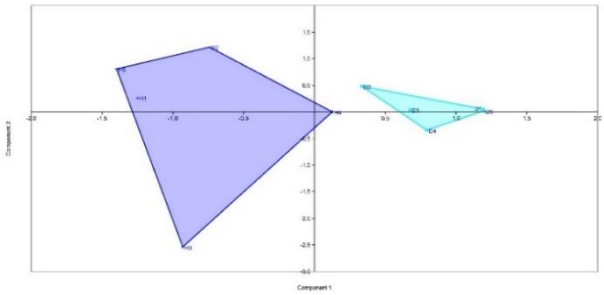

Figure 3. Distribution of samples on the graph over the first principal component (PC1), Light blue: Donkey's incus (D), Blue: Horse's incus (H)

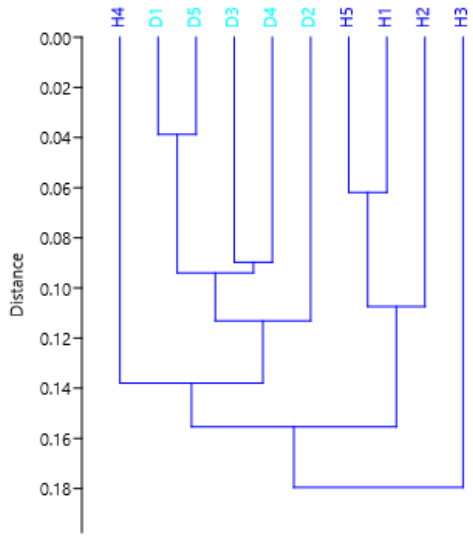

Figure 4. Graph of hierarchical proximity of individuals. Light blue: Donkey's incus (D), Blue: Horse's incus (H).

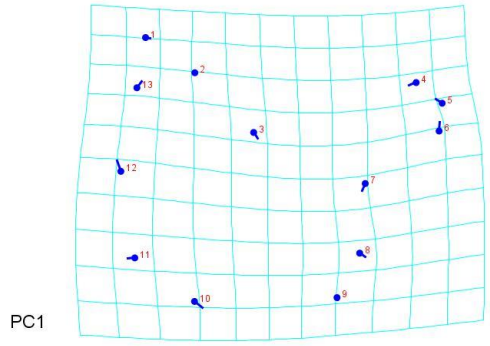

Figure 5. Landmark representation of shape differences of incus between donkey and horse for the first principal component (PC1). (Set scale factor: 0.05).

The graphs showing the shape differences at which landmarks (LM) according to PC1 were shown in Figure 5. Accordingly, shape differences for PC1 became evident in the landmarks except for LM1, LM2 and LM9.

Canonical variance analysis defined the between-group difference within a canonical variable (CV1). Shape variations with respect to CV1 were similar to anatomical points according to PC1. Mahalanobis and Procrustes distances values were determined as 3.2349 and 0.1098 (p: 0.0052), respectively. Shape differences and frequencies according to groups in incus' wire-frame warp graph were shown in Figure 6 and Figure 7. Accordingly, it was observed that the frequencies were homogeneously distributed between the groups.

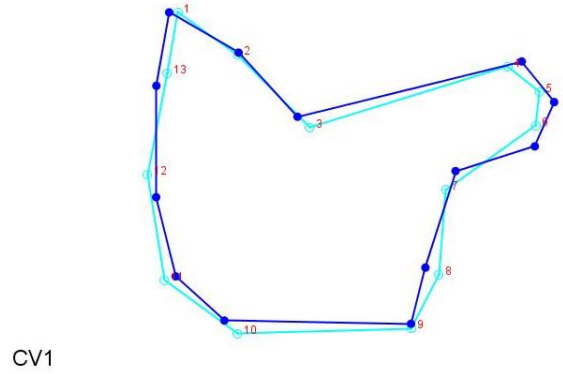

Figure 6 . Canonical variance analysis. Wire-frame warp plot (Set scale factor: 3.0). Dark blue: donkey, Light blue: Horse.

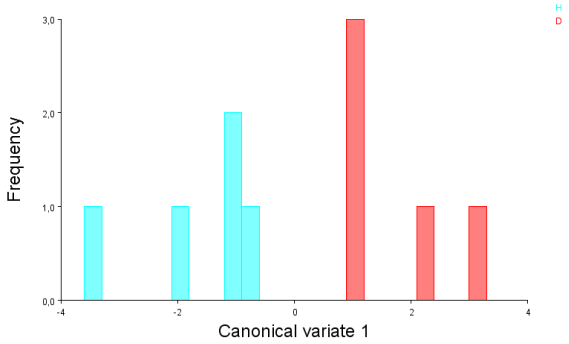

Figure 7 Grouping by Canonical Analysis of Variance. Light blue:Horse, Red: Donkey.

According to canonical variance analysis, in the wire frame warp graphic, corpus incudis edges (right, left and bottom) were flatter in donkeys. Angle at the LM13 level was more pronounced on the crus breve. The apex of the crus longum (Landmark 4, 5, and 6) was wider in donkey.

DISCUSSION

The most important factor in the difference in bone shapes is genetic structure (Seeman, 2003). Anatomically, the bones of different members of a family subgroup are similar. However, the bone shape and size are different. Therefore, many studies have been carried out on the geometric morphometry of various bones (Demircioglu et al., 2021; Duro et al., 2021; Gundemir et al., 2020; Gundemir et al., 2021; Gurbuz et al., 2020; Szara et al., 2022). In this study, it was aimed to reveal the anatomical features and differences of the incus between the donkey and horse belonging to the same family subgroup. In the study, the left incus of horse and donkey was examined by geometric morphometric method. The study contains restrictions in terms of the number of materials. However, it was determined that the incus shape of the horse and donkey were different from each other with the geometric morphometric method.

The incus hangs medial to the malleus and lateral to the stapes and connects these ossicles with synovial joints. Anatomically, the horse's and donkey's incus have a large body called the corpus incudis and two projections, the crus longum and the crus breve, which are separated from the body.The crus longum has a projection called the proccessus lenticulare, which articulates with the stapes (Demiraslan et al., 2015; Gürbüz et al., 2016). Incus length on the left side is 2.53 mm, corpus incudis width is 1.25 mm in donkeys (Demiraslan et al., 2015), these lengths are 3.92 mm and 3.68 mm in horses (Gürbüz et al., 2016), respectively. According to the results of the previously reported morphometric study (Demiraslan et al., ; Gürbüz et al.,), the horse's incus was larger than the donkey's incus, that is, their sizes differ from each other. In the study, although the incus of the horse and donkey were similar anatomically, it was determined that there were some differences in shape by detailed geometric morphometric analysis. Accordingly, the corpus incudis margins (right, left, and bottom) were flatter in donkeys. Angle at the LM13 level on the crus breve was more pronounced in horse. The apex of the crus longum (Landmark levels 4, 5 and 6) was wider in donkeys.

CONCLUSIONS

As a result, the shape differences of horse's and donkey's incus were determined by geometric morphometric method. Accordingly, in the PC1 graph, the principal component analysis, the samples were significantly clustered according to the race factor. The points of shape differences were determined by canonical variance analysis. It is important in that it is the first geometric morphometric study performed on the ossicula auditus. We think that this study will contribute to the morphology of the ossicula auditus.

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THE EFFECT OF HEXAFLUMURON ON RENAL BLOOD FACTORS IN ADULT WISTAR RATS

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Abstract

Utilizing insecticides in a vast range lead to creating many issues in nature and contamination of the material cycle in the ecosystem with these kinds of chemicals appears to be harmful and devastating for humans and other livestock, especially in industrial and developing areas of countries. Hexaflumuron is a systemic pesticide of the benzoyl urea group that removes living insects and reduces their population by blocking the chitin synthesis cycle. Chitin is the main component of the insect´s exoskeleton. Poisening with this kind of chemical can cause several systematic disorders in living tissues. kidney because of being susceptive to pesticide poisoning was surveyed in this study. The present study was performed to recognize the effect of Hexaflumuron on renal health indicators such as creatinine and Urea in adult Wistar rats. Twenty rats were divided into five groups of 4 each. Group A presented normal control. Group B, C, D and E were administered hexaflumuron in technical form by gavaging with different doses (22, 16.5, 11 mg/kg respectively, corresponding 20% of LD50, 15% of LD50, 10% of LD50) for 28 days. The plasma levels of Urea and creatinine were measured. The highest amounts of Urea, Creatinine were detected in 20% of LD50 group(B) and that was noticeable. In conclusion, with regards to these results and the effect of hexaflumuron on the most important renal blood factors in poisoning, this insecticide seems to be harmful at least for the kidney tissue.

Key words: *Hexaflumuron, Urea, Creatinine, Wistar, Rats*

THE EFFECT OF HEXAFLUMURON ON TESTOSTERONE LEVELS IN ADULT MALE WISTAR RATS

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Abstract

Excessive use of insecticides is one of the main causes of material cycle pollution in nature and has many harmful effects on the ecosystem. hexaflumuron is a systemic insecticide from the benzoylurea (BPU) group. Considering that chitin is the main part of the exoskeleton of insects, this insecticide inhibits the synthesis of chitin in insects, which disrupts the molting process in insects and leads to the death of insects in immature stages. During studies on the toxic effects of hexaflumuron on adult albino rats, mild tremors, bleeding from the nose, hypoglycemia, methemoglobinemia, and tissue damage in the liver and spleen were observed. The present study was conducted to investigate the effect of hexaflumuron on testosterone levels in adult male Wistar rats. This experiment began with 32 male Wistar rats, divided into four groups of eight (A, B, C, and D). During the 28-day study, groups A and B received a placebo as a control group, and groups B through D received 11, 16.5, and 22 mg/kg of technical hexaflumuron as daily gavage, which is equivalent to 10%, 15%, and 20% of the LD50, respectively. In this test, the testosterone levels of the groups that got hexaflumuron were the lowest, and there was no significant difference between these groups, but there was a significant difference between these groups and the control group. According to the findings of this study hexaflumuron seems to have an adverse effect on the male reproductive system in Wistar rats and causes a decrease in testosterone levels in these animals.

Key words: *Hexaflumuron, Testosterone, Wistar rat, Male reproductive system*

THE RELATIONSHIP BETWEEN ANOGENITAL DISTANCE AND CONCEPTION RATES IN EWES DURING THE BREEDING SEASON

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Abstract

Evaluation of reproductive productivity in ewes is important for an economic sheep breeding. Genomic, hormonal and progeny tests are used in the evaluation of reproductive productivity in ewes. The aim of this study is to investigate the relationship between anogenital distance and conception rates in nulliparous and multiparous ewes during the breeding season. The study was carried out in a commercial farm on a total of 121 Hungarian merino sheep, 44 nulliparous and 77 multiparous, in the breeding season. Anogenital distances of all ewes were measured and recorded with a digital caliper before ram insertion to herd. After adding proven Merino rams to the herd (ewe:ram ratio of 6:1), the matings were followed and recorded. Ultrasonographic pregnancy examinations were performed on day 35 following the insertion of the ram. Animals that did not become pregnant at the first mating were formed in a separate group and followed their mating in a similar way. Ultrasonographic pregnancy examinations of these animals were also repeated at 35 days after the insertion of rams (on day 70). The conception rates of the ewes were calculated and compared in nulliparous and multiparous animals. In addition, the correlations between anogenital distance and conception rate in nulliparous and multiparous animals were calculated. The mean anogenital distance was 43.34 mm in nulliparous ewes and 45.91 mm in multiparous ewes (P>0.05). In nulliparous ewes, there was no significant difference in the comparison of the anogenital distances of the ewes that conceived at the first mating (43.53 mm), conceived in the subsequent matings (43.87 mm) and did not become pregnant during the breeding season (40.91 mm) (P>0.05), while a significant difference was found between the mean anogenital distance in multiparous ewes 44.77 mm, 46.59 mm and 50.86 mm, respectively (P<0.05). The conception rates in the first mating of nulliparous and multiparous ewes were 18% and 49.3%, respectively, (P<0.05), and in subsequent matings, it was calculated as 80.5% and 89.7%, respectively (P>0.05). It was detected that 7 of the nulliparous ewes and 4 of the multiparous ewes did not become pregnant during the breeding season. According the results of the study, it was concluded that anogenital distance is not a valid parameter for estimating the reproductive efficiency in nulliparous ewes, the anogenital distance is shorter in multiparous ewes conceived early, however, the hypothesis should be tested with similar studies on more animals and in different sheep breeds.

Key words: *Anogenital distance, Breeding season, Reproductive parameters, Ewes*

POTENTIAL ACTIVITY OF IRANIAN CARUM COPTICUM ESSENTIAL OIL ON FUNGAL PATHOGENS ISOLATED FROM ANIMALS

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Abstract

Fungal agents are widespread and can be isolated from a wide range of animals, from the soil and the environment. This makes fungal diseases as a group of transmissible infections in which animals can represent important reservoirs and asymptomatic carriers for people in close contact with them. The important role of farm and pet animals as carriers and spreaders is well known. The increasing resistance to antifungal drugs and the reduced number of available drugs led to the search for therapeutic alternatives among aromatic plants and their essential oils, empirically used by antifungal effects. The purpose of the current study was to evaluate the antifungal activity of Carum copticum essential oil (EO) against the most frequent pathogenic fungi including Candida, Aspergillus, Chrysosporium and Trichophyton species. EO from the seeds of the plant was obtained by hydrodistillation. Susceptibility tests were expressed as growth inhibition zone (diameter) using disk diffusion method and minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) using broth microdilution method. Results of susceptibility tests showed that Carum copticum EO was effective against all the tested strains. The diameters of growth inhibition zone of the EO were between 11 mm and 60 mm. The EO was also the most active, with MIC and MFC values ranging from 0.3 to 2.5 mg/ml and 0.6 to 5 mg/ml, respectively. The EO of Carum copticum showed a significant degree of antifungal activity against different Candida species in comparison with other fungi ($p<0.05$). The present study indicated that Carum copticum EO has considerable antifungal activity, deserving further investigations for its clinical application for treatment of fungal infections

Key words: *Carum copticum, Antifungal susceptibility, Fungal pathogens*

A SURVEY OF THE PARASITIC INFESTATIONS BY THE CILIATED PROTOZOANS AND NEMATODES IN FRESHWATER ORNAMENTAL ANGELFISH (Pterophyllum scalare Schultze, 1823) IN TEHRAN, IRAN

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Abstract

Freshwater angelfish (Pterophyllum scalare), one of the most beautiful and popular aquarium fish, is an egg-laying species in the Cichlidae family. The purpose of this research was to conduct a survey of ciliated protozoan and nematode parasites in freshwater ornamental angelfish (P. scalare) in Tehran, Iran. From September to October 2020, 50 freshwater angelfish were collected at random from ornamental fish farms in Tehran and transported alive to the aquatic animal disease laboratory in Tehran (Tehran, Iran). To investigate the parasitic contamination of the fish, samples were first examined macroscopically. The fish´s body surface, fins, and gills were then prepared as wet smears for microscopic examination. The fish were then euthanized and autopsied, and internal organs such as the intestines were examined for parasitic infections. In the current study, several protozoan parasites, including Ichthyophthrius multifiliis (12%), Chilodonella spp. (8%), as well as Trichodina spp. (16%) were isolated from the body surface and gills of the fish. In addition, infection with Capillaria spp. (18%) was discovered during a microscopic examination of the intestines. However, no other internal organs were infected with parasites. Salt + formalin (short bath) and levamisole (oral) were used to treat the fish for ciliated protozoan and nematode infections, respectively. After a month, the treated fish were re-examined and showed no signs of infection.

