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AR-GE & İNOVASYON

INTERNATIONAL CENTER FOR LIVESTOCK RESEARCH AND TRAINING

3rd International Livestock Science Congress

NOVEMBER 14-15, 2023 | ONLINE



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3rd International Livestock Science Congress

CONGRESS PROCEEDINGS BOOK

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PREFACE

The International Livestock Research and Training Center, which is one of the well-established institutes, where research and development (R&D) studies are carried out in the field of animal husbandry with its institutional and strong infrastructure within the General Directorate of Agricultural Research and Policies (TAGEM), started its R&D activities in 1930 with the breeding and breeding of Angora goats. Today, it continues with its studies in the fields of Genetics and Breeding, Reproductive Biotechnology Feeds and Animal Nutrition, Animal Production and Breeding. In addition, our institute is one of the important R&D centers in the protection and breeding of domestic animals genetic resources, with its animal gene bank and livestock protection herds. We are very happy to announce that the third of our "International Livestock Science" congress, which has been organized since 2019 with great responsibility, desire and honor by our institution, which has carried out important studies in the field of animal husbandry, will be held online on "14-15 November 2023".

Since the day it was founded, our Institute, which has adopted the mission of providing access to reliable and high-quality animal products that our country and world markets need and the sustainable use of resources, welcomes individuals and institutions who devote their time and effort to the livestock industry through the "3rd International Livestock Science" Congress, where the most up-to-date information and experiences will be shared. It aims to bring together respected scientists and institutions. As valuable stakeholders of the industry, we are honored and happy to invite you to our congress, and we thank you in advance for your valuable contributions and participation in the realization of our congress.

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- Animal Biotechnology
- Animal Breeding and Genetics
- Animal Genetic Resources
- Reproductive Biotechnology
- Animal Nutrition
- Livestock Economics and Management
- Climate Change and Livestock Production
- Animal Husbandry

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- Dr. André EGGEN

- Assoc. Prof. Todd Riley CALLAWAY

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TABLE OF CONTENTS

INVITED SPEAKERS	1
Current Synchronization Methods for Cows and Heifers	2
Nutrition and Microbiology: Complementary Goals and Demands	3
Strategies to Improve the First Service Conception Rate in High Producing Dairy Cows	4
Impact of Genomics in Livestock	5
ORAL PRESENTATIONS	6
A Comprehensive Review of Machine Learning, GBLUP, and Bayesian Methods for Genomic Selection Accuracy in Livestock	7
Estimation of Variance components and Genetic Parameters for Milk Yield and Fertility Traits of Simmental Cows	8
Possibilities of Using Buga Software in Breeding Value Estimation within the Scope of Community Based the Breeding Program of Anatolian Water Buffalo Population of Istanbul	9
Unraveling the Genetic Landscape of Neonatal Arthritis/Polyarthritis Resilience in Karacabey Merino Lambs: A Dual-Model GWAS Investigation	10
Revealing Genetic Markers for Neonatal Diarrhea Resilience in Karacabey Merino Lambs Using Single and Multi-Locus GWAS Models	11
Circadian Genes and Economic Traits in Livestock Animals	12
Effect of Environmental Factors on Prewaning Growth Traits of Central Anatolian Merino Lambs	13
Effects of Treatment with Prostaglandin F2 α , Carbetocin and Cephapirin Combinations on Fertility in Holstein Cows with Clinical Endometritis	14
Nanopurification Techniques for Sperm Selection: A Promising Alternative to Conventional Methods	15
Phenomenon Additives in <i>In Vitro</i> Development: Antioxidants and Their Functions	16
Determination of Semen Characteristics of Locally Adapted Anatolian Black Bulls with CASA and Flow Cytometry	30
The Effects of Some Antioxidants Participating in Embryo Culture Media on <i>In vitro</i> Embryo Development	31
Some Reproductive Characteristics of Hair Goats and Survival Ability Rate of Their Kids At Breeders Conditions	32
Effects of Lysine and Organic Chromium Addition to Breeder Laying Hen Diets at Different Environmental Temperatures	33
Effects of Pomegranate Peel Powder Added to The Feed of Laying Hens Exposed to Heat Stress on Egg Quality Characteristics	34
Pomegranate By-Products as an Alternative Feed Material in Ruminant	35