Key words: *Angelfish, Ichthyophthrius multifiliis, Chilodonella spp., Trichodina spp., Capillaria spp*

STUDY OF THE CAUSE OF BANDED CICHLID (Heros severus Heckel, 1840) MORTALITIES IN AN ORNAMENTAL FISH BREEDING CENTER IN TABRIZ, IRAN

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Abstract

The banded cichlid (Heros severus) is a popular freshwater ornamental fish of the Cichlidae family that is widely cultivated in Iran. The aim of this study was to investigate the causes of banded cichlid losses in an ornamental fish breeding center in Tabriz, Iran. During the period from January 2022 to February 2022, following the chronic mortalities of banded cichlids with signs such as white feces hanging from the anus, the fish were packed in plastic bags filled with water, oxygenated, and transferred to the aquatic animal disease laboratory in Tehran (Tehran, Iran). Macroscopic examinations revealed that the fish was thin, weak, and pale. Wet smears were prepared and examined under a light microscope to investigate parasitic contamination of the fish´s body surface and gills. The fish were then euthanized and necropsied. Bacterial culture yielded negative results. Internal organs such as the intestines were checked for parasitic infections. There was no parasitic contamination on the fish´s skin, fins, or gills. However, a microscopic examination of the intestine revealed an infestation with Capillaria sp. eggs and mature parasites with high severity. In the present study, the severity of infection was very high and this parasite was identified as the main cause of losses. In order to treat the rest of the fish, levamisole was administered for 48 hours every 7 days for 21 days. The losses were completely stopped, and no infection was found after 21 days of re-examination.

Key words: *Banded cichlid, Nematode, Light microscope, Capillaria sp., Levamisole*

EVALUATION OF DIFFERENT DOSES OF TRICYCLAZOLE ON AMYLASE AND LIPASE IN FEMALE WISTAR RAT

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Abstract

One of the most important factors that pollute soil and water is agricultural pesticides. Considering that Tricyclazole is a widely used pesticide to deal with rice blast disease, it is important to investigate its effects on Amylase and Lipase levels as indicators of body health. Methods: This study is a case-control study that was performed on 24 female Wistar rats in the weight 200±20 g. Wistar rats were divided into 4 groups of 6 under the treatment groups consisting of group A, group B, group C, and the control one. The treatment groups (A, B, and C) were placed on gavage with 25, 37.5, and 50mg/kg body weight of Tricyclazole over 28 days. Serum samples were obtained from all groups and were used to measure Amylase and Lipase. Results: Biochemical evaluations demonstrated that there is a dose-dependent relation between elevated Tricyclazole and amylase ($p<0.05$) but not a meaningful one with lipase values ($p>0.05$). In other words, by increasing the dose of Tricyclazole, the amylase value drops. In addition, the Tricyclazole dose (50 mg/kg/day) was the lowest, while the highest levels were linked to the control group. Conclusion: Through this research, can be manifested by decreased levels of amylase, which can cause adverse effects on rats and pancreatic factors should be considered as subsequences of this pesticide intake so it´s better to control the use of this pesticide in the future.

Key words: *Wistar rat, Tricyclazole, Pancreas, Amylase, Lipase*

THE STUDY OF DIFFERENT DOSES OF COMBINATION OF METHYLTIOPHANATE + TRICYCLAZOLE ON INSULIN AND GLUCOSE IN FEMALE WISTAR RAT

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Abstract

One of the most important factors that pollute soil and water is agricultural pesticides. Considering that combination of Methyltiophanate and Tricyclazole is a widely used pesticide to deal with rice blast disease, it is important to investigate its effects on insulin and glucose levels as indicators of body health. Twenty-four adult female Wistar rats weighing 200 ± 20 were distributed semi-randomly into 4 groups of six. Group Control, group A that received the combination of Tricyclazole (12.5 mg/kg b.w./day, PO) and Methyltiophanate (332 mg/kg b.w./day, PO); group B, were treated with the combination of Tricyclazole (18.75 mg/kg b.w./day, PO) and Methyltiophanate (498 mg/kg b.w./day, PO) and group C, were treated with the combination of Tricyclazole (25 mg/kg b.w./day, PO) and Methyltiophanate (664 mg/kg b.w./day, PO). The study period was 4 weeks. Blood samples were taken from all rats. Serum samples were obtained from all groups and were used to measure Amylase and lipase. There was a meaningful dose-dependent relationship between the increase in levels of the combination of Tricyclazole and Methyltiophanate and insulin value ($p<0.05$) but not a meaningful one with Glucose value ($p>0.05$). In addition, Methyltiophanate (664 mg/kg/day) and Tricyclazole (25 mg/kg/day) cause the highest, while the lowest levels were related to the control group. According to mounting levels of insulin and glucose, it ought to be reckoned that Tricyclazole and Methyltiophanate have negative impacts on human and animal health and toxicity effects on pancreas tissue.

Key words: *Methyltiophanate, Tricyclazole, Pancreas, Glucose, Insulin*

EVALUATION OF DIFFERENT DOSES OF THIOPHANATE METHYL ON HISTOPATHOLOGIC EFFECTS OF PANCREAS IN FEMALE WISTAR RAT

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Abstract

With the existence of various plant diseases such as rice blast and the fact that the health of agricultural products is important, the use of pesticides is increasing. Among these toxins is Thiophanate methyl. Recent studies indicate that the residue of these toxins has the ability to be digested in living organisms, which can have harmful effects on different body tissues, including the pancreas. Twenty-four female rats were divided into Four groups, group 1, control group; group 2, rats that received Thiophanate methyl (664 mg/kg b.w./day, PO); group3, were treated with Thiophanate methyl (996 mg/kg b.w./day, PO); group4, were treated with Thiophanate methyl (1328 mg/kg b.w./day, PO). Then, the tissue of the pancreas was obtained, and pancreatic changes were examined. The examination of histopathologic lesions showed mild hyperemia in all groups and necrosis in groups 3 and 4 and it became more significant by elevating the dose of Thiophanate methyl. It is obvious that elevating levels of the Thiophanate methyl can cause destructive effects on rats and nutrition health and it should be considered as a subsequence of this pesticide intake. So its recommended some Propose to review and implement serious measures for the consumption of such pesticides.

Key words: *Thiophanate methyl, Wistar rat, Pancreas*

EVALUATION OF DIFFERENT DOSES OF CONSULT ON THE BLOOD INSULIN LEVEL OF FEMALE WISTAR RAT

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Abstract

As a matter of fact, increasing the use of a number of pesticides is clear to us. One of the serious worries about this fact is their residue in the soil and environment. The Consult is one of the common pesticides which is used against agnoscena pistaciae, kermania pistacia, phyllocnistis citrella and etc. It is a benzoyl phenyl urea insecticide growth regulator that inhibits chitin synthesis. This study is a case-control study that was performed on 24 female Wistar rats in the weight 200±20 g. Wistar rats were divided into 4 groups of 6 under the groups of treatment consisting of group A which received Consult (11 mg/kg b.w./day, PO); group B, which was treated with Consult (1.5 times the dose of group A PO); group C, were treated with Consult (2 times group A, PO) and the control one. The treatment groups (A, B, and C) were placed on gavage over 28 days. Serum samples were obtained from all groups and were used to measure the blood Insulin level. Data obtained from the measurements were compared using statistical analysis the one-way ANOVA and the Duncan technique. There was a meaningful dose-dependent relation between the increase in levels of Consult and insulin value and the highest level of it was obtained in group C which had a significant difference from the control and group A and B ($P<0.05$). There was not any meaningful difference between Group A and B to one. Through this research, it can be manifested by elevated levels of Consult can cause adverse effects on rats´ health and it should be considered as a subsequence of this pesticide intake. More education to the farmers who use this pesticide and harder consideration is recommended for further use of this pesticide.

Key words: *Wistar Rat, Insulin, Consult, Pancreas*

THE STUDY OF DIFFERENT DOSES OF HEXAFLUMURON ON THE BLOOD GLUCOSE LEVEL OF FEMALE WISTAR RAT

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Abstract

Among environmental pollution, excessive exposure to pesticides is considered one of the factors that cause various diseases in humans and animals. Pesticides are considered the main source of environmental pollution. Pesticides have found wide applications in horticulture and veterinary medicine and have the potential for accidental effects on natural life, humans, and domestic animals. One of the most common insecticides is hexaflumuron poison. Twenty-four adult female Wistar rats weighing 200 ± 20 were distributed semi-randomly to 4 groups of six. Group Control, group A that received Hexaflumuron 11 mg/kg b.w./day, PO); group B, were treated with Hexaflumuron 16.5 mg/kg b.w./day, PO) and group C, were treated with Hexaflumuron (22 mg/kg b.w./day, PO). The study period was 4 weeks. Blood samples were taken from all rats. Serum samples were obtained from all groups and were used to measure the Glucose level. Data obtained from the measurements were compared using statistical analysis the one-way ANOVA and the Duncan technique. There was a meaningful dose-dependent relation between the increase in levels of Hexaflumuron and glucose value and the highest level of it was obtained in group C which had a significant difference from the control and group A (P<0.05). According to mounting levels of glucose, it ought to be reckoned that Hexaflumuron has negative impacts on human and animal health and toxicity effect on pancreas tissue and further serious considerations are recommended.

Key words: *Wistar Rat, Hexaflumuron, Pancreas, Glucose*

HISTORY OF VETERINARY SURGERY IN ANCIENT ARABIC MANUSCRIPTS

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Abstract

The old Arabic manuscripts were very rich with valuable information in most fields of veterinary medicine, anatomy, physiology, internal and infectious diseases, gynecology, obstetrics, surgery, poultry diseases, wild animals and birds, and zoonotic diseases. Some works about ancient Arabic veterinary medicine were done mainly by Germany, French, and English. Turkish. Spanish, Persian, and Arabic, but do not cover many manuscripts, and most textile criticism on it is not good. The ancient Arabic veterinary medicine is part of the world history of vet. med., if carried out good studies will obvious and explain some roots and relations of old veterinary medicine. Some of the ancient Arabic veterinarians were genus, and ancient they knew diagnosis of many surgical problems in animals especially horse castration, hernia, internal and external surgical operations, surgical on eyes ophthalmology, limbs, etc.., and disinfectants, sterilization, internal and external sutures, treatment of bleeding, astringents, ointments. Many ancient Arabic manuscripts without or with colored pictures are rich with surgical information and some of their surgical methods and treatment used until now.

Key words: *History of veterinary, Veterinary surgery, Arabic civilization, Islamic civilization*

POSTER PRESENTATIONS

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ANALYSIS OF THE COW GENOME, DETERMINATION OF MILK-RELATED SNPs FROM TEST-DAY MILKINGS

Magdalena KOLENDA [1]*, **Beata SITKOWSKA** [1], **Justyna RZĄSIŃSKA** [1], **Dariusz PIWCZYŃSKI** [1]

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Abstract

In dairy farming, access to phenotypic and genotypic data is an opportunity to improve the understanding of the impact of, for example, individual SNPs on cow's milk yield. Such knowledge may allow a more precise selection of animals for breeding in terms of the preferred variants of genes. The aim of the study was to identify the SNP sequences highly affecting the level of traits related to milk yield obtained from test-day milkings of Polish Holstein-Friesian cows.
The research took into account the results concerning 1,224 cows. A total of 9,394 SNPs were analysed. SNPs determining cow's milk performance were investigated by two alternative statistical methods, i.e. linear regression and random forest. For this purpose, the R program and the following packets were used: bigstep (linear regression with mBIC criterion) and ranger (random decision forest). Both models used different approaches and different sets of SNP sequences selected by mBIC and random forest were obtained (results were limited to a maximum of 10 most important SNPs according to each model). In linear bigstep regression, a model limited the number of SNPs to those it considered most important. Since linear regression assumes linear dependencies and the lack of interactions between the studied features, it may lead to the loss of important SNPs. Therefore, we also decided to conduct a random forest analysis. We analysed the results of test-day milkings, including milk yield, percentages of fat, protein, dry matter, urea, lactose contents in milk and logarithmic somatic cells in milk.
It was observed that levels of more than one of the examined traits were affected by 6 SNPs according to linear regression and 10 SNPs according to random forests. While comparing the results of both statistics for each trait individually it can be noticed that 12 SNPs were indicated by both models. Most relevant SNPs were found on chromosome 14. Comparing both models in the case of milk yield, it was found that only 1 SNP (also found on chromosome 14) was shown in both models. The linear model selected only 3 significant SNPs, and the last of them was also classified in the random forest model (4^{th} position). In the case of protein content in milk, both models selected the same 4 SNPs, while the same 3 SNPs affecting urea content in milk were indicated by both models.
It can be stated that depending on the proposed statistical model, various SNP sets, indicated as significantly affecting the milk performance during test-day milkings, were obtained. At the same time, it was observed that, despite the different approaches and criteria, several SNPs were included in both groups of results. More importantly, the models also indicated SNPs that probably affect several analysed features simultaneously.
Source of funding: Implementation doctorate ARRANGEMENT No DWD/5/0264/2021

Key words: *SNP**,** milk yield, Polish Holstein-Friesian*

IDENTIFICATION OF HUB GENES AND MIRNAS LINKED TO BOVINE SUBCLINICAL STAPHYLOCOCCUS AUREUS MASTITIS USING BIOINFORMATICS ANALYSES

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Abstract

Background: Bovine mastitis, with a high incidence rate, is one of the multifactorial inflammatory diseases for dairy cattle worldwide with regard to incurred economic losses, and Staphylococcus aureus is a leading cause of bovine mastitis. However, despite all the scientific and technological developments in animal husbandry, effective methods for the prevention and treatment of mastitis have not been developed. Identifying the core genes and miRNAs is essential for early diagnoses of subclinical mastitis, and also it could provide a more precise method for treating subclinical mastitis. The main purpose of this research is to identify the target genes associated with the Staphylococcus aureus pathogen and the bovine species-specific innate immune system in subclinical mastitis infections in cows, and the target miRNAs and/or miRNAs of these genes using bioinformatic analyses. Methods: In this research, to determine the genes that play a role in the innate immune system of the cattle InnateDB website was used, and then the pathway analyses were performed to which genes are related were determined by the Reactome and KEGG websites. The genes that play a role in the response to Gram-positive bacteria invasion in the innate immune system were determined by Gene Ontology (GO). According to the GO result, genes belonging to the bovine species were determined in the accession number of GO:0050830: defense response to Gram-positive bacterium. Among these, specific genes belonging to the innate immune system against the Staphylococcus aureus pathogen were selected by combining the information of pathway analysis and gene-gene interactions. The protein-protein interaction (PPI) network analysis was performed using STRING. Also, DAVID bioinformatics database was used for functional annotation analysis. Cytoscape tools were used to visualize the interactions between hub genes. Prediction of the miRNAs of the determined hub genes was performed by miRNet online analysis tool. The miRNet database was used to identify experimentally validated mammary tissue-specific target genes in the mirTarbase and Tarbase v.8 tools. Results: A total of 76 genes were identified for the cow species specific to the GO:0050830 access number. Among 76 genes, TLR2, MYD88, CASP4, and NOD2 were found as hub genes. Furthermore, bta-miR-19a-3p, bta-miR-149-5p, bta-miR-122-5p, and bta-miR-192-5p were found as the target microRNAs for determined hub genes, respectively. Conclusion: The determined hub genes and their target miRNAs may be involved in the mechanisms of bovine subclinical Staphylococcus aureus mastitis. They could be new potential targets for further functional studies of bovine subclinical Staphylococcus aureus mastitis.

Key words: *Subclinical mastitis, Staphylococcus aureus, miRNA, hub genes, bioinformatics*

TRENDS IN MILK PRODUCTION AND THE COUNTRY OF ORIGIN OF FATHER-BULL USED IN DAIRY FARM

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Abstract

The milk yield level determines the breeder´s economic profit, which is why increasing it in subsequent production seasons is so important. This can be achieved by appropriately selecting male breeders in the herd. Poland is one of the most important countries in the EU in terms of milk production; also, the animals represent a high genetic value. In Poland, in 2020, the average milk yield per cow was 8,823 kg, with a fat content of 4.07% and protein content of 3.41%. The aim of the study was to analyse changes in milk production in the high-producing herd of Polish Holstein-Friesian cattle as well as to identify the country of origin of bulls with the largest group of daughters born between 2008-2018. The analysed data concerned animals calving in the years 2010 – 2021. A total of 9,240 lactations from 9,597 cows were taken into consideration. Time trends for changes in the production level were estimated. In the examined farm, the average production results significantly exceed the national average. Moreover, it was a large herd with an average of about 900 cows. The highest milk yield in a 305-day standard lactation was achieved by cows whose calving year was in the years 2020-2021, and their yield exceeded 12,200 kg and 11,542 kg throughout the tested working period. There is an upward trend in the herd in terms of this trait. At the same time, the level of fat in milk decreased (from 3.82 to 3.38%). When analysing the share of daughters of Polish sires, it was noticed that they constituted less than 12% of the population. The most numerous group was the group of daughters of sires from the USA (over 44%), and Dutch (almost 29%), while the groups of daughters of German (11%) and Italian bulls (4.11%) were less numerous. Throughout the years, there were large fluctuations in the amount of semen used. In the case of Polish bulls, their offspring born until 2015 constituted an average of a dozen or so per cent of the population (maximum 22.02% among females born in 2008). After this period, it fell to a few per cent (the lowest was among females born in 2018). The share of daughters of Dutch bulls born in the analysed period ranged from 5% in 2011 to 45.07% in 2018, while the offspring of American bulls from 17.14% in 2019 to 53.09% in 2017. On average, the offspring of Polish, Dutch and US bulls accounted for nearly 70% of all animals on the farm. Summing up, it can be stated that the studied herd was characterised by a very good level of milk production during the studied period of time. It was also confirmed that the breeder extensively used imported bull semen, mainly from the USA.
Source of funding: Implementation doctorate ARRANGEMENT No DWD/5/0264/2021.

Key words: *cattle, milk production, bull, herd*

REPRODUCTIVE ABILITIES OF THREE COLOR VARIETIES OF CHINCHILLA: STANDARD, BLACK VELVET, AND POLISH BEIGE

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Abstract

One of the most critical sectors of the Polish agri-food industry is the production of fur animals, including chinchillas. In 2021, the percentage of female chinchilla breeding stocks covered by the utility value and genetic evaluation was 10.80%. It is the third place to other species of fur animals. The main factors determining the profitability of chinchilla production are the reproductive parameters of a herd, including fertility. A large quantity of offspring allows for obtaining a higher amount of skin. At the same time, it is necessary to care for their high quality during rearing, mainly due to the relatively low fertility of chinchillas, characterized by strong seasonality. Data for three color varieties of chinchillas: standard, black velvet, and beige Polish were obtained from the annual studies of the National Center for Animal Breeding. The obtained results included data from the years 2000-2020 and concerned the following reproductive indicators: the average quantity of litter obtained from one female, the average number of offspring obtained from a female per year (born), the average number of offspring obtained from one litter per year (born) and the percentage of rearing. All parameters are presented as a first-degree function (linear trend) with the formula yt = at + b. The highest mean quantity of litter from 1 female was obtained in 2007 for the standard and black velvet variety (R2 = 0.313). In the case of the beige variety, the highest value of this indicator (1.6) was found in 2007, 2010-2012, and 2014 (R2 = 0.504). In both cases, a decreasing trend line was found. In the standard and black velvet cultivars, the highest average number of offspring obtained from 1 female was demonstrated in 2007 (3.4) and 2010-2016, and 2018 (3.3) (R2 = 0.1343). For the Polish beige variety, this parameter at the level of 3.3 occurred only in 2011 (y = -0.165x +36.197). The average number of offspring born from 1 litter was stable (2.1) from 2010 to 2020, except for 2017 (2.2). In the case of the beige variety, high variability with an upward trend was found (y = 0.0092x-16.586). In chinchillas of all cultivars, the percentage of young chinchillas rearing was 88.5% - 92.1% from 2007-2020. It can be concluded that more favorable reproductive indicators in 2000-2020 were found in chinchillas of the following varieties: standard and black velvet. In all cases, the average number of litter and the average number of born from one female per year shows a downward trend. A negative trend line was found in the average number of born from one litter. The rearing percentage of approximately 90% makes it possible to obtain a high quantity of skins for fur production, which is an essential economic factor.

Key words: *chinchilla, color varieties, reproduction, fur animals*

THE EFFECT OF DIFFERENT FORMS OF SELENIUM ON BLOOD PARAMETERS, HEPATIC ENZYMES AND IMMUNE SYSTEM OF HOLSTEIN SUCKLING CALVES

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Abstract

Selenium is one of the low-consumption micronutrients that can be included in the diet from various sources. This study aimed to investigate the effect of different forms of selenium on blood parameters, hepatic enzymes, and the immune system of Holstein suckling calves. This study was performed using 40 male and female Holstein calves with an average age of 1 to 8 days and an average weight of 39±6 kg with 4 treatments and 10 replications as a completely randomized block design. Experimental treatments include: 1) Selenium-free base diet (control), 2) Basic diet with 3 mg / kg nano-selenium micelles, 3) Basic diet with 3 mg / kg nano-selenium colloid, 4) Basic diet with 3 mg / kg sodium selenite. The results showed that the supplementation of various forms of selenium did not have a significant effect on blood metabolites including glucose, cholesterol, triglycerides, urea, albumin, total protein, and beta-hydroxybutyrate in suckling calves. The blood concentrations of aspartate aminotransferase, alanine aminotransferase, and superoxide dismutase could not make a significant difference by adding different forms of selenium in the diet of suckling calves, while blood concentrations of glutathione peroxidase and catalase were significantly increased by adding nano-selenium micelles and selenium mineral form, respectively (P <0.05). It is generally concluded that the use of nano-selenium micelles can improve the immune status of suckling calves.

Key words: *different forms of selenium, health, growth performance, dairy calves*

THE EFFECT OF PHYTONUTRIENTS ON CALF HEALTH

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Abstract

Newborn calves have the risk of exposure to pathogens and some stress factors such as heat/cold stress in the first few days of life. Furthermore, some problems can be experienced in milk feeding programs, especially in the provision of passive immunity in herd management. For this reason, good management is required to support the health and performance of the calves. After the prohibition of the use of antibiotics as feed additives in animal nutrition in the European Union countries and in our country, the interest in alternative feed additives such as probiotics, prebiotics, essential oils, feed enzymes, phytobiotics, organic acids, etc. has increased. The main goal of the use of alternative feed additives including nutraceuticals is to reduce the use of antibiotics and promote calf health. Nutraceuticals are a large group of compounds that can be classified in various. Especially, nutraceuticals have been widely searched in animal nutrition in recent years due to their natural nature and are generally accepted as safe. In this paper, the effects of phytonutrients used in calf nutrition and included in nutraceuticals on calf health were emphasized.

Key words: *Calf, phytonutrients, performance*

INTRODUCTION

As the newborn calves in dairy farms will ensure the continuity of the herd and form the genetic material of the future, care and feeding conditions in the calf period should be carefully considered. Newborn calves have the risk of exposure to pathogens and some stress factors such as heat/cold stress in the first few days of life. Furthermore, some problems can be experienced in milk feeding programs, especially in the provision of passive immunity in herd management. For this reason, good management is required to support the health and performance of the calves. Newborn calves are disadvantaged in the gastrointestinal and immune systems both anatomical structure and functionality. Therefore, they show sensitivity to many infectious factors (Meale et al., 2017). After the prohibition of the use of antibiotics as feed additives in animal nutrition in the European Union countries and in our country, the interest in alternative feed additives such as probiotics, prebiotics, essential oils, feed enzymes, phytobiotics, organic acids, etc. has increased. The main goal of the use of alternative feed additives including nutraceuticals is to reduce the use of antibiotics and promote calf health. Reducing the pathogen exposure of newborn calves and/or supporting the development of the gastrointestinal tract can reduce the morbidity and mortality of infections. For this reason, various nutraceuticals added to liquid feeds attract a lot of attention as they are delivered directly to the targeted area (Ballou et al., 2019). Nutraceuticals are a large group of compounds that can be classified in various. Especially, nutraceuticals have been widely searched in animal nutrition in recent years due to their natural nature and are generally accepted as safe. In this paper, the effects of phytonutrients used in calf nutrition and included in nutraceuticals on calf health were emphasized.

Definition and Benefits of Nutraceuticals

The term nutraceutical is derived from "nutrition" and "pharmaceutical". “Pharmaceuticals” are drugs used primarily to treat diseases, while “nutraceuticals” are designed to prevent these diseases. Nutraceuticals are non-specific natural compounds used to improve health, prevent malignant processes and control symptoms of various diseases (Radhika et al., 2011). They consist of natural compounds or microorganisms that offer potential effects in ruminant nutrition related to increasing milk yield and disease resistance (Benchaar et al., 2008; Oh et al., 2017).

Among the most commonly used nutraceuticals in ruminant nutrition are tannins, saponins, rumen regulators, oils obtained from various plants and fruits, phenolic and polyphenolic compounds, flavonoids. Vitamins, minerals and fatty acids generally have a synergistic effect. In addition, by supplementing the ration with a quality nutraceutical, optimum health, vitality, immune status, growth, fertility and improvement in muscle structure can be achieved (Lata M., 2021). Nutraceuticals in general; can be classified as probiotic, prebiotic, phytonutrient (e.g. polyphenol, spices/essential oils) or polyunsaturated fatty acid (Ballou et al., 2019).
Nutraceuticals support health with their regulatory effects on the digestive system and metabolism in animals. Some of these effects are;

- Adding cinnamon, cloves, cardamom, bay leaf or mint to the diet changes the sensory properties of the diet (Czech et al., 2009).
- Echinacea supplement contributes to the strengthening of the immune system (Grela et al., 2003).
- They reduce susceptibility to stress during weaning, diet change and transportation (Windisch et al., 2008).
- It positively affects the digestive system of animals by inhibiting the development of pathogenic factors (Si et al., 2006).
- Provides modification and improvement of the sensory and nutrient content of meat (GrelaandKowalczuk., 2007).
- It can reduce methane gas production in ruminants (Bodas et al. 2008).
- They contain many biologically active compounds, primarily polyphenolics, which are found to have antimicrobial, antioxidant, antiparasitic, antiprotozoal, antifungal and anti-inflammatory properties (Christaki et al. 2012).

The Effect of Phytonutrients on Calf Health

Rich sources of phenolic compounds used in ruminant nutrition include numerous industrial fruit and vegetable by-products such as citrus, pomegranate, green tea, grapes, and green vegetable processing residues. After consumption, the rumen microbiota can significantly degrade polyphenols and reduce host bioavailability. However, some of the dietary polyphenols can pass through the rumen intact and reach the intestinal system. Following absorption from the gut, phenolic compounds enter the systemic circulation where they can exert their bioactive effects on inflammation, oxidative status, and immune function (Gessner et al., 2017). Chemically, flavonoids are hydroxylated polyphenolic compounds consisting of a 15-carbon chain attached to an oxygenated heterocyclic ring structure (Kalantar., 2018).
Studies on the effects of flavonoid metabolites in ruminant nutrition are mainly limited to grape, pomegranate and green tea derivatives. By-products from the wine and juice industries include residual grape pulp, which consists of seeds, skins and stems. Grape pulp contains high concentrations of flavonoid derivatives and tannins, thus serving as a potential source of antioxidant, anti-inflammatory and antimicrobial phenolic compounds (Nielsen and Hansen.,2004). Pomegranate by-products (for example, seeds, pomace and skins) are a potent source of polyphenols, predominantly containing stereoisomers of vitamin E as well as flavonoids, phenolic acids and tannins (Colitti and Stefanon., 2006; Safari et al., 2018).
Some researchers (El-Ashry et al. 2006; Khir and Ibrahim 2007; Aiad et al. 2008, Pankaj et al. 2008 and Sirohi et al. 2012) stated that the beneficial effects of essential oils and saponins obtained from medicinal plants on rumen fermentation, nutrient utilization, and growth rate of calves.
Greathead et al. (2000) added a mixture of cinnamaldehyde, cinol and eugenol essential oil components to the milk replacer feed at the level of 200 g/t and that the feed consumption increased with the addition of essential oil in the Holstein calves between 2-8 weeks, but it was reported that this increase was not statistically significant. Ünlü and Erkek (2011) determined that the daily addition of 250 mg of thyme and garlic essential oilsto the whole milk consumed by the calves did not have any effect on the daily live weight gain, feed and dry matter consumption, feed efficiency, body measurements, stool bacteria count and blood values. They also observed that the total number of coliforms in stool decreased with the addition of thyme essential oil to whole milk, while the addition of garlic essential oil reduced blood serum cholesterol levels.
In another study, Cardozo et al. (2006) determined that the addition of anise to calf diets decreased the acetate/propionate ratio, ammonia nitrogen concentration and the number of protozoa as well as improving feed consumption in calves. Bampidis et al. (2006) in their study on 30 Holstein calves, they added dried thyme plant to milk instead of antibiotics against *E.coli* pathogen bacteria that cause calf deaths in the postpartum period. The researchers stated that the dried thyme plant is

both effective on *Escherichia coli* and prevents diarrhea.

Hill et al. (2007) who conducted on 220 Holstein calves in the study, determined the effects of oils added to the calf diet during the neonatal period. The results of the study showed that the stool score in the canola or coconut oil group was better than the calves in the other groups.

Table 1 presents some studieson the effects of some phytonutrients on calf health, performance and immune functions.