The Effects of Fumaric Acid on The Developmnet of digestive System and Growth Performancein Broilers	39
Using Diverse Carbohydrate Source to Improve Legume Silage Quality	40
Determination of Calf Development Pasture and Intensively Fed Anatolian	46
Optimizing Poultry Production through Microencapsulated Feed Additives	47
Determination of Lactation Parameters and Shape of Anatolian Buffaloes for Three Lactations	53
Bio – Economic Modeling Approach in Livestock Enterprises	54
Growth and Development Characteristics of Karakaş Breed Lambs	55
Cattle Population in Eastern Anatolia and Southeastern Anatolia Regions	56
Effects of Calving Age and Calf Sex on Colostrum Composition and Its Changes After Calving in Anatolian Buffaloes	57
Growth of Awassi Sheep in Şanlıurfa Province	58
Evaluation of Productivity Characteristics of Karacabey Merino in Balıkesir Province Between 2016 amd 2020 (3rd Five Years) within The Scope of The National Sheep and Goat Breeding Project	59
Investigation of Some Parameters of Cocons Obtained from Commercial Hybrid (MxN) Silkworms Fed in Different Numbers	60
Investihgation of Growth, Birth Type and Sex Ratio Characteristics of Akkaraman – Şavak Lambs in Breeder Conditions	61
Growth Characteristics, Mohair Yield and Fineness of Angora Goats Raised in Ankara Province	62
Lactation Characteristics of Awassi Sheep in Şanlıurfa Province	63
Investigation of the Structural Characteristics of Central Anatolian Merino Sheep Farms and Effectiveness of the breeding Project in Ankara Province	64
Growth and Development Characteristic of Mahalli Goats Cubs Raised in Diyarbakır Rural Area	65
Determination of Some Fertility and Growth Characteristics of Akkaraman Breed Sheep Raised in Konya Province: The Example of Yağlıbayat Neighborhood	66
Estimation of Breeding Value and Genetic Trends for Milk Yield Traits of Simental Cattle	67
Bottleneck Analysis of Brown Stud Bulls Used in Turkey	68
Morphological Characterization of Cyprus Fat – tailed Sheep Breed Using Quantitative and Qualitative Trait Analysis	69
An Investigation of {OLFML3} Gene Polymorphism Related to Meet Quality in Berrichon Du Cher Sheep	70
Examination of Some Environmental Factors Affecting Birth Weight in Awassi Lambs Using Exhaustive Chaid Determination Tree Algoritm	76
Effect of Coat on the Physiological Response of Heat – Stressed Late – lactation Dairy Cows	77
Growth and Development Characteristics of Karakaş Breed Lambs	78
Investigation of Some Maternal Effects on Lamb Birth Weight in Akkaraman and Lalahan Sheep (Kıvrıcık x Akkaraman G1)	79
Examination of Some Breeding and Welfare Proctise in Akkaraman Sheep Farms	80
POSTER PRESENTATIONS	81
Presence of Small Ruminants in the Provinces in the GAP International Agricultural Research and Training Center (GAPUTAEM) Study Area	82
Freezing Possibilities of Rooster Sperm	83

The Use of Non-Protein Nitrogenous Compounds in Ruminant Feding	84
Some Growth Traits of the Kids in Maltese Breed	85
Investigation of Some Breeding and Welfare Practices in Farmer Condition Angora Goat Farms	86
Some Characteristics Obtained from Denizli and Gerze Chicken Breed Conservation Flocks	87
Evaluation of Pelemir (<i>Cephalaria syriaca</i>) as a Feed Source	88
A Case of Atresia Ani in a Kivircikx Akkaraman Crossbred Male Lamb	89

3rd International Livestock Science Congress

INVITED SPEAKERS

Current Synchronization Methods for Cows and Heifers

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Estrus synchronization is a widely used method to improve reproductive efficiency and fertility rates in cattle. This technique involves the manipulation of the estrous cycle in cows and heifers using pharmacological agents to optimize reproductive processes such as mating or artificial insemination. This study investigates the effects of three commonly used agents, namely GnRH, PGF_{2α} and progesterone, in estrus synchronization. GnRH stimulates the initiation of estrus and the process of ovulation. Buserelin, a GnRH analogue, is widely employed for estrus synchronization. PGF_{2α} assists in initiating estrus by inducing regression of the corpus luteum, and this can be achieved using prostaglandin analogs such as dinoprost, which are commonly used in synchronizing estrus cycles. Progesterone is a hormone that regulates different stages of the estrous cycle. A high level of progesterone helps suppress estrus and prepares for the pregnancy process. Basally, progesterone is necessary for a successful pregnancy outcome. There are several products containing progesterone available and widely used in synchronization protocols. GnRH, PGF_{2α}, and progesterone are popular agents used effectively in estrus synchronization in cattle. GnRH analogues control the initiation of estrus and the process of ovulation. PGF_{2α} assists in the regression of the corpus luteum, enabling the start of a new cycle. Progesterone contributes to the regulation of the estrous cycle and prepares for the pregnancy process. These agents are essential components of synchronization protocols used in reproductive management. However, each cow may respond differently, so careful implementation of protocols is crucial for optimal results.

Keywords: Estrus, synchronization, Cow, Heifer

Nutrition and Microbiology: Complementary Goals and Demands

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Our understanding of the function of the gut microbial population has long been limited by only being to understand the biochemical activity of microbes that we can grow in the laboratory. “You have to grow it, to know it” was long an axiom in microbiology; however the development in recent years of Next Generation Sequencing (NGS) has allowed a deeper understanding of which microbes were present in any sample. The advent of NGS has allowed us to see microbial population shifts, but there is still a lack of connection between the microbial population and host animal physiology. Unfortunately, simply equating populations with impacts on the host animal does not work, but there is increasing research to determine endproducts from each of the microbial population members, and how these can impact animal efficiency, sustainability, carcass quality, and animal health. The NGS revolution has focused our attention on the effects of diet changes, addition of eubiotics (prebiotics, postbiotics, and probiotics), and impact of changing the microbial population by probiotic treatment on host animal gene expression. So our recent research has focused on attempting to identify important microbes in microbial environmental niches and their endproducts and degradative activity, especially in the rumen. The future impacts of NGS is going to be broad and transformative, with widespread impacts on farm profitability, environmental impact, food safety, and animal health.