Table 1.Effects of phytonutrients on theimmunefunction, health, andperformance of calves

Phytonutrient	Dose and application	Outcome	Reference
Macleaya Cordata extract	10 g/ head/ d in milk replacer	Reduced the incidence of respiratory diseases.	Köroğlu and Kocabağlı (2019)
Mulberry Leaf Flavonoids and *Candida tropicalis*	3 g/d Flavonoids and 5.0x10^{9}cfu/d *Candida tropicalis*- in milk replacer (21 d-56 d)	Reduction of fecal scours, increased feed efficiency and average daily gain, total volatile fatty acide concentration and molar proportion of propionate	(Kong et al. 2019)
Oregano	1%-1.5% or 2% oregano oil in milk replacer (4 d to weaning)	Increased IgG concentrations, decreased fecal score, reduced Enterobacteriaceae shedding	Ozkaya et al. (2018)
Essential oils	1 g/kg of starter dry matter (3- 80 d)	Improved weight gain, growth and feed efficiency	Kazemi-Bonchenari et al. (2018)
Greek Oregano essential oil	12.5 mg/kg (10 d) orally Preweaning period	Decreased incidence, severity, and duration of scours	Katsoulos et al. (2017)
Medical plant(9% tyme,25% mint,12% oregano,25% cumin,10% camel thorn, 7% garlic and 12% Eucalyptus) + Probiotic	1.5% medical plant in control diet and 2 g probiotic (60 d)	Increased dry matter intake, improves performance and immunesystem, reduces the weaning age.	(Seifzadeh at al. 2016)
Oregano	100 ppm/head/d (120 d)	No reduction in Eimeria oocyst shedding	(Grandi et al. 2016)
Pomegranate extract	**5 and 10 g/d Pomegranate extract; top-dressed On to the grain (70 d)**	**Increased peripheral cytokine synthesis (IFN-g, IL-4), improved IgG response to ovalbumin vax, no effect on fecal scores or rectal temperatures**	**(Oliveira et al. 2010)**

CONCLUSION

As a result, studies have shown that phytonutrients, one of the nutraceuticals used as an alternative with the prohibition of antibiotics, have positive effects on performance and health in calves. At the same time, nutraceuticals is a rapidly evolving field and yet these compounds lack formal regulatory oversight. They are classified as "dietary supplements" by the Food and Drug Administration. Therefore, they are not standardized in terms of composition, dose, efficacy, etc. Although these natural compounds have positive effects, there is a need for detailed studies on the use levels, duration of use and active ingredients in calf nutrition.

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MACROSCOPICAL AND MICROSCOPICAL STUDY ON SKIN LESIONS OF REFERRED FISHES

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Abstract

The aquaculture industry is a growing trend all over the world and Iran. Identification and study of the pathogenic species of fishes end up sound management maintenance and restocking of the fishes. Skin lesions (bacteria, viruses, fungi and parasites) in fish breeding industry are of high importance to avoiding high mortality rate and to properly manage them. In the present study, a total number of 173 fishes with skin lesions were referred. They were studied macroscopically and microscopically. Based on the results, of 173 fishes, 40 Red Mark Syndrome, 5 neoplasia, 61 parasitic diseases, 33 bacterial diseases, 9 fungal diseases and 25 other diseases were identified. Most fishes showed parasitic diseases and Ichthyophthirius multifiliis was the most predominant pathogen. In addition, the lowest prevalence rate was associated with benign skin tumors (one fish). Among infectious diseases, the highest rate of infection was related to parasitic diseases, bacterial diseases and fungal diseases, respectively. Skin lesions are predisposing factors for affection with secondary infections, economic losses, increased healthcare costs and growth reduction. Therefore, early diagnosis and conducting studies on these lesions are of high importance in fishes.

Key words: *fish, skin, pathology*

GENETIC PARAMETERS FOR METHANE EMISSION AND ITS RELATIONSHIP WITH MILK YIELD AND COMPOSITION

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Abstract

Methane is a major source of greenhouse gases. Ruminants are one of the main methane emitters from anthropogenic sources. Measuring methane emitted by a large number of animals is expensive and requires specialized equipment, therefore direct selection on methane emission trait for reducing methane production by dairy cow in large-scale is difficult. A total of 38342 raw milk yields (MY) and milk composition traits (dry matter (DM), protein (MP), fat (MF) and lactose yields (ML)) of 17468 Polish Holstein-Friesian cows in parity from 1 to 6 were used in the study. Three methane production (g/lactation per cow) equations (MPE) developed by Niu et al. (2018) were used to estimate indirect methane production based on milk yield and composition: MPE1 = 299 + 2.73 × MY; MPE2 = 259 + 3.86 × ECM (energy corrected milk); MPE3 = 150 + 4.31 × ECM + 28.3 × MP. The AIC of the models indicate that the MPE1 model was superior to MPE2 and MPE3 for estimating genetic parameters of methane production. The heritability estimated for methane production based on MPE1 was 0.29, which was greater than MPE2 and MPE3 (0.24). The repeatability of methane production for all models was similar (0.15 for MPE1 and 0.16 for MPE2 and MPE3). For all traits and models, except MF and MPE1, high positive genetic (0.76 to 0.82) and phenotypic (0.85 to 0.98) correlations were estimated between methane production, milk yield and milk composition. The estimated genetic and phenotypic correlations with MPE2 were similar to obtained using MPE3.

Key words: *dairy cattle; methan emission; genetic parameters*

PARTIAL PURIFICATION OF CATALASE ENZYME FROM MUSCLE TISSUE OF DUSKY SPINEFOOT (*Siganus luridus*)

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Abstract

Catalase, one of the antioxidant enzymes, decomposes hydrogen peroxide into water and oxygen. From the discovery of catalase to this day, several studies have been carried out to reveal its importance in health, food and cosmetics industries etc., and these studies are still ongoing. In this study, the catalase enzyme was partially purified from muscle tissue of Dusky spinefoot (*Siganus luridus*). Homogenate was prepared for purification, and ammonium sulfate precipitation and dialysis were applied. The enzyme was precipitated in the range of 40-60 % Ammonium Sulphate concentration. The optimum buffer was determined as 200 mM phosphate buffer, pH 8.0, and substrate H_2O_2 120 mM.

Key words: *purification, catalase, characterization, enzyme*

INTRODUCTION

Oxidative substances such as reactive oxygen species and free radicals and their undesirable biological effects are eliminated by enzymatic and non-enzymatic antioxidant defence systems. Enzymatic defence is provided by many enzyme systems such as glutathione peroxidase, glutathione reductase, superoxide dismutase, glutathione S-transferase, catalase and DNA repair enzymes. Non-enzymatic antioxidant defence systems include transferrin, lactoferrin, ceruloplasmin, uric acid, cysteine, GSH, and cysteine. Antioxidants are substances that can scavenge for free radicals and prevent cell damage (Shinda et al., 2012).

The defence systems that function in the organism to prevent the formation of reactive oxygen species ward off the damage caused by these substances, and provide detoxification are called "antioxidant defence systems" or "antioxidants" (Sener et al., 2009).

Catalase (CAT: EC 1.11.1.6) is a characteristic enzyme abundant in protein structure. This enzyme is widely present in animals, plants and microorganisms. It also plays a essential role in eliminating toxic hydrogen peroxide from cells (DeDuva et al., 1983; Master et al., 1977).

Catalase enzyme catalyzes the conversion of H_2O_2, one of the reactive oxygen species, which causes cellular damage, has a toxic effect and is one way to water. The enzyme catalase uses H_2O_2 as a substrate, both as an electron acceptor and electron donor (Lanır et al., 1975; Jones et al., 1976).

Siganus luridus is one of two species of siganus on Israel's Mediterranean coast. Both are migratory fish from the Red Sea described by Ben-Tuvia. This species migrated from the Red Sea along the Suez Canal to the Mediterranean, where it was first recorded in 1956 along the respective coasts of Israel. Later, they spread rapidly to the west and north and became widespread in Lebanon, the Turkish Republic of Northern Cyprus, the shores of Turkey, Rhodes and the Central Aegean Sea. Since then, they have established significant populations in the Mediterranean and have acquired high economic importance. There are two species belonging to the *Siganidae* family in our country (*S. luridus* and *S. rivulatus*). The primary food of these two species in the *Siganidea* family is algae. The most defining feature that distinguishes these two types from each other is; *that S. luridus* has a caudal fin close to a flat shape, while *S. rivulatus* has a forked caudal fin. The length of both species can be around 25-30 cm. *S. luridus* has spines on its dorsal and ventral fins. These sharp and strong spines are covered with mucus mixed with venom and can cause painful wounds. They may lose their colour at dusk and change colour if threatened, but usually have a brown back, light brown abdomen and fine yellow stripes on both sides. They generally prefer coastal waters that are not deeper than 40 meters and on the rocks covered with vegetation. They live their entire lives without migrating to distant places or leaving their areas (Castriota et al., 2008).

MATERIALS AND METHODS

In this study, 8 grams of muscle tissue of the used Dusky spinefoot was weighed and used to prepare homogenate. The muscle part taken from the fish was thoroughly pounded in a mortar with the help of liquid nitrogen and turned into flour, and the prepared homogenate was taken into a 50 ml tube and made up to 40 ml by adding 200mM KH_2PO_4+0.5mM EDTA+%PVP (pH:8). It was then centrifuged for 20 minutes at 15000 g at +4°C.

Solid ammonium sulphate precipitation process was applied to homogenates obtained from muscle tissue of Sokar fish in the range of 0-20%, 20-40% and 40-60%, respectively. In this process, the homogenate is kept in ice and placed on a magnetic stirrer. Then, solid ammonium sulfate was carefully added to the homogenate at intervals of 45 seconds, not exceeding 1 gram. Centrifugation was performed for 10 minutes at 10000 g at +4°C at each saturation interval.

Dialysis is performed to remove salts from the protein solution. In this study, dialysis was applied to remove the salt around the precipitated proteins at 40-60% ammonium sulfate saturation. For dialysis, 200mM phosphate buffer was prepared, and the sample containing the precipitated proteins was placed into the membrane and left in the prepared buffer solution for 2 hours to remove the salts around the proteins.

Tris and phosphate buffers were prepared at 10mM, 50mM, 100mM, 200mM, and 300mM concentrations, and activity measurements were made with these buffers at different concentrations.

In order to determine the optimum pH after buffer characterization, phosphate buffer was prepared at pH ranges of 5.5-6-6.5-7-7.5-8-8.5-9 and activity measurement was made at these intervals.

İn order to determine the optimum substrate concentration, activity measurements were made for optimum substrate concentration using 25µl, 50µl, 100µl, 150µl, and 200µl H_2O_2 substrate.

Determination of the optimum enzyme concentration was done by observing activity measurements which were made using 25µl, 50µl, 100µl, 150µl, and 200µl enzymes.

RESULTS AND DISCUSSION

Catalase is one of the important antioxidant enzymes that significantly reduces oxidative stress by destroying cellular hydrogen peroxide to produce water and oxygen. It is assumed that catalase deficiency is associated with the pathogenesis of many age-related degenerative diseases such as diabetes, hypertension, anaemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder, cancer, and schizophrenia. Therefore, efforts are being made in many laboratories to investigate its use as a potential drug in treating such diseases (Nandi et al., 2019).

Ammonium sulphate was precipitated in the ranges of 0-20%, 20-40% and 40-60%, respectively, into the homogenate prepared for purifying catalase enzyme from the muscle tissue of Dusky spinefoot. In the results obtained after the activity measurements, it was determined that the value giving the highest activity was between 40-60% ammonium sulfate saturation.

KH_2PO_4 measurements were made at different concentrations to purify catalase enzyme from Dusky spinefoot muscle tissue. As a result of the activity measurements, it was determined that the most suitable ionic strength for catalase enzyme from stingray tissue was in 200mM KH_2PO_4 buffer.

Catalase enzyme activity in shaded Dusky spinefoot muscle tissue was measured by spectrophotometer at pH 5.5-9.0 using 200 mM phosphate buffer. The optimum pH value of catalase enzyme obtained from Dusky spine foot muscle tissue was determined as pH 8.0 in 200mM phosphate buffer.

The optimum substrate amount for catalase enzyme was measured using 200mM KH_2PO_4 (pH 8.0) between 25-200 µl from Dusky spinefoot tissue. The optimum amount of substrate was determined as 120 µl.

CONCLUSIONS

This study is for purification, determination of structural properties and characterization of catalase enzyme from Dusky spinefoot. Hoping that the results of this study will contribute to the elucidation of the function of the catalase enzyme in Dusky spinefoot, we think that our research can help future studies on catalase enzyme and Dusky spinefoot.

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COMPARISON OF SHREDDING TRAITS IN MEAT AND DAIRY CATTLE BREEDS

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Abstract

Ten body meats obtained from 12-24 months old angus and holstein crossed male cattle were used in the study. Quartering of carcasses was done at the eleventh intercostal space. As a result of the fragmentation process; It was determined that the ground and cubed meat ratios of Angus cattle were higher, and the ratio of meat (round, tenderloin, ribeye steak, sirloin), round meat and bone ratios was lower.

Key words: *carcass shredding, bone ratio, meat ratio*

EFFECTS OF MIXING BUFFALO MILK AND COW MILK ON VISCOSITY AND COLOR CHARACTERISTICS OF YOGHURTS

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Abstract

Buffalo yoghurt is considered the health promoting effect. However some costumers not prefer due to its taste and appearance. The objective of this study was to evaluate yoghurt made from buffalo milk and a mixture of buffalo and cow milk. In the experiment pure buffalo milk and buffalo+cow milk (50/50) mixture were used. Milks obtained from Kayseri Buffalo Association's pooled tank. The milks were pasteurized at 90oC for 15 minutes and then cooled to 40 to 42oC, then inoculated with a yogurt starter culture contained Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Yoghurts were transferred for cooling unit after 4 hours fermentation. The samples were then analyzed for 14 days. The result shows that the viscosity values of the mixture of buffalo+cow milk yoghurt's viscosity values were significantly higher than buffalo yoghurt (P<0.05). The samples of the buffalo yoghurt had a higher colour value L than the mixture of buffalo+cows milk yoghurt during all storage days. Redness (a*) values were not significantly changed both types of yoghurt. Yellowness (b*) values of the mixture of buffalo+cow were higher than that of buffalo yoghurt (P<0.05); the buffalo yoghurt had a whiter colour. In conclusion, cow milk addition to buffalo milk caused change on viscosity and color values of yoghurts.*

Key words: *Yoghurt, buffalo, cow, viscosity, appearance, sustainability*

PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF CAMEL MILK YOGURT

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Abstract

Camel milk may comprise all of the necessary nutrients as well as a variety of chemicals that be beneficial to health due to its anticancer, antihypertensive, hypocholesterolemia, antidiabetic, and antioxidant properties. Among fermented dairy products, yogurt is one of the most widely consumed. Camel milk boosts metabolism and lowers blood cholesterol levels, as well as prolonged shelf life. Camel milk contains 2.9 to 5.5% fat, 2.5 to 4.5% protein, 2.9 to 5.8% lactose, 0.35 to 0.90% ash, 86.3 to 88.5% water, and 8.9 to 14.3% solid-non-fat (SNF). In yoghurts made from bovine milk, as the storage time increases, total bacteria, yeasts, molds, Lactobacillus and Streptococcus spp. while increasing; there is a decrease in the number of these microorganisms in yoghurts made from camel milk. Yogurt produced using good manufacturing techniques have a shelf life of 3–4 weeks at 5°C and contains no more than 10 yeast cells per g. The fundamental cause of yogurt´s sourness and refreshing flavor is its acidity. Yogurt produced from camel milk scored substantially higher for sourness than yogurt made from cow milk. The camel milk plain yogurt had a strong scent and salty flavor and has a solid texture. Camel yoghurt's pH value change between 4.59 to 4.63 and titrable acidity of 0.71 to 0.87 % lactic acid. pH values of camel milk yoghurt is decreasing a longer storage period however better The viscosity of camel milk is lower than that of cow milk, so, it is look like solidness is higher in camel yoghurt. Due to insufficient denaturation of whey protein, excessive acidification, mechanical shaking of the gel network, an excessively long incubation period, or insufficient acidification (pH>4.5), this quality flaw occurs in camel milk yogurt. The observed syneresis value for camel milk yogurt is approximately 33.5. In conclusion, there are some advantages in camel milk yoghurt compared to cow milk yoghurt.