Strategies to Improve the First Service Conception Rate in High Producing Dairy Cows

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The ovsynch protocol was designed to control and optimise the time of ovulation and consequently time insemination can perform. To achieve the greater milk production, dairy herd's owners must increase the percentage of cows in early lactation and the percentage of cows in later lactations (older cows). It has been reported that cows inseminated following fertility programs (double ovsynch) produce more P/AT. The original ovsynch protocol had 3 hormonal injections to control ovarian functions (day 0=GnRH, day 7=PGF2a, day 9=GnRH and day 10=TAI)

Ovsynch is more successful if:

- 1- Low p4 concentration near TAI
- 2- High p4 concentration during follicle development
- 3- Adequate p4 concentration after TAI

TAI: Timed artificial insemination

In this presentation, the physiological and nutritional aspects of improving fertility in high – yielding cows will be discussed.

Impact of Genomics in Livestock

André EGGEN¹

¹Agrigenomics, Illumina Inc.

For centuries, conventional animal breeding, making use of the fact that natural variations exist within a species, within a breed, and within a population, has been very successful. Breeders have enhanced production traits in their herds by selecting superior individuals as progenitors for the next generations. So-called enhanced "breeding values" have been achieved by combining phenotypic recording of individual performance with genealogical information.

Over the past two decades, the rapid development of genomics has opened new paths to address the scientific basis of livestock biology and breeding, resulted in new production methods to achieve sustained increases in animal feed yields and long-term improvements in the efficiency of livestock production. A new era, the "genomics era", promises to enable the objective prediction of consequences based on direct access to the full DNA sequence of many individuals and therefore a renewed and more objective view of the genetic value of animals that is not limited to a few production traits.

The biggest (r)evolution is taking place in the application of genomics to the design and implementation of livestock breeding programs, promising gains across the value chain. For breeders, breeding organizations and the livestock industry, genomics is expected to increase efficiency and productivity of animal breeding while for consumers and the processing sector it should enhance security and quality of animal products. New insights into growth, nutrition, health and protection of animals are expected, enabling a better understanding of the molecular mechanisms of traits of interest. Therefore, genomics proposes further opportunities to improved selection accuracy while decreasing the costs, reducing generation intervals and exploiting new sources of polymorphisms. Genomic selection builds on existing breeding programs where the collection of pedigree information together with phenotypic data is already routine; it provides a new level of information that can be integrated in the decision-making process to identify and to select the most promising animals. The principal advantages of genomic selection are that it can be implemented very early in life, is not gender limited and can be extended to any traits that are recorded in a reference population. It provides, especially for difficult to improve traits, better selection accuracy while reducing the generation interval, therefore increasing the intensity of selection.

As genomic information will continue to provide hugely valuable biological information, the key for further success of genomic selection and genomic approaches will be to collect the most pertinent phenotypes, identify the causal mutations and the exact mechanisms by which phenotypes are produced, and bring the different superior variants together in breeding lines in as few generations as possible. We are entering a truly exciting era fueled by genomics.

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ORAL PRESENTATIONS

A Comprehensive Review of Machine Learning, GBLUP, and Bayesian Methods for Genomic Selection Accuracy in Livestock

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Genomic selection (i.e., genome-wide selection, genomic prediction) has gained widespread recognition and has been effectively integrated into animal breeding programmes. It offers various advantages over conventional selection methods in livestock production with increased genetic gain, early selection opportunity, cost efficiency as well as other opportunities. So far, multiple models and methods, including machine learning, gBLUP (genomic best linear unbiased prediction) and Bayesian methods, have been employed for the estimation of genomic breeding values to enhance the accuracy of genomic selection. Machine learning methods have gained significant attention in Genomic Selection due to their capacity to capture complex, nonlinear relationships within genomic data. Techniques like Random Forests, Support Vector Machines, and deep learning models can efficiently handle high-dimensional genotypic data and improve prediction accuracy. On the other hand, GBLUP is a widely adopted linear model for Genomic Selection, building upon the foundations of infinitesimal model. Finally, Bayesian approaches such as Bayesian Ridge Regression and Bayesian Lasso, provide a flexible framework for Genomic Selection. To assess the superiority and efficiency of these methods, it is essential to conduct a comprehensive comparison. Thus, this study aims to provide a thorough review of the techniques used for genomic breeding value estimation with a focus on enhancing accuracy. We concluded that, the choice between machine learning, gBLUP, and Bayesian methods for Genomic Selection depends on the specific characteristics of the dataset and programme objectives. Machine learning is suitable for large datasets with complex genetic architectures but demands computational resources. gBLUP is a robust and computationally efficient choice for routine breeding programs. Bayesian methods are flexible and beneficial when prior knowledge is available, but they may require more computational resources and expertise. In conclusion, our study suggests to implement a combination of these methods in practice to leverage the strengths of each approach, thereby improving Genomic Selection accuracy. Ultimately, the choice of method should be informed by the available data, the genetic architecture of the trait of interest, and computational capabilities, with the goal of achieving the highest prediction accuracy for genomic breeding and selection programmes.

Keywords: Genomic selection, Machine learning, GBLUP, Bayes

Estimation of Variance components and Genetic Parameters for Milk Yield and Fertility Traits of Simmental Cows

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This current examination was carried out with determining the variance components and genetic parameters related to milk and fertility characteristics of Simmental cows raised in Gökhöyük State Farm. For this aim, the data collected in the period of 2007-2014 of a Simmental herd raised at Gokhoyuk state farm utilized. Minitab (Version 12) statistical program was used to determine significant fixed effects on milk yield and fertility traits. Variance components and genotypic parameters for milk yield traits and reproductive traits were estimated by using “MTDFREML” statistical program. Lactation milk yield, 305 day milk yield (305-DMY) lactation length, calving interval and service period were detected as 4363.01 ± 64.790 kg, 4181.47 ± 60.122 kg and 322.92 ± 2.432 days, 367.37 ± 2.840 and 116.45 ± 3.433 days respectively. The heritabilities of lactation milk yield, 305 day milk yield (305-DMY) lactation length, calving interval and service period were estimated as 0.17 ± 0.080 , 0.18 ± 0.015 , 0.02 ± 0.035 , 0.02 ± 0.010 and 0.01 ± 0.0035 , respectively. The heritabilities of lactation milk yield, 305 day milk yield (305-DMY) lactation length, calving interval and service period were estimated as 0.17 ± 0.080 , 0.18 ± 0.015 , 0.02 ± 0.035 , 0.02 ± 0.010 and 0.01 ± 0.0035 , respectively. Repeatabilities of lactation milk yield, 305 day milk yield (305-DMY) lactation length, calving interval and service period were calculated as 0.19, 0.20, 0.03, 0.02 and 0.02 respectively. In conclusion, it can be said that the determined variance components and genetic parameters could be utilized as selection criteria and to increase the success of the selection in breeding studies for Simmental herds in Turkey.

Keywords: Cow, Genetic trend, Heritabilities, Repeatabilities, Simmental

Possibilities of Using Buga Software in Breeding Value Estimation within the Scope of Community Based the Breeding Program of Anatolian Water Buffalo Population of Istanbul

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In this study, variance components and breeding value estimations of Anatolian water buffaloes were estimated from the data obtained within the scope of community based water buffalo improvement program of Anatolian water buffalo population of Istanbul. The "BUGA" named software, which was started to be developed with the support of Harran University Scientific Research Project Unit (HUBAP) for use in the estimation of (co)variance components and breeding value were used in this study. The BUGA software used in the research can predict genetic and environmental effects together with variance components as Best Linear Unbiased Prediction (BLUP) with the Restricted Maximum Log likelihood (REML) method. Unlike its counterparts of another software of foreign origin, it has a visual interface. The language of use is Turkish. The dataset includes records such as city, district, village, genealogy information, date of birth, gender, calving date, lactation parity, number of test day milk yield, lactation milk yield and previous calving date of 442 Anatolian buffaloes raised in this population. The records were analysed using the repeatability model with BUGA software and the additive genetic and permanent environmental effects of all animals in the data set were estimated. Random environment variance, genotypic variance and permanent environment variance values were estimated as 107112.60 kg², 20045.22 kg² and 1259.50 kg², respectively, while heritability (h^2) and degree of repeatability (r) were determined as 0.1561 and 0.1659, respectively. The results obtained as an example of case study applied for the possibility of using the BUGA software in the estimation of genetic parameters and breeding values showed that BUGA software can be easily used in farm recording conditions with the advantage of friendly user features.

Keywords: (Co)variance components, Computer software, Restricted maximum log likelihood

Unraveling the Genetic Landscape of Neonatal Arthritis/Polyarthritis Resilience in Karacabey Merino Lambs: A Dual-Model GWAS Investigation

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Neonatal arthritis in lambs is a complex condition that can be caused by various infectious agents, including *Cryptosporidium parvum*, rotavirus, coronavirus, *Escherichia coli*, *Streptococcus dysgalactiae*, *Erysipelothrix rhusiopathiae*, and *Histophilus somni*. These infectious agents can lead to joint inflammation and subsequent lameness in lambs. Genetic factors and inadequate passive immunity transfer may also contribute to the development of neonatal arthritis. To understand the underlying host genetic factors for resilience to neonatal arthritis, two GWAS analyses (single-locus and multi-locus) were performed. In the project material, out of 741 Karacabey Merino lambs, 58 were diagnosed with neonatal arthritis/polyarthritis. The prevalence of neonatal arthritis/polyarthritis was determined to be 7.83%. Genotype data were initially tested using the non-parametric multi-locus method, pKWmEB. In the pKWmEB GWAS, 4 SNPs (oar3_OAR1_94053785, OAR9_61405448.1, oar3_OAR16_17924245, and Chr19:48330391) were identified, meeting the significance criterion of $LOD \geq 3$. Subsequently, the same dataset was reanalyzed using the classical single-locus method with the GEMMA software under the Mixed Linear Model (MLM). Following Bonferroni correction on the "p" value in the MLM analysis, SNP Chr3:215726613 was found to be genomewide associated. Lastly, McNemar's test was conducted on all the SNPs that met the significance criteria in both the multi-locus pKWmEB and single-locus MLM GWAS methods. For this purpose, 57 matched pairs were formed considering gender, birth type, and dam's age. As a result of the McNemar test, only SNP Chr3:215726613, which was identified in the MLM model, passed the McNemar test, and this SNP was determined to be associated with a 4-fold genetic susceptibility in lambs to neonatal arthritis/polyarthritis infection.