Key words: *camel milk, yogurt, Physico-chemical, Sensory*

SPOILAGE MICROORGANISMS IN MILK AND MILK PRODUCTS

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Abstract

Milk quality depends on chemical parameters as well as microbial and somatic cell counts and affects the price of milk. When milk is stored as raw or processed into various products, it is exposed to spoilage due to the proliferation of some micro-organisms and change their taste which makes impossible to use for human consumption. These microorganisms can take place during milking, handling, storage and other pre-processing activities additionally they can produce heat-stable proteases and lipases which remains active after pasteurization and thus can spoil the milk during prolonged storage. They are also responsible for visible or non-visible defects such as off-odor and flavor and cause wide range of milk and milk product wastes as well as economic losses. Spoilage microorganisms include aerobic psychorotrophs, gram-negative bacteria, hetero-fermentative lactobacilli, yeasts, molds and spore-forming bacteria. Control of these spoilage microorganisms is extremely important for the industrial production of milk and products and the health of human population. Inspite of quality control, pasteurization and ultra-high temperature (UHT) treatments, numerous outbreaks of foodborne illnesses due to the consumption of spoiled dairy products were reported. According to the World Health Organization (WHO) report in 2015,every year as many as 600 millions people in the world fall ill after consuming contaminated food. Of these, 420,000 people die, including 125,000 children under theage of 5 years. Health experts estimate that the yearly costs of all the milk spoilage are approximately 5 to 6 billion USA dollars. On the other hand, bacterial contamination in processed food has led to many large-scale recalls of food. Several methods are practiced to prevent the contamination of these microorganisms to the milk and milk products which includes good manufacturing and hygiene practices, air filtration and decontamination systems. Also, the decrease of spoilage microorganism population can be achieved by decreasing the PH via fermenting of lactose to lactic acid, and adding desirable microflora that restricts the growth of undesirable microorganisms, and adding sugar or salt to reduce water activity, also packaging and freezing to limit available oxygen.

Key words: *spoilage microorganism, economic loses, food borne diseases, sustainability*

COMPARISON OF VARIOUS SEROLOGICAL TESTS, CULTURE AND PCR TO DIAGNOSE *BRUCELLA MELITENSIS* IN DOG

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Abstract

The objective of this study was to compare different tests to detect *B. melitensis* in dogs. The samples (whole blood samples for PCR and culture, vaginal swab samples for PCR test, and serum samples for serological tests) were taken from 14 bitches at a kennel, in which a bitch had been aborted due to *Brucella melitensis* infection. Also, whole blood sample and semen sample were also obtained from the only male dog of this Kennel. The results of our study showed that in suspected cases of *B. melitensis* (recently abortion, dead fetuses, or other signs suspected to brucellosis) in dogs, the cheapest and most reliable test for diagnosis is the Rose Bengal test. In the next step, the PCR test of the vaginal swab sample can be used to confirm the diagnosis.

Key words: *dog, brucellosis, culture, PCR, serological test*

INTRODUCTION

Brucellosis is one of the most prevalent bacterial zoonotic diseases causing significant economic losses due to the livestock abortion, and also it is possibly a life-threatening multi-system disease in human(Pappas., 2010, Seleem et al., 2010).

B. canis is the most common cause of canine brucellosis , although occasional infections with B. melitensis, B. abortus, or B. suis occur in dogs that have close contact with tissues or secretions of infected livestock animals, especially raw milk, aborted fetuses, and placentas (Woldemeskel et al., 2013; Baek et al., 2003).

Canine brucellosis, that is caused by *Brucella* other than *B. canis,* is increasingly gaining importance as a significant source of disease for other animals and human beings. There are reports from many countries that pregnant bitches have aborted their foetuses due to infection by *B. melitensis* as well as *B. abortus* or *B. suis* (Morse *et al.,* 1953; Taylor *et al.,* 1975; Srinlvasan *et al., 1992).*

Considering that infected dogs can shed organisms into the environment via urine and vaginal discharges and secretions, aborted materials or feces, they play a significant role in the maintenance of *Brucella* spp. and its possible transmission to other dogs, cattle, and humans (Baek et al., 2003; Forbes et al., 1990).

Rose Bengal plate test (RBT) is a routine qualitative test for brucellosis in both humans and animals, which could detect *B. abortus*, *B. melitensis,* and *B. suis.* Definitive diagnosis is supported by bacterial isolation followed by PCR, but culture is not widely available because it is time-consuming and requires a laboratory with proper biosafety conditions given that this pathogen poses a potential health risk to laboratory workers as a result of aerosol transmission. (Carmichael et al., 1660; Sam et al., 2012; Wallach et al., 2004; Wanke, 2004)

The aim of the present study was to compare different tests to detect *B. melitensis* in dogs.

MATERIALS AND METHODS

In January 2022, a 4-year-old German shepherd was brought to the Small Animal Hospital of Veterinary Medicine, University of Tehran, Iran. The bitch was aborted one day before coming to the hospital (at day 57 of pregnancy). The bitch had no clinical symptom but vaginal discharge. The previous pregnancies of this dog had culminated to parturition without any problems. This animal came from a little kennel, without brucellosis and reproductive control.

Serum, whole blood and vaginal swab samples were obtained. Initially the serum were screened for the presence of anti- *Brucella* antibodies using Rose Bengal plate test (RBT), which is a routine qualitative test for brucellosis in both humans and animals (Razi Vaccine and Serum Research Institute, Iran). The result of RBT was positive in this bitch. Then *Brucella* species DNA has been detected in blood and vaginal swab samples (Sinaclon DNA kit, Iran). The results showed that the case encountered *Brucella*

melitensis. As the *B. melitensis* is the most pathogenic species of *Brucella* for humans, we decided to test the kennel for this pathogen. So, the samples (whole blood samples for PCR and culture, vaginal swab samples for PCR test, and serum samples for serological tests) were taken from 13 other bitches at this kennel. Also, whole blood sample and semen sample were also obtained from the only male dog of this Kennel.

RESULTS AND DISCUSSION

Sera from 8 out of 14 dogs were found positive by Rose Bengal test (RBT) and vaginal swab PCR test. The RBT positive samples were further evaluated using Wright serum agglutination test and 2-ME. The results showed that in standard tube agglutination test (STA) or Wright Test and also the 2-Mercaptoethanol (2-ME), just 5 out of 14 bitches were positive. As shown in the table 1, the vaginal swabs of 8 bitches were positive in PCR (comparable with the results of RBT), but 7 bitches showed positive results in whole blood PCR. The only positive whole blood culture of *B. melitensis* was belonged to the recently aborted bitch. Our report was based on data derived from bacteriological culture, RBT, wright test and 2-ME test. Further confirmation was by positive PCR using rigorously tested *B. Melitensis*-specific primers.

The result of PCR test of whole blood on male dog was positive for *B. melitensis,* although the culture of whole blood and also the PCR test on semen sample were negative.

Brucellosis is one of the most important zoonotic diseases worldwide (Wanke, 2006; Pappas, 2010), and most of Brucella species are capable of infecting humans, although they have a highly variable zoonotic potential. *B. melitensis* is the most pathogenic species of Brucella for humans, with the exposure to only 1–10 CFU(colony forming units) being sufficient for establishment of infection, whereas B. suis and B. abortus have intermediate zoonotic potential. (Young, 1983; Xavier et al., 2010).

Bacteremia typically persists for months, but it may be intermittent, so serial blood cultures should be performed if the initial culture is negative. This is may be the reason in which the blood culture of 7 cases were negative in our study.

Although, it was reported that the only detection tests for detecting *B. canis* with reasonable agreement were PCR and 2ME-MAT (Juliana et al; 2020), the results of our study showd the RBT and PCR of vaginal smear sample were superior to other tests to detect *B. melitensis* in bitches.

CONCLUSIONS

It is reported that in screening to detect *B. canis* in kennels, the use of a single laboratory method, or even the use of different laboratory methods, may not be sufficient to reach a definitive diagnosis. (Juliana et al; 2020).

The results of our study showed that in suspected cases of *B. melitensis* (recently abortion, dead fetuses, or other signs suspected to brucellosis) in dogs, the cheapest and most reliable test for diagnosis is the Rose Bengal test. In the next step, the PCR test of the vaginal swab sample can be used to confirm the diagnosis.

Table 1. Results of different tests on 14 bitches suspected *to B. melitensis* infection

WBC	B. PCR	V. PCR	RBT	WT & 2ME
$1/14^{a}$	$7/14^{b}$	$8/14^{b}$	$8/14^{b}$	$5/14^{b}$

[a,b,c] Different superscript letters in the row indicate significant difference ($P<0.05$).
WBC = Whole Blood Culture, B. PCR = Blood PCR, V. PCR = Vaginal smear PCR, RBT = Rose Bengal Test, WT = Wright test, 2ME = 2- Mercaptoethanol test

ACKNOWLEDGEMENTS

We would like to thank Dr. Ashrafi-temay, I., at the laboratory of Microbiology section at the Faculty of Veterinary Medicine, University of Tehran for their overall cooperation in this project.

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INFLUENCE OF HOUSING CONDITIONS ON THE WELFARE OF INDOOR CATS

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Abstract

The domestic cat is becoming more and more popular as a companion animal. Currently, the number of cats in Europe (110 million) exceeds that of dogs (about 90 million). It is estimated that about 6 million cats are kept in Poland. As the kept animal population grows, their keepers´ sensitivity to ensuring proper welfare increases. This is primarily a consequence of the "A Universals Declaration of Animal Rights" adopted in 1977 by the International League for Animal Rights and Affiliated National Leagues, which among others, includes animal's right to be taken care of (in Poland, the act on the protection of animal rights was introduced in 1997). The research aimed to determine the influence of demographic factors, particularly housing factors, on the welfare of indoor cats. The study was carried out using an electronic questionnaire published on Facebook. The survey questionnaire consisted of a total of 63 questions, and 1000 respondents answered these questions. The welfare of cats kept in households was determined based on answers to the following questions: "Does the cat have the ability to move around the entire area of the house/flat?"; "Does the cat have the opportunity to go out to the balcony or terrace?"; "Does the cat have a shelter?"; "Does the cat have a place to rest (e.g. a vertical scratching post)?"; "Does the cat have a cat lair/bed in the house/flat?"; "Does the cat have toys?"; "How would you define a relationship between animals?"; "Do you let your cat go outside?". Based on the obtained responses, animal welfare was classified as: very good (380 people), good (616 people) and bad (4 people). The last class (well-being = bad) was omitted in the final statistical study due to a low number of responders. In order to select variables significantly related to the welfare of cats (very good, good), multiple logistic regression was used in conjunction with the method of selecting forward variables. The statistical analysis was performed with the use of the SAS computer package using the LOGISTIC procedure. The statistical analysis showed a highly significant impact of the size of the household area, the maintenance expenses, and the age of the owners on the cat´s welfare. The analysis of the determined odds ratios allows the conclusion that the very good welfare of cats was observed in premises that had more than 50 m2, for which monthly maintenance expenses exceeded EUR 15.5 and whose owners were above 18 years of age.

Key words: *indoor cats, welfare, housing conditions, prediction;*

POLYCYSTIC KIDNEY DISEASE IN A PERSIAN CAT

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Abstract

Autosomal dominant polycystic kidney disease (AD-PKD) is a genetic feline disease characterized by fluid-filled cysts formation in one or both kidneys. Persians and Persian-related cats can be affected. The renal cysts are congenital, can arise from any part of the nephron. In August 2021, a five-year-old male Persian cat weighing 6.5 kg with severe bilateral abdominal swelling and a clinical history of lethargy, anorexia, polyuria, polydipsia and vomiting was referred to the Clinic. Physical examination, blood cell counts, serum biochemistry tests, urinalysis radiography and ultrasonography were performed. Hematology and biochemistry results showed mild leukocytosis, mild anemia and severe azotemia. Dorsoventral radiograph showed bilateral abdominal distention. Ultrasonography revealed significant enlargement of both kidneys with multiple anechoic or hypoechoic cysts. Finally, the cat was euthanized due to the severity of clinical signs, and necropsy was performed. At necropsy, on cut surface, the sonographically diagnosed polycystic structure was apparent, and the cystic cavities were contained varying amounts of water-like fluid. Histopathological examination of the kidneys revealed cystic structures. The cystic tubules were lined by cuboidal or squamous epithelium and separated by a fibrous connective tissue. Some cysts were filled with eosinophilic and amorphic material. Birefringent crystals were observed with polarized and non-polarized light. Based on ultrasonographic images, clinical, radiological and histopathological findings, this disease was diagnosed polycystic kidney disease. Similar to our case, ultrasound as a noninvasive technique is the most commonly used imaging modality for diagnosis of PKD in cat. PKD is considered a progressive disease, and the prognosis is guarded.

Key words: *Cat, Necropsy, Pathology and PKD*

AGGRESSION IN INDOOR CATS

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Abstract

Aggression is a common behavioural disorder seen in cats. Indoor cats show aggression not only towards their owners but also towards other household members and animals. Aggression towards other animals can cause conflicts and even a lack of acceptance between animals living in harmony. Aggression in indoor cats is a severe problem for their owners, which may result in the decision to return the cat to the shelter. The aim of the study is to analyze the occurrence of aggressive behaviour among indoor cats and to try to determine their causes. The data for the analysis came from a survey in which 422 respondents, owners of cats from Poland, participated. The survey consisted of 42 single- and multiple-choice questions. In addition to closed-ended questions, there were also open-ended questions to which the respondent had to provide a short written answer. The chi-square test was used to determine the factors statistically related to aggression in indoor cats. The studies showed that 43% of indoor cats showed aggressive behaviour. The chi-square test found that the aggression of indoor cats was determined by the following factors: castration, breed, sex, health problems, regularity of feeding, time spent on their own, changing toys and diversifying the environment. It was observed that males showed more aggressive behaviour than females. Purebred cats showed aggressive behaviour more often than non-purebred cats. Among the tested domestic breed cats, aggressive behaviour was most frequently observed in British Main Coon cats. On the other hand, it is the least common in Bengal and Russian cats. It was observed that healthy cats were less aggressive than cats with health problems, and neutered cats were less aggressive than non-neutered cats. Cats fed irregularly at different times of the day were shown to be more aggressive than cats fed regularly at fixed times. On the other hand, cats that during the day spend a long time alone more often exhibited aggressive behaviour. It was found that cats whose owners did not change toys showed aggression more often than cats whose owners changed toys. Moreover, a more diverse environment decreased the chance of aggression in indoor cats. The result of the study shows that while aggression is common in indoor cats, it can be minimized by appropriate actions of the owners, such as castrating aggressive cats, providing regular feeding at a fixed time, providing a diverse environment, changing toys etc.