Keywords: Genetic resistance/ susceptibility, Joint ill, McNemar, MLM, pKWmEB

Revealing Genetic Markers for Neonatal Diarrhea Resilience in Karacabey Merino Lambs Using Single and Multi-Locus GWAS Models

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Neonatal diarrhea is a condition that occurs in the first month of life and is typically caused by complex and multifactorial factors, with viral, bacterial, and parasitic pathogens playing a role in its etiology. Even when treated, neonatal diarrhea often leads to growth retardation in animals due to persistent damage to the intestinal epithelium and increased susceptibility to other infections. To assess potential genetic resistance or susceptibility to neonatal diarrhea in Karacabey Merino lambs, a Genome-Wide Association Analysis was performed. There were 527 controls and 180 cases available for statistical analysis. The prevalence of neonatal diarrhea in Karacabey Merino lambs was calculated to be 24.5%. Genotype data were initially tested using the non-parametric multi-locus method pKWmEB. Based on the pKWmEB GWAS results, 13 SNPs meeting the significance criterion of $LOD \geq 3$ were identified. Subsequently, the same dataset was reanalyzed with a Mixed Linear Model (MLM). After Bonferroni correction, SNP s61781.1 was found to be genome-wide associated. Finally, a McNemar's test was performed on SNPs that met the statistical significance criteria for both the non-parametric multi-locus pKWmEB and MLM models. For this purpose, 180 matched pairs were constructed from neonatal diarrhea data based on gender, birth type, and dam's age ($n = 360$). Three SNPs passed the final McNemar test. It was observed that the s61781.1 SNP provided 2.7 times genetic resistance to neonatal lamb diarrhea, while SNPs oar3_OAR1_122352257 and OAR17_77709936.1 were found to cause 3.1 and 2 times genetic susceptibility, respectively. According to our results, these three SNPs are suggested as strong candidate markers associated with genetic resistance/susceptibility to neonatal diarrhea in Karacabey Merino lambs. As a significant finding, the s61781.1 SNP, which passed all three statistical tests (pKWmEB, MLM, and McNemar), emerges as the strongest candidate SNP associated with genetic resistance/susceptibility to neonatal diarrhea infections.

Keywords: Genetic resistance/susceptibility, McNemar, MLM, Neonatal diarrhea, pKWmEB

Circadian Genes and Economic Traits in Livestock Animals

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Circadian rhythms are endogenous autonomous oscillators of physiological activities in all living creatures and regulates economical traits like reproduction, milktrait and growth performance in livestock animals. They are controlled by the circadian clock directly or indirectly in a 24 h cycle. It is an adaptation system during evolution in living organisms for a cyclic natural environment. A regular circadian rhythm can be linked to good health, wellbeing, strong immunity, and high economical traits. The interconnection between circadian rhythmicity and livestock physiology is becoming one of the major focuses of the field of animal science. Understanding circadian genes (CLOCK, BMAL, PER1, PER2, CRY1, CRY2) and actions modulating circadian oscillation in livestock is important to improve management and increase economical traits. In the study, Research conducted on the topic up to the present day has been complied. The aim of this study is to summarize research on the effects of circadian rhythm genes on productivity in farm animals, highlighting the significance of the topic.

Keywords: Circadian genes, Circadian rhythm, Economical traits, Livestock

Effect of Environmental Factors on Preweaning Growth Traits of Central Anatolian Merino Lambs