Key words: *Cat, Behaviour, Aggression, Indoor*

CHEMICAL COMPOSITION AND FATTY ACID PROFILE OF THE MEAT OF PIGS FED WITH THE ADDITION OF LEGUMES

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Abstract

Proper feeding of fattening pigs contributes to the increase in body weight, i.e. the growth of muscle and fat tissue, and the development of the skeletal system, as well as the composition of individual tissues. It plays an important role in shaping the chemical composition of meat and influences the formation of the fatty acid profile. Therefore, a lot of attention is paid to ensuring that fatteners have the appropriate nutrient content in the ration, especially protein. Most often it is soybean protein, but in order to become independent from this feed component, it is more and more often replaced with seeds of other legumes, which, unlike soybean, have not been genetically modified. The aim of the study was to analyze the chemical composition and fatty acid profile of meat of pigs fed with fodder containing a different proportion of legumes (pea and lupine). The meat was obtained from 45 fatteners of hybrids F1(Polish Latrge White x Polish Landrace) x F1(Pietrain x Duroc). Fattening was carried out in two phases with the use of complete mixtures. The animals were divided into three equal groups (n=15), due to the different source of protein in the feed: control group K - I phase protein in the form of post-extraction soybean meal (100%); experimental group: D1 - fattening phase I soybean protein / pea and lupine protein (50% / 50%), phase II soybean protein / pea and lupine protein (25% / 75%); experimental group D2 - phase I soybean protein / pea and lupine protein (50% / 50%), phase II peas and lupins (100%). After finishing the fattening, the animals were slaughtered. After a 24-hour cooling of the carcasses, meat samples were taken from the dorsal part of Longissimus lumborum muscle. The basic composition of meat was determined: the content of water, protein, fat and collagen, the content of minerals and the share of individual fatty acids. Meat from all analysed groups was characterized by a high protein content (K-25.14%, D1-24.71% and D2-25.09%) with a simultaneously low fat content, within the limits of D 21.59% - D1 1.22%. The content of macro- and microelements in the studied groups of pigs was very even. Among the analyzed fatty acids, no significant differences were found between the studied groups, which proves their very balanced level. The content of saturated fatty acids (SFA) was quite high in all the studied groups (K-44.02%, D1-43.86%, D2-42.27%). The presence of polyunsaturated fatty acids (PUFA) was recorded in all the groups at an even level (within 12.09 (D2) to 13.54 (D1). The share of omega-3 acids in this experiment ranged from 1.66% (K), through 1.74% (D2), to 1.88% (D1), which allowed to obtain a PUFA n-6 / PUFA n-3 ratio at the level of 6.24 - 7.03. The use of various proportions of legumes in the nutrition and the use of domestic sources of protein in the form of peas and lupins did not affect the value of meat, resulting from the chemical composition and content of appropriate fatty acids.

Key words: *soy bean, peas and lupine, fatteners, chemical composition, fatty acids*

EFFECT OF DIFFERENT LIGHTING PROGRAMS ON EGG PRODUCTION OF TURKISH NATIVE GEESE

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Abstract

The demand for goose production is tending to increase both in the World and in Turkey. The geese are seasonal breeders and start laying in February-March in Northern Sphere. This limits the number of produced eggs. For example, Turkish native geese lay 10-13 eggs annually, and 7-9 goslings hatch from these eggs. Lower productivity of native geese inhibits the improvement of production. Therefore, one of the most critical topics in goose breeding must be increasing the productivity of native geese. In this study, different lighting programs were used to determine the effect on egg production, because lighting has a significant role in the egg production of poultry species. Four different programs were used in four different windowed barns between November-June of 2019. In two of barns a fixed 14 and 20 hour lighting was applied, while one has no lighting as control group. In the control group windows of the barn allowed daylight to enter inside. Variable lighting program was used for the last group. Geese start laying in March, so we tried to make the lighting program of March artificially in November. From the first day of November, artificial lighting was applied between the sunrise and sunset of the first day of March in the region and changed every day according to the changes of March. Each lighting group had four replicates containing 3 females and one male at two years of age. The geese were randomly chosen from the flock and allocated to groups. On contrary to expected results, lowest egg production was obtained from 20 hours lighting group with 9,76 mean eggs per goose, while highest was obtained in control group with 14,68 mean eggs per goose. The mean eggs per goose in 14 hours lighting group was 11.58 while 10.82 in variable lighting group.

Key words: *Goose, lighting, egg production, native*

HATCHING EGGS, CHICKS, AND EGGSHELL WEIGHT ANALYSIS AND PILOT STUDY OF INCUBATION ENVIRONMENTAL PARAMETERS MONITORING WITH THE USE OF A FORCE AND TEMPERATURE TESTER

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Abstract

Hatching eggs are the basis of all poultry production. Its effectiveness depends on the parent flocks and the incubator´s environmental conditions. The changes in the egg during incubation are believed to depend on the egg´s initial weight and the hatching chamber temperature and humidity. The eggs are also exposed to mechanical damage. It is caused by the vibration of the incubator and during routine work in the hatchery. The study aimed to assess the egg´s weight during incubation, chicks, and eggshells and to analyze the hatch quality. As part of the pilot study, environmental conditions and the forces acting on the egg were also monitored using the Wireless Egg Node® (WEN). 1011 eggs obtained from Rosa 1 hens were used. The eggs were divided into two groups (1, <60 g; 2, >60 g). Incubation lasted 21 days. The eggs were weighed before incubation and on days 7 and 18. After hatching, the chicks and eggshells were weighed. The quality of the brood was analyzed. The temperature and humidity were controlled during the incubation. Data on the egg, chick, and eggshell characteristics were statistically processed using the Student´s t-test with a P <0.05. On the selected days, the forces acting on the egg were recorded using a WEN. Continuous changes in incubation parameters were demonstrated, which could affect the quality of the obtained chicks. The influence of the initial egg weight on the weight of the hatched chicks was found (P <0.001). Eggs from group 2 were characterized by higher weight (P <0.001) and eggshell thickness (P =0.013). Group 1 showed higher water loss by day 18 (P =0.040). It could be due to more evaporation through the thinner eggshell. It was found that in group 2, more abnormalities led to a lower brood. The WEN device gave information on the forces affecting the egg during the incubation and activities related to its proper course. It was shown that incubator forces could not cause mechanical damage to the eggshell. The analyses suggest that the control of incubation parameters is a crucial factor influencing the efficiency of poultry production.

Key words: *hatching egg, hen, incubation, brood quality, environmental parameters*

VARIATION IN GROWTH PERFORMANCE AND FEED CONVERSION RATIO IN SELECTED FUNAAB ALPHA (Dual purpose and layer line) CHICKENS

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Abstract

A study was conducted to examine variations in growth performance and feed conversion ratio in FUNAAB Alpha dual-purpose and FUNAAB Alpha layer line chickens. A total of 200 birds, comprising 100 FUNAAB Alpha dual purpose and 100 FUNAAB Alpha layer lines, were sampled. The data obtained were subjected to a one-way analysis of variance (ANOVA) and the Duncan Multiple Range tests were used for mean separation. Results showed that FUNAAB Alpha dual-purpose birds exhibited superior growth performance when compared to the FUNAAB Alpha layer line. Chicken genetic lines and sex differences significantly influence growth performance, average feed intake, and feed conversion ratio ($p<0.05$). Dual-purpose FUNAAB Alpha lines consistently weighed heavier (942.22±21.48) than the layer lines (765.30±15.88). Body weight and morphometric indices of both FUNAAB Alpha genetic lines increase as birds advance in age, with the widest mean breast girth value recorded among dual-purpose chickens. Male FUNAAB Alpha birds weighed heavier than their female counterparts in both dual-purpose and layer genetic lines. Irrespective of chicken genetic lines, the quantity of feed consumed increased significantly per bird per week continuously with age. The FUNAAB Alpha dual-purpose line consumed more feed in weeks 1, 2, 3, 5, 6, 7, and 8, except in week 4. The dual-purpose FUNAAB Alpha chicken line exhibits superior body weight, feed intake, and feed conversion ratio as compared to the layer lines.

Key words; *Variation, growth performance, feed conversion, and FUNAAB Alpha chickens*

INTRODUCTION

All over the world, livestock keeping constitutes essential support for the livelihood of nearly 20 to 40 billion people in developed and developing countries. In particular, poultry production is believed to be one of the major industries supporting the nutritional needs of the vast population (Malpotra *et al.,* 2017). In Nigeria, the poultry production industry comprises over 180 million birds (FAOSTAT, 2019), representing the second largest chicken population in Africa after South Africa (SAHEL, 2015) and accounting for 650 000 tonnes of eggs and 300 000 tonnes of poultry meat annually (FAOSTAT, 2018). The sub-sector remains one of the most common and economically rewarding means of providing animal base proteins that contribute to food security and nutrition supply.

Nigerian indigenous chickens are fast-growing species, reared significantly to supply both meat and eggs for human consumption and as a source of income. They are believed to be hardy and capable of thriving in tropical environments on little or no feed inputs (Odah *et al.*, 2019), unlike conventional broiler strains. Although modern broiler selection programs have produced rapidly growing birds with low feed conversion and high meat yield (Gonzales *et al.,* 1999), these incomparable features make the improvement of indigenous stock imperative and give rise to the development of FUNAAB Alpha chicken lines (Etim and Udoh, 2014; Adebambo, 2017).

The demand for chicken meat is fast evolving globally (Fesseha *et al.*, 2021), indicating the need to breed birds with a rapid growth rate and high feed conversion ratio. Amakiri *et al.* (2011) identified the main indices for economic poultry production as fast growth rate and efficient feed conversion. These factors can be achieved through good management practices that ensure effective disease prevention and control, coupled with the availability of quality feed, fed

ad libitum. Previous studies on chicken growth rate and feed conversion ratio have focused on growth patterns under continuous and restricted lighting (Amakiri *et al.*, 1992; Buyse *et al.*, 1996). Also, there is limited information on the variation in growth performance and feed conversion ratio of improved indigenous Nigerian birds. This study is therefore designed to investigate the variation in growth performance and feed conversion ratio in FUNAAB Alpha chickens, especially (dual-purpose and layer genetic lines).

MATERIALS AND METHODS

This research was carried out at the Programme for Emerging Agricultural Research Leadership (PEARL) farm of the Federal University of Agriculture Abeokuta, Nigeria. Abeokuta is in Southwestern Nigeria's rainforest zone, with a latitude of 7° 13′, 49° 46′ N, a longitude of 3° 26′, 11° 98′ E, and an elevation of 76 mm above sea level. Amujoyegbe *et al.* (2008) found the annual mean temperature and humidity to be 34 °C and 82%, respectively. The mean monthly ambient temperature of the site ranges from 28˚C in December to 36˚C in February, with a yearly average of 34˚C. Relative humidity ranges from 60% in January to 94% in August, with a yearly average of about 82%.

A total of 200 birds were used. The population was comprised of two lines of improved Nigerian indigenous chickens; 100 FUNAAB Alpha dual-purpose birds (NDL) and 100 FUNAAB Alpha layer line (NLL) respectively. The birds were generated via artificial insemination from parent stock kept at the Poultry Breeding Unit of the farm. Birds were fed *ad libitum* on a broiler super starter mash that supplies 23-25% crude protein and 3000Kcal/Kg metabolized energy from 0 to 4 weeks. Thereafter, they were subjected to a broiler finisher diet of 21% crude protein and 2900Kcal/KgME metabolized energy for 5-8 weeks. Clean water was supplied *ad libitum* throughout the period of the experiment.

Data collection

The initial live weights of the chicken were observed for each line at the commencement of the experiments using a sensitive scale with 0.01g sensitivity. The average live weight per bird was measured weekly by weighing the chicken in each pen per line for 8 weeks, and the total live weight was divided by the total number of birds in the pen to obtain the average live weight per chicken per line, which was then applied to determine the chicken feed efficiency. The weekly mean feed intake, body weight measurements, daily feed intakes, and efficiency were determined accordingly until the termination of the experiment.

$$Feed\ efficiency\ (FE) = \frac{\text{average body weight per bird per day (g)}}{\text{Feed consumed per bird per day (g)}}$$

Growth data

Day-old chicks (DOC) were wing-tagged appropriately and weighed using a 0.01 g sensitive balance. The birds were reared together, and differences due to sex, age, and chicken lines were noted. Body weight indices measured on a weekly basis for 8 weeks are body weight (BW) and breast girth (BG); these were measured with the use of a weighing scale and measuring tape.

Statistical analysis

Data on feed intake, feed conversion ratio, and feed efficiency of all lines were analyzed using one-way analysis of variance (ANOVA) to determine the mean amongst strains and the differences between the means of strains. Mean separation was carried out using the Duncan multiple range test in SAS (2009). A general linear model (G.L.M.) was used;

$Y_{ij} = \mu + G_i + S + GS_{ij} + \varepsilon_{ijk..}$

Where;

Y_{ijkl} = Observed variable,

μ = Population mean,

G_i = Effect of i^{th} chicken lines (FUNAAB Alpha dual purpose and FUNAAB Alpha layer lines)

S_j = Effect of j^{th} chicken sex (Male and female)

GS_{ij} = Effect of interaction of the i^{th} chicken genotype and sex

ε_{ijkl} = Residual error.

RESULTS AND DISCUSSION

Effect of chicken genetic lines on their growth performance

Table 1 represents the effect of chicken lines on the growth performance of FUNAAB Alpha birds. Chicken lines exhibited significant effects on growth performance ($P<0.05$). FUNAAB Alpha dual-purpose strain consistently showed heavier body weight (942.22±21.48), compared to layer lines with (765.30±15.88) body weight at the 8^{th} week. At week 4, comparable body weight values were observed (354.82±8.01, and 308.32±5.02) for both lines. Chicken breast girth measures increase as birds advance in age. The influence of f chicken line on breast girth was significant ($P<0.05$) with the widest breast girth observed

among dual-purpose strains. However, breast girth values at early growth stages were significantly different across strains.

Table 1: Effect of chicken line on the growth performance

Age (week)	Genotype	N	Body weight (g)	Breast Girth (cm)
	NLD	101	31.97±0.37^{b}	-
0	NLL	102	32.15±0.35^{ab}	-
	NLD	101	67.22±1.23^{a}	8.75±0.08
1	NLL	102	62.98±0.79^{b}	8.24±0.04
	NLD	100	133.79±3.23^{a}	11.27±0.11
2	NLL	102	118.22±1.88^{b}	10.57±0.11^{ab}
	NLD	100	235.94±5.35^{a}	13.52±0.11
3	NLL	102	193.44±3.19^{b}	13.17±0.09
	NLD	100	354.82±8.01^{a}	15.72±0.13
4	NLL	102	308.32±5.20^{b}	15.90±0.11
	NLD	99	493.85±11.76^{a}	18.52±0.15
5	NLL	102	397.09±7.35^{b}	17.11±0.12^{b}
	NLD	99	591.32±14.02^{a}	19.29±0.17
6	NLL	101	497.14±9.08^{b}	18.08±0.13^{b}
	NLD	99	779.04±17.87^{a}	20.86±0.17
7	NLL	101	587.21±11.84^{b}	19.15±0.14^{ab}
	NLD	99	942.22±21.45^{a}	21.83±0.18
8	NLL	101	765.30±15.88^{b}	20.34±0.16^{b}

^{ab} Means with different superscripts on the same column and week differ significantly(P<0.05), N= Number of individuals, NLD= FUNAAB Alpha chicken (dual-purpose), NLL= FUNAAB Alpha chicken (layer line).

Effect of sex on growth performance of FUNAAB Alpha chickens

In Table 2, the effect of chicken sexes on body weight and breast girth was found to be significant. The
body weight and breast girth values recorded from week 1 to week 8 indicated that males were weighed heavier than their female counterparts at the same age under similar conditions. This trend of superiority was maintained throughout the period of the experiment. Although the breast girth measurement of males was not significantly different from females at weeks 1, (8.86±0.07, 8.71±0.07), 2 (11.68±0.13, 11.41±0.12), and 3 (14.46±0.16, 14.19±0.15).

Table 2: Effect of sex on growth performance of FUNAAB Alpha chickens

Age (week)	Sex	N	Body weight (g)	Breast Girth (cm)
Day-old	Male	149	33.01±0.29^{a}	-
	Female	161	31.97±0.31^{b}	-
1	Male	146	72.49±1.21^{a}	8.86±0.07
	Female	160	67.91±1.08^{b}	8.71±0.07
2	Male	143	154.28±4.02^{a}	11.68±0.13
	Female	159	138.37±3.29^{b}	11.41±0.12
3	Male	143	284.08±8.14^{a}	14.64±0.16
	Female	159	251.23±7.05^{b}	14.19±0.15
4	Male	143	492.36±17.18^{a}	18.12±0.26^{n}
	Female	159	429.18±15.02^{b}	17.49±0.25^{b}
5	Male	143	652.48±22.11^{a}	20.46±0.30^{n}
	Female	158	563.06±19.19^{b}	19.67±0.28^{b}
6	Male	142	824.38±29.19^{a}	21.73±0.33^{n}
	Female	158	700.07±24.93^{b}	20.76±0.31^{b}
7	Male	142	1088.40±42.43^{a}	23.76±0.39^{n}
	Female	156	912.07±36.59^{b}	22.39±0.37^{b}
8	Male	142	1365.92±51.87^{a}	25.19±0.43^{n}
	Female	156	1127.20±44.71^{b}	23.76±0.41^{b}

^{ab} Means with different superscripts on the same column and week differs significantly (P<0.05), N= Number of individuals.

Effect of chicken lines by sex interaction on growth performance

Table 3 shows the effect of chicken genetic lines by sex interaction on growth performance. The effect of chicken genetic lines by sex interaction is significant on growth performance ($p>0.05$). Male FUNAAB Alpha dual-purpose birds exhibited the heaviest body weight and had the best average weight gain compared to female birds.

Table 3: Effect of chicken genetic lines by sex interaction on their growth performance

Age (week)	Parameters	Chicken genetic lines			
		NLD		NLL	
		Male	Female	Male	Female
0	Body weight (g)	32.08±0.43^{b}	31.87±0.58^{b}	32.80±0.53^{ab}	31.63±0.46^{b}
1	Body weight (g)	68.75±1.78	65.83±1.69^{ab}	64.40±1.12^{ab}	61.86±1.08^{b}
	AWG (g)	36.67±1.58	33.96±1.35^{ab}	31.60±0.89^{d}	30.23±0.86^{d}
2	Body weight (g)	140.04±5.10	128.25±3.96^{ab}	122.13±2.58^{ab}	115.12±2.63^{b}
	AWG (g)	71.06±3.57^{a}	62.42±2.57^{ab}	57.73±1.79^{d}	53.26±1.77^{da}
3	Body weight (g)	248.40±8.42^{a}	224.89±6.48^{b}	203.71±4.22 ^{ab}	185.33±4.3b^{b}
	AWG (g)	108.36±4.74	96.64±3.81^{b}	81.58±2.16^{a}	70.21±2.01^{f}
4	Body weight (g)	379.43±12.79^{b}	333.00±9.09^{b}	327.13±6.95^{d}	293.47±6.94^{b}
	AWG (g)	131.02±5.71	108.11±4.13	123.42±3.54^{b}	108.14±2.92^{b}
5	Body weight (g)	533.98±19.79	457.58±11.51^{b}	421.89±11.0b^{d}	377.51±9.13^{a}
	AWG (g)	154.55±9.44	120.46±5.81	94.76±6.01^{e}	84.04±3.6
6	Body weight (g)	639.98±23.63^{b}	547.35±13.56	534.93±13.78	467.96±10.66
	AWG (g)	106.00±9.65^{a}	89.77±5.90	110.05±5.29	90.46±4.52
7	Body weight (g)	846.85±29.09	717.75±17.99^{a}	632.00±18.74^{a}	552.63±13.6
	AWG (g)	206.87±9.02	170.40±6.78	97.07±6.85^{b}	84.67±5.30
8	Body weight (g)	1036.83±33.61	856.71±21.47	835.09±25.52	711.42±17.13
	AWG (g)	189.98±9.2	138.96±6.34	203.09±8.85	158.79±5.47

^{abcd} Means with different superscripts on the same column and week differs significantly ($P<0.05$), NLD= FUNAAB Alpha Dual-purpose chicken, NLL= FUNAAB Alpha layers line, and AWG= Average weight gain.

Effect of chicken genetic lines on feed intake and feed conversion ratio

In table 4, the effect of chicken genetic lines on the feed intake and feed conversion ratio of FUNAB Alpha birds is presented. The result showed that irrespective of chicken genetic lines, the quantity of feed consumed increases significantly per bird per week as birds advance in age. The chicken genetic lines significantly ($p<0.05$) influence average feed intake and feed conversion ratio.

Table 4: Effect of chicken genetic lines on feed intake and feed conversion ratio

Age (Week)	Parameters	N	Genotype	
			NLD	NLL
1	Feed Intake (g)	4	65.55±0.39^{b}	61.09±0.38^{c}
	FCR	4	1.90±0.07^{a}	1.97±0.06^{n}
2	Feed Intake (g)	4	141.93±0.27^{b}	109.59±1.49^{c}
	FCR	4	2.18±0.08^{a}	1.97±0.04^{ab}
3	Feed Intake (g)	4	210.60±2.88^{b}	158.64±1.01^{c}
	FCR	4	2.09±0.06^{a}	2.09±0.08^{n}
4	Feed Intake (g)	4	241.19±4.24b	269.57±1.28^{n}
	FCR	4	2.04±0.07^{b}	2.34±0.08^{n}
5	Feed Intake (g)	4	321.62±2.11^{b}	254.72±5.81^{c}
	FCR	4	2.48±0.20	2.90±0.23
6	Feed Intake (g)	4	382.6±13.93^{b}	348.38±7.00^{b}
	FCR	4	4.39±0.56^{a}	3.56±0.22^{ab}
7	Feed Intake (g)	4	535.23±13.73^{b}	394.39±1.92^{c}
	FCR	4	2.92±0.17^{b}	4.41±0.31^{n}
8	Feed Intake (g)	4	575.73±14.56^{b}	475.32±3.03^{c}
	FCR	4	3.64±0.29^{a}	2.66±0.14^{b}

^{abc} Means with different superscripts on the same row differs significantly ($P<0.05$), N= Number of replicates, FCR= Feed conversion ratio, NLD= FUNAAB Alpha dual-purpose chicken, and NLL= FUNAAB Alpha layer lines.

The dual-purpose line consumed more feed than layer lines in weeks 1, 2, 3, 5, 6, and 8. At week 4, layer lines consumed more (269.57±1.29g) than dual-purpose birds (241.19±4.24g) at week 4. Also, a level of superiority existed between FUNAB Alpha dual-purpose chicken and the FUNAB Alpha layer lines in terms of feed conversion ratio. Feed conservation ratio increases in both chicken genetic lines with age from week 1 to week 8. The dual-purpose line had a better feed conversion ratio at weeks 2, 6, and 8 in relation to layer lines.

DISCUSSION

This study reveals the existence of variations in body weight, breast girth, and feed conversion ratio among the different genetic lines where FUNAAB Alpha dual birds exhibited superior results than layer lines. The result is consistent with body weight posited by several studies for dual-purpose chickens kept for meat production in sub-humid and tropical zones (Fisher, 2016; Muller, 2018; Dawud, 2019; Fesseha *et al.*, 2021). The overall mean body weight of dual and layer lines recorded in the present study is higher than the report for indigenous Horo breeds from Ethiopia at 8 weeks of age (Biazen *et al.*, 2021). This could be ascribed to the mass selective breeding that has been undertaken to improve the performance of the strains for generations. The significant difference in the genetic lines of FUNAAB Alpha birds on growth performance indicates a level of variation in inherited genetic effect on chickens. The differences in chicken genetic lines might be considered an attractive choice for selection and breeding because of their unique constitution, genetic distinctiveness, and influence on mean performance.

The increase in body weight and breast girth measurements across chicken lines observed from day old to eight weeks may be attributed to the fact that growth results in increased size and changes in the functional capability of animal tissues and organs (Peters *et al.,* 2005; Adebambo *et al.,* 2006; Adedeji *et al.,* 2008). Consequently, the increase in cell size due to cell division and enlargement could be responsible for changes in body conformation that affected breast girth measures observed in the study.

Body weight and breast girth of both chicken lines increased continuously as birds advanced from early growth phases up to week 8 with the widest breast girth measures observed among the dual-purpose genetic line. A similar result was reported by Ige (2013), who recognized that the age of an animal influences its growth pattern. The body weight of the FUNAAB Alpha dual-purpose line was compared favorably at weeks 4 and 8 with those of Shaobo, Huaixiang, and Youxi chickens of China (Zhao *et al.*, 2015). This may be associated with the genetic constitution of the individual line assessed as well as some environmental influence, suggesting that a dual-purpose line could be of more genetic importance for selection towards the development of broiler strains from Nigerian local chicken. The current study is also in agreement with Iraqi *et al.* (2002), who concluded that genetic selection at early ages may give a rapid improvement in the growth of local strains. Yunusa and Adeoti (2014) further buttressed the fact that an increase in growth traits associated with body morphometric indices such as chest girth, body length, and shank length can serve as indicators of good growth.

Sexual dimorphism in favor of males accounted for higher body weight values for male birds recorded at weeks 1 to 8 for both chicken lines examined. The superior performance of males compared to female birds could be ascribed to high testosterone secretion in males (Adeleke *et al.*, 2015). This agreed favorably with Muller and Aline (2015), who suggested that apart from the inherited genetic line difference, environmental indices such as feeding and management conditions are critical factors affecting the body weight of chickens. The effect of sex on breast girth remained insignificant during the early growth phase (weeks 1 to 3). However, at 4, 5, 6, 7, and 8 weeks of age, sex significantly influenced mean breast girth values, with male birds measuring greater than females. The combined effect of bird genetics and feeding management may be responsible for the variation in average breast girth records among different chicken lines (Biazen *et al.*, 2021).

Recently, feed conversion ratio (FCR) has been described as the most common method applied to evaluate efficiency in poultry production (Biazen *et al.*, 2021). The existence of significant differences in feed intake and feed conversion ratio among FUNAAB Alpha genetic lines has been reported in previous studies (Ekka, 2015; Dawud, 2019). Higher FCR in the present study was observed among dual chickens with the best feed conversion obtained at 8 weeks. This result is contrary to Muller (2018), who reported that Ross PM3 commercial broilers grew faster, consumed more feed, and converted feed into

gain more efficiently compared with dual-purpose chickens. The average FCR of FUNAAB Alpha dual and layer chickens recorded was much higher than the result indicated for local Nigerian chickens reared under similar conditions (Bamidele *et al*., 2019). This might have emanated from the fact that FUNAAB Alpha chicken strains have been subjected to genetic improvement via multiple selections and cross-breeding.

CONCLUSION

Genetic lines and sex significantly influence chicken performance. The FUNAAB Alpha dual-purpose chicken line was superior in body weight, feed intake, and feed conversion ratio as compared to layers. Selective breeding of improved Nigerian indigenous strains in poultry production may improve feed efficiency and growth performance.

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COMPARATIVE GROWTH PERFORMANCE OF TWO CHICKEN GENOTYPES (KUROILER AND INDIGENOUS NIGERIAN NAKED NECK) KEPT IN A TROPICAL ENVIRONMENT

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Abstract

Chicken farming provides a major contribution to both animal protein and household income. This study compared the growth performance of Kuroiler with indigenous Nigerian naked neck chicken genotypes. Two chicken genotypes comprising 345 indigenous Nigerian naked necks and 193 Kuroiler were sampled. Bodyweight and morphometric traits were observed at the 2nd, 4th, 6th, 8th, and 10th weeks of age. Data on body weight and morphometric traits obtained were subjected to a 2-way analysis of variance using the GLM procedure of SAS. Results showed significant variation in genotype with chicken growth. Kuroiler birds exhibited superior mean body weight (p<0.05) at the 2nd, 4th, 6th, 8th, and 10th weeks than the indigenous Nigerian naked neck chickens reared under the same settings. Throughout the early stages of chicken growth, the effect of genotype and sex on mean body weight and breast girth measures was not significant. Both chicken genotypes exhibited differential growth patterns and sexual dimorphism in favor of male populations. The introduction and cross-breeding of Kuroiler genotype with indigenous Nigerian naked neck birds may improve performance and adaptability.

Key words: *Comparative, growth performance, Kuroiler, and chickens*

INTRODUCTION

Poultry species, particularly chickens, constitute a unique and valuable component of the world's animal genetic resources. Chicken farming has evolved into a globally recognized practice appraised for regular household income creation, and a rich source of animal protein for human and industrial use (Ogbu, 2021). Chicken products, particularly meat and eggs, are the most cost-effective source of animal-derived protein, consumed by billions of people worldwide. With a total population size of 180 million birds, chickens are considered the most widespread avian species in the Nigerian poultry production industry (FAOSTAT, 2019).

Previous studies have assessed the growth performance of several indigenous and exotic farm animal species, such as; Cattle, (Denis *et al.*, 2019), sheep, (Yashim, *et al.*, 2016) goats, (Worku *et al.,* 2019), rabbits, (Gasco *et al.*, 2019) and chickens, (Malpotra *et al.*, 2018; Mueller *et al.*, 2020). According to Lawrence and Fowler, (2002) growth describes an increase in the number of cells or body size per unit of time. Growth in chickens is a complex trait that is believed to be regulated by both genetic and non-genetic factors such as genotype, sex, breed, adaptability, management profile, and environmental variables.