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One of the first domesticated species, sheep, has been used throughout history not only as a source of animal protein through its meat and milk but also for clothing and shelter with the production of its skin and wool (Demiryürek et al., 2017). Sheep have several advantages over cattle farming due to their ability to adapt to harsh climate conditions and use low-quality forage (Joy et al., 2020). Furthermore, sheep farming is an important component of rural economies and cultures due to its consistent output and contribution to food security (Alhamada et al., 2017). Turkey holds a significant position in the world in sheep farming with approximately 41 million sheep, including 91.14% native sheep breeds and 21.08% Merino crossbreeds (TUIK, 2023). In this study, the effects of some environmental factors on the birth weight, weaning weight, daily live weight gain, and Kleiber ratio of Central Anatolian Merino sheep raised in the Ankara province were determined. To achieve this, the effects of the factors were estimated, and least square means for the traits were established. According to the results obtained, the birth weight was 4.00 ± 0.02 , weaning weight was 28.55 ± 0.17 , daily live weight gain was 271.78 ± 1.82 , and the Kleiber ratio was 21.92 ± 0.06 . In terms of birth weight, males had a higher birth weight than females, single-born lambs had higher birth weights than multiple births, lambs born from two-year-old mothers had higher birth weights than those born from mothers of other ages, lambs born in 2021 were heavier than those born in other years, lambs born in farms with 500 or more sheep had higher birth weights than those born in other groups, and lambs born in winter months were heavier than those born in other seasons. As for weaning weight, males had higher weaning weights than females, single-born lambs had higher weaning weights than twin-born lambs, lambs born from three-year-old mothers had higher weaning weights than those born from mothers of other ages, the highest weaning weight year was 2019, lambs born in farms with 500 or more sheep had higher weaning weights than other types of farms, and lambs born in the spring had higher weaning weights than those born in other seasons. In general, this study found that the pre-weaning growth traits in Central Anatolian Merino sheep are influenced by various environmental factors and can be used for herd management practices aiming for higher productivity. Improvements can be achieved by optimizing environmental conditions and determining the most suitable birth months according to feeding strategies in the project.

Keywords: Sheep, Central Anatolian Merino, Environmental factors, Growth, Kleiber's ratio

Effects of Treatment with Prostaglandin F_{2α}, Carbetocin and Cephapirin Combinations on Fertility in Holstein Cows with Clinical Endometritis

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Postpartum uterine diseases are one of the important problem reducing reproductive performance in dairy cows. The aim of this study was to investigate the effects of different combinations of PGF_{2α} with carbetocin and/or benzathine cephapirin on the clinical recovery and fertility parameters in Holstein cows with clinical endometritis. Clinical endometritis was diagnosed in Holstein cows (n=60) with metricheck device between 21-40 days postpartum. Cows were randomly divided into 5 groups as 12 cows in each group and medicated in group 1 with double dose PGF_{2α} (days 0 and 14); in group 2 double dose PGF_{2α} (days 0 and 14) and carbetocin (day4); in group 3 double dose PGF_{2α} (days 0 and 14) and benzathine cephapirin intrauterine (day4); in group 4 double dose PGF_{2α} (days 0 and 14) and both benzathine cephapirin intrauterine and carbetocin (day4). Cows in control group was not treated. Additionally, cows were examined gynecologically including vaginal examination, rectal palpation, ultrasonography and endometrial cytology at day of diagnosis (day0) and after treatment (day21) to evaluate clinical cure level. Blood plasma concentrations of progesterone and malondialdehyde (MDA) were measured before and after treatment. Subsequently fertility parameters were recorded. Among the groups, no significant differences (P>0.05) were observed in terms of gynecological examination findings on day 21. Vaginal discharge score and PMN proportion in all treatment groups were significantly lower (P<0.05) on day21 compared to day0, except control group (P<0.05). Blood MDA levels were higher on day0 than day21 in all groups. Regarding the fertility parameters, no statistically significant (P>0.05) differences were observed among all groups. In conclusion; although the used treatments have no positive effect on the fertility parameters of cows with clinical endometritis, treatments exhibited favourable effects on the clinical recovery process. It is recommended to investigate various antibiotic and hormone combinations. This study was supported by Erciyes University Scientific Research Projects Coordination Unit (TDK- 2019-9364).

Keywords: Cattle, Infertility, Postpartum uterine diseases, Reproduction, Therapy

Nanopurification Techniques for Sperm Selection: A Promising Alternative to Conventional Methods

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Sperm purification involves isolating healthy spermatozoa by eliminating non-motile, acrosome-damaged, and abnormal spermatozoa. Extensive research has led to the discovery of various methods, including swim-up, Percoll gradient separation, Sephadex filtration, and glass wool filtration. The swim-up technique separates motile spermatozoa with progressive motility from washed pellets, allowing them to swim into a fresh culture medium. Percoll gradient filtration measures semen motility by leveraging its movement at different densities. Sephadex and glass wool filtration techniques aggregate non-motile and dead spermatozoa based on changes in surface charges. While these techniques yield motile spermatozoa, they are associated with a significant reduction in total sperm count. Consequently, the limitations, technical difficulties, and suboptimal outcomes of these methods have spurred researchers to explore new approaches. This has led to the emergence of nano purification techniques as a promising field of study. Studies on nanopurification of sperm have shown a rapid increase since 2010. Nano purification techniques primarily utilize highly magnetic Fe₃O₄ nanoparticles. Magnetic nanoparticles, enhanced with Anti-Ubiquitin antibodies and Annexin V (serving as apoptosis indicators), as well as Lectin (Peanut Agglutinin, PNA) as an acrosome damage detector, have demonstrated potential for successful nanoselection. Although studies differ in terms of animal species, administration dosages, and rationale, researchers concur that nanopurification represents a promising alternative to current sperm purification methods. This presentation will delve into how nanopurification techniques can overcome the limitations of traditional methods, enhance the efficiency of sperm selection, and offer a new alternative in assisted reproductive technologies.

Keywords: Fe₃O₄, Nanotechnology, Purification, Reproduction, Sperm

Phenomenon Additives in *In Vitro* Development: Antioxidants and Their Functions

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The use of antioxidants in in vitro embryo production is an increasingly important and studied topic. Antioxidants can improve the development of in vitro embryos by reducing cellular oxidative stress with the help of molecules such as resveratrol, ergothioneine, melatonin, lycopene, quercetin and others. Antioxidants are used in in vitro fertilization for reproductive and infertility treatments, in scientific research, in the preservation of genetic material of valuable breeding animals and in embryonic stem cell studies in biotechnology. The antioxidants used in the production of in vitro embryos neutralize free radicals and reduce oxidative stress. They prevent possible damage to DNA by rendering free radicals that can cause damage ineffective. They also support the healthy development of embryo cells by inhibiting the apoptosis process and reducing oxidative stress at the same time. Antioxidants also help to maintain mitochondrial functions and contribute to embryonic development. Antioxidants have been shown to improve embryo viability and have positive effects on embryo morphology, cell proliferation and gene expression. Numerous studies have highlighted the positive effects of antioxidants on in vitro embryo production. Antioxidants have great potential in the field of in vitro embryo production and can be used in future studies for better results. However, further research is needed to determine the optimal concentrations and application methods.

Keywords: Embryo, Antioxidants, *In Vitro* development

1. Introduction

Assisted reproductive technologies (ART) have greatly enhanced the field of animal reproduction in recent decades, resulting in the creation of valuable tools. The main objective of Assisted Reproductive Technology (ART) is to enhance the reproduction process in animals with superior genetics and promote the global dissemination of their favorable genetic characteristics (Berglund, 2008).

Several essential assisted reproductive technologies (ART) employed in animal agriculture include artificial insemination, embryo transfer, in vitro fertilization, semen sexing, and cryopreservation. Artificial insemination enables the reproduction of genetically superior males to produce a significantly higher number of offspring compared to natural mating. Embryo transfer allows females with advantageous genetics to generate several offspring simultaneously. In vitro fertilization and embryo culture procedures enable the production of embryos under regulated laboratory conditions.

Aside from genetic enhancement, Assisted Reproductive Technologies (ARTs) offers crucial reproductive solutions. They allow for the use of donors who have certain anatomical constraints or conditions that may hinder natural reproduction. ART, or Assisted Reproductive Technology, can be employed in cattle that have a tiny stature which hinders natural mating. Additionally, they contribute to conservation initiatives by preserving genetic material from endangered species in gene banks. Cryobanks store germplasm, oocytes, embryos, and sperm, enabling the preservation of genetic diversity from endangered breeds and communities over extended periods of time.

Moreover, ART plays a crucial role in tackling the obstacles associated with illness management. Disease-resistant stock can be more easily tested and selected with their assistance. Methods such as semen sexing offer a way to selectively breed offspring of a preferred gender in order to prevent the spread of diseases through specific parental lineages. The implementation of Assisted Reproductive Technologies (ART) in animal agriculture has resulted in significant progress, leading to several advantages such as enhanced productivity, improved animal welfare, and increased sustainability.

Oxygen is prone to the production of reactive oxygen species (ROS) due to its electron configuration, which includes two unpaired electrons in its outer orbit. Most reactive oxygen species (ROS) begin as superoxide, a by product of oxygen's incomplete conversion. Some examples of reactive oxygen species (ROS) are hydroxyl

radicals ($\text{OH}\cdot$), hydrogen peroxide (H_2O_2), and superoxide anion ($\text{O}_2\cdot^-$). Metabolic oxygen-containing processes are the usual culprits in aerobic metabolism, which is how these compounds are generated (Mauchart et al., 2023).

2. What exactly free radicals?

Anything with an unpaired electron is considered a free radical (Halliwell and Gutteridge, 2015). Hyperoxide ($\text{O}_2\cdot^-$), hydroxyl ($\text{OH}\cdot$), peroxy ($\text{RO}_2\cdot$), and hydroperoxyl ($\text{HO}_2\cdot$) radicals are all examples of oxygen radicals; nonradical oxidizing agents like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3) are also part of the reactive oxygen species (ROS) (Halliwell and Gutteridge, 2015). The progression of sepsis is influenced by reactive oxygen species (ROS) (Salvemini and Cuzzocrea, 2002). Enzymatic activities, electron transport in mitochondria, signal transmission, activation of nuclear transcription factors, gene expression, and the antimicrobial activity of neutrophils and macrophages are all impacted by reactive oxygen species (ROS), which are not only produced during normal metabolic processes but also play a role in these processes.

The existence of two unpaired electrons in its ground state allows the oxygen molecule, also called dioxygen and represented as O_2 , to be categorized as a radical. Dioxygen has a ground state electron spin quantum number of three, which, according to quantum mechanics, means that oxygen is a triplet species. A singlet state, where each electron has a quantum number of one, is the most common and stable configuration for organic compounds. Even though oxygen is a powerful oxidizing agent, the reaction between ground-state oxygen and cellular components is "spin forbidden" because of this.