The indigenous Nigerian naked neck chickens are a tropically adapted breed of fowl naturally devoid of feather coverage on the neck and vent region. The naked neck trait results from an autosomal gene in chickens (Warren, 1933) and is controlled by an incompletely dominant allele located near the middle of Chromosome 3. The indigenous Nigerian naked neck chicken genotype possessed robust adaptation ability and can thrive efficiently in the tropics and hot humid zones. They are widely distributed across different agroecological zones of Nigeria, reared by the majority of the rural farmers. Ismail, (1997), revealed that indigenous fowls are to exhibit sigmoid growth and are also considered inferior to improved species due to their inherent lower genetic potential. Some improved

chicken varieties like; Shika brown, FUNAAB Alpha, and Kroiler) are substantially contributing to the overall chicken egg and meat output (Adebambo *et al.,* 2018; Islam *et al*., 2021). The Kuroiler chicken genotype is an improved dual-purpose, multicolored chicken, capable of producing more eggs and meat than the indigenous chickens.

Rearing naked neck chickens in a warm or hot humid environment might support adaptation, growth performance, and egg production. Understanding the growth performance of chicken genotypes becomes imperative to identify populations with superior merit. As a result, this study aims at comparing the growth performance of Kuroiler and indigenous Nigerian naked neck chicken genotypes.

Pictorial representation of indigenous Nigerian naked neck and Kuroiler chickens' genotypes

Figure 1. Matured indigenous Nigerian naked neck cock and hen

Figure 2. Matured normal feather indigenous Nigerian naked neck cocks

Figure 3. Matured naked neck Kuroiler cocks

Figure 4. Matured normal feather Kuroiler hens

METHODOLOGY

This study was carried out at the Programme for Emerging Agricultural Research Leadership Poultry Breeding Unit of the Federal University of Agriculture Abeokuta, Nigeria. Characterized by tropical rainforest vegetation, mean temperature of 33.7℃, relative humidity of 80%, rainfall of about 1037mm, and altitude of 76 mm above the sea, the site lies within latitude 7° 13′, 49° 46′ N, longitude 3° 26′, 11° 98′ E Google Earth, 2018). Eggs from parent stock Kuroiler and indigenous Nigerian naked neck chickens were collected at the poultry breeding unit and hatched for this experiment. A total of 538 birds comprising 345 Nigerian indigenous naked neck and 193 Kuroiler chickens genotypes were sampled. Bodyweight and linear body measurements (breast girth) were observed at the 2^{nd}, 4^{th}, 6^{th}, $8^{th,}$ and 10^{th} weeks using a sensitive weighing scale (grams) and graduated measuring tapes (centimeters) respectively. Data obtained on body weight and the morphometric trait data were subjected to a two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS 9.2 software (SAS, 2010, SAS Institute Inc. Cary, North Carolina, USA). Significant differences were separated using turkey-Kramer.

RESULTS

Effect of genotype on chicken growth traits at different growth ages

Table1. Effect of chicken genotype on growth traits at 2nd, 4th, 6th, 8th, and 10th week of age

Weeks	N	Genotype	Breast girth (cm)	Bodyweight (g)
2	**532**	**KLR**	18.95±3.98	154.42±2.0[a]
		NKN	11.64±0.11	127.65±6.2[b]
4	**529**	**KLR**	17.46±0.15[a]	396.45±5.60[a]
		NKN	14.37±0.12[b]	220.27±7.48[b]
6	**512**	**KLR**	20.97±0.16[a]	614.19±8.26[a]
		NKN	15.66±0.16[b]	356.95±9.68[b]
8	**503**	**KLR**	23.65±0.20[a]	800.57±19.50[a]
		NKN	18.53±0.15[b]	499.85±9.80[b]
10	**496**	**KLR**	26.80±0.23[a]	959.72±0.07[a]
		NKN	20.51±0.18[b]	623.69±12.32[b]

[abc]Mean with different superscripts on the same column are significantly different (p<0.05). **KLR**=Kuroiler, **NKN**=Nigerian indigenous Naked neck, **N**=Number of observations, **g**=grams and **Cm**= Centimeter.

Table 1 shows the effect of genotype on chicken growth characteristics at the 2nd, 4th, 6th, 8th, and 10th week of age. There exist significant genetic differences in chicken growth. Kuroiler chickens maintain significantly higher mean body weight (P<0.05) at various growth stages than indigenous Nigerian naked neck chickens raised in the same settings. The Kuroiler chicken genotype had the greatest mean body weight (959.72±0.07[a]) during the 10th week of growth when compared to the indigenous Nigerian naked neck genotype (623.69±12.32[b]) at the same age. During the early stages of growth, the effect of chicken genotype on breast girth remains largely insignificant. Kuroiler birds, on the other hand, had a significantly higher mean breast girth (26.80±0.23[a]) than indigenous Nigerian naked neck birds (20.51±0.18[b]) when the birds approached the 10th week of age.

Effect of sex on chicken growth traits at different growth ages

Table2. Effect of Sex on chicken growth traits at 2nd, 4th, 6th, 8th, and 10th week of age

Weeks	N	Sex	Breast girth (Cm)	Bodyweight (g)
2	**532**	♂	12.38±0.11	143.99±2.38
		♀	18.20±4.40	138.08±5.19
4	**529**	♂	16.03±0.17	315.44±7.23
		♀	15.82±0.16	301.27±8.60
6	**512**	♂	18.65±0.2[a]	502.99±10.85[a]
		♀	17.98±0.23[b]	468.15±11.84[b]
8	**503**	♂	21.58±0.22[a]	691.05±18.97[a]
		♀	20.60±0.24[b]	609.38±18.6[b]
10	**496**	♂	24.49±0.27[a]	849.42±14.56[a]
		♀	22.82±0.29[b]	733.99±15.33[b]

[abc] Mean with different superscripts on the same column are significantly different (p<0.05).
♂=male**,** ♀=female, **N**=Number of observations, **g**=grams and **Cm**= Centimeter.

Table 2 presents the effect of sex on chicken growth parameters. Sex did not significantly affect chicken growth traits at the 2nd and 4th weeks. However, at the 6th, 8th and 10th weeks of age, the males were generally superior (p<0.05) to both females of Kurioler and indigenous Nigerian naked neck chickens genotypes in mean body weight and average breast girth measures under similar management profile and environmental conditions.

Effect of sex and genotype on chicken growth traits at different growth stages

Table 3. Effect of Sex and Genotype interaction on chicken growth traits at different growth stages

Week	N	Genotype	Sex	Breast girth (Cm)	Bodyweight (g)
2	**532**	**KLR**	♂	12.94±0.13	154.43±2.62
			♀	24.97±12.23	154.40±3.28
		NKN	♂	11.83±0.15	133.54±4.94
			♀	11.44±0.13	121.75±7.76
4	**529**	**KLR**	♂	17.55±0.22	399.07±6.92
			♀	17.37±0.19	393.82±9.52
		NKN	♂	14.69±0.18	323.81±10.89
			♀	14.09±0.15	208.72±9.02
6	**512**	**KLR**	♂	21.05±0.20	632.46±10.46
			♀	20.89±0.27	604.93±13.21
		NKN	♂	16.25±0.34	382.52±16.03
			♀	15.08±0.18	331.38±11.41
8	**503**	**KLR**	♂	23.76±0.23^{a}	809.41±21.76^{a}
			♀	23.54±0.37^{a}	791.74±39.75^{a}
		NKN	♂	19.41±0.24^{b}	572.69±22.1^{b}
			♀	17.67±0.17^{bc}	427.02±9.62^{bc}
10	**496**	**KLR**	♂	27.35±0.26	982.60±14.22^{a}
			♀	26.26±0.47	936.85±16.57^{a}
		NKN	♂	21.63±0.34	716.25±29.49^{b}
			♀	19.38±0.19	531.15±11.7^{bc}

abc Mean with different superscripts on the same column are significantly different ($p<0.05$).
KLR=Kuroiler, **NKN** = Nigerian indigenous Naked neck, ♂=male, ♀=female, and **N**= number of observations, **g**=grams and **Cm**= Centimeter.

Table 3 shows the effect of genotype and sex interaction on chicken growth parameters. Sex and chicken genotype combination showed no significance in early birds' growth traits. When compared concerning genotype and sex interactions, Kuroiler sexes differ significantly in breast size and body weight from naked neck sexes only at the 8th and 10th weeks. At the 8th week, the mean breast girth of the Kuroiler sexes was statistically alike (23.76±0.23^{a} and 23.54±0.37^{a}), and a similar observation was recorded for their mean body weight (809.41±21.76^{a} and 791.74±39.75^{a}). Also, at 10th week, both Kuroiler sexes weighed similar values (982.60±14.22^{a} and 936.85±16.57^{a}) but were significantly heavier than naked neck males and females.

DISCUSSION AND CONCLUSION

The superior growth performance of the Kuroiler chicken genotype observed in this study compared to the indigenous Nigerian naked neck genotype is consistent with the findings of Islam *et al.* (2017) in Northern India, who found that the Kuroiler genotype had a significantly higher mean body weight and egg production at various ages than the indigenous chicken variety. These variances in chicken growth with genotype might be ascribed to exotic species having greater genetic potential due to a series of selection and multiple cross-breeding, which has genetically improved their growth potentials. This shows that the indigenous Nigerian naked neck genotype reared in the topics has not yet experienced complete gene mixing with exotic species. Hence, they can be harnessed for present and long-term genetic improvement for human sustenance (Ogbu, 2021).

This report's significant sex effect on mean body weight and linear measures from the 6th to 10th week of age is consistent with Ajayi and Ejiofor (2009). Similarly, the heavier mean body weight of male birds compared to females in this study is similar to the findings of Islam and Nishibori, (2009), Odah *et al.* (2019), who also revealed variations in mean body weight with both sexes of indigenous chickens. These findings agreed with those of Deeb and Lamont (2002), who found variation in chicken growth patterns within species, as well as Ajayi and Ejiofor, (2009) and Madilindi *et al.* (2018), who studied Ross and Anak strains and commercial broiler chickens, reared in Nigeria and subtropical South Africa zone. Superior male weights in this study might be attributed to greater feed intake abilities and a high level of testosterone secretions in males, which influence the growth dynamics of body components not directly related to muscle mass, reproduction, and

sexual development (Lawrence and Fowler, 2002).

The sexual dimorphism in favor of males found in both genotypes in this study is consistent with the findings of Adeleke (2005) and Isidahomen *et al.* (2012), who also showed differential growth patterns in chickens. The variation in the growth pattern between Kuroiler and indigenous Nigerian naked neck chicken genotypes in this report might be attributed to strain differences and bird genetics.

The significant genotype and sex interaction difference between Kuroiler genotype and indigenous Nigerian naked neck birds are in line with the findings of Razuki *et al.* (2011), who observed a significant genotype and sex interaction effect on the mean body weight of Ross and Anak Broiler Strains. The findings of this study contradict those of Ojedapo *et al.* (2008), who reported no significance in the genotype-sex interaction on chicken body weight. Because organ and tissue functions drive body weight in farm animals (Adedeji *et al.,* 2006), differences in mean body weight and breast girth measurements of chicken genotypes with age in this study could be due to their genetic makeup, sexual dimorphism, and avian physiology, which may have influenced organ and tissue functions at different ages.

In conclusion, the growth performance of Kuroiler and indigenous Nigerian naked neck chickens differ due to genetic and strain variances. The Kuroiler genotype is superior to the indigenous Nigerian naked neck chickens genotype. Hence, introducing the Kuroiler birds and crossing them with indigenous Nigerian naked neck chickens may improve growth and adaption in the tropics.

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DETERMINING THE EFFICIENCY OF LAYING HEN FARMS BY DATA ENVELOPMENT ANALYSIS IN KAYSERI PROVINCE

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Abstract

In this study, it is aimed to determine the optimum scales of farms in egg production of laying hen farm in Kayseri, which is one of the important egg production centers of Turkey, and to make productivity and economic/econometric analyzes with Data Envelopment Analysis (DEA). Data envelopment analysis (DEA) is a powerful knowledge-based analytical method for evaluating the relative performance of a set of homogeneous decision-making units consisting of multiple inputs and outputs, developed by Charnes, Cooper, and Rhodes in 1978. The study material consisted of 16 laying hen farms operating in the province of Kayseri. Input-oriented Slack Based Model (SBM) data envelopment analysis was used to determine the economic efficiency of these farms. In the applied DEA, input variables, production and general administration cost, maintenance, repair and depreciation, feed cost, health and labor cost; output variables were determined as egg income and discarded chicken income. According to the results of the analysis, 8 of the 16 farms (1st; 2nd; 8th; 10th; 11th; 14th; 15th; 16th farms) were found to be efficient. 15th farm was determined as the most effective business by being referred to 8 times by other businesses. It has been observed that inefficient farms need to reduce their input costs at different rates in order to be effective. With this study, the usability of data envelopment analysis in determining the efficiencies in laying hen farms has been demonstrated, but it has been seen that the use of more decision-making units is necessary for the application of the analysis.

Key words: *Data Envelopment Analysis, Hen, Kayseri, Poultry*

GILL ECTOPARASITES (PROTOZOAN) IN FOUR SPECIES OF IMPORTED FRESHWATER ORNAMENTAL FISH IN IRAN

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Abstract

The domestic cat is becoming more and more popular as a companion animal. Currently, the number Parasitic disease is the most common disease in fish. Gills as a vital and sensitive organ, have a high importance to pathogens and parasites investigations. In common parasites in fish, protozoans are highlighted because of non-specificity in host. In this study, 400 fish gill archs of 4 species of imported ornamental fish; Goldfish (Carassius auratus), platyfish (Xiphophorus maculatus), Dwarf gourami (Colisa lalia) and Catfish (Hypostomus plecostomus) for gill ectoparasites were inspected. In this study four prorozoan species were identified that including: 1.Trichodina sp., with the highest prevalence (35%) in dwarf gourami. 2. Ichthyophthirius multifiliis, with the highest prevalence (47%) in platyfish. 3. Ichthyobodo sp. (Costia sp.), with the highest prevalence (6%) in dwarf gourami. 4. Cryptobia branchialis, with the highest prevalence (8%) in goldfish were detected. By the way, Trichodina and Ichthyophthirius were found in all four species fish. Ichthyobodo, was just found in dwarf gourami and Cryptobia was seen only in goldfish.

Key words: *Ectoparasite, Ornamental fish, Gill, protozoa*

Author Index

Nationality of Presenters

Country	n	%
Algeria	3	1.97
Brasil	1	0.66
Cameroon	1	0.66
Egypt	1	0.66
France	1	0.66
Germany	2	1.32
Hungary	4	2.63
Indonesia	1	0.66
Iran	34	22.37
Italy	1	0.66
Kazakhstan	2	1.32
Nigeria	8	5.26
North Macedonia	1	0.66
Pakistan	1	0.66
Poland	13	8.55
Somalia	3	1.97
Türkiye	75	49.34
TOTAL	152	100