To get over this restriction on rotation, oxygen prefers to take electrons one at a time. Oxygen species that are reactive are created when electrons are added one by one. An oxygen molecule becomes a superoxide radical ($\text{O}_2\cdot^-$) with one unpaired electron when it absorbs just one electron. Because of its low reactivity, superoxide can directly deactivate just a small subset of enzymes. The electron transport chain of mitochondria has a specific target that superoxide attacks: the reduced nicotinamide adenine dinucleotide phosphate dehydrogenase complex.

The production of reactive oxygen species (ROS) is inhibited by cellular defense mechanisms that prevent oxidative damage under normal physiological conditions. When the levels of reactive oxygen species (ROS) exceed what the body can handle, a condition known as oxidative stress occurs. According to Covarrubias et al. (2008), biomolecules such as lipids, proteins, carbs, and nucleic acids can be damaged by an excess of reactive oxygen species (ROS) due to oxidation processes.

According to Rikans and Hornbrook (2007), hydroperoxides are produced when reactive oxygen species (ROS) initiate lipid peroxidation. Proteins acquire carbonyl groups when they undergo oxidative modification (Dalle-Donne et al., 2003). As an additional DNA modification inducer, reactive oxygen species (ROS) can oxidize DNA to form 7,8-dihydro-8-oxoguanine. These kinds of oxidative changes disrupt the normal behavior and structure of molecules and cells (David et al., 2007).

Both male and female infertility have been associated with long-term or severe oxidative stress. Intensive research is currently underway to determine the precise role and basic processes of oxidative stress (OS) in reproductive physiology and illness. Understanding the dynamics of oxidative stress (OS) more thoroughly may help improve fertility and reproductive outcomes.

A hydroxyl radical ($\text{OH}\cdot$), hydrogen peroxide (H_2O_2), and a superoxide anion radical ($\text{O}_2\cdot^-$) are produced during the intermediate stages of oxygen reduction, which are corresponding to the one, two, and three electron reduction steps, respectively. "Reactive oxygen species" describes these free radicals. Coexistence of reactive oxygen species (ROS) with other components can lead to the formation of alkoxyl ($\text{RO}\cdot$) or peroxy ($\text{ROO}\cdot$) radicals in some compounds, such as lipids (Guerin et al., 2001).

3. Sources of Free Radicals

Free radicals are generated both endogenous and exogenous.

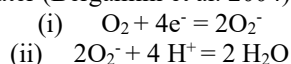
3.1. Endogenous sources of Free Radicals

3.1.1. NADPH oxidase

According to Thompson et al. (2000), adenosine triphosphate (ATP) is produced by oxidative phosphorylation (OXPHOS) and glycolysis in a pre-implantation embryo. Lowering ROS generation with OXPHOS inhibition improves in-vitro embryo development in pigs (Machaty et al., 2000) and cows (Thompson et al., 2000). At the blastocyst stage, OXPHOS metabolizes 70% of the oxygen in the mouse embryo, but at the 2- to 4-cell stages, that number drops to less than 30% (Trimarchi et al., 2000). When tested on embryos in a lab setting, high glucose levels are harmful. According to Forsberg et al. (1996), embryos derived from diabetic rats display an upregulation of mitochondrial (Mn)-SOD mRNA and a downregulation of catalase mRNA. Based on

these findings, it appears that ROS originating in mitochondria via OXPHOS plays a significant role in embryos exposed to high glucose concentrations.

Under aerobic conditions, oxygen plays a critical role in the existence of higher organisms by serving as the final acceptor for the electrons generated during biological oxidations. The standard process for energy synthesis in animal cells is aerobic respiration, as mentioned in all major biochemistry textbooks. The higher energy yield is the basis for the superiority of aerobic metabolism over anaerobic metabolism. Glycolysis is a mechanism by which some mammalian tissues, such as erythrocytes and skeletal muscle, are able to derive energy from carbohydrates even when oxygen is unavailable. Within the mitochondria, the so-called cytochrome C oxydase reaction reduces oxygen to the dianion at complex IV, demonstrating oxygen's capacity to accumulate electrons produced in oxidative reactions as the respiratory chain's last acceptor. Subsequently, in processes (i) and (ii), the dianion combines with protons to produce water (Bergamini et al. 2004).



NAD(P)H oxidases are complex enzymes consisting of many subunits. Among them, p40phox, p47phox, p67phox, and rac are primarily found in the while p22phox, cytosol, and gp91phox, the primary catalytic subunit, are attached to the membrane (Geiszt, 2006). Various signaling molecules and environmental changes, such as platelet-derived growth factor, shear stress, angiotensin II, thrombin, tumor necrosis factor α , metabolic changes, and interferon γ can activate the NADPH oxidase complex and cause it to produce O_2^- . (Finkel, 2011). On the surface of a rabbit blastocyst, NADPH oxidase has been reported as producing superoxide anion and hydrogen peroxide (Manes and Lai, 1995). Additionally, a concentration-dependent decrease in H_2O_2 generation is induced by incubating 2-cell mouse embryos with an inhibitor of NADPH oxidase (Nasr-Esfahani and Johnson, 1991).

3.1.2. Xanthine Oxidase (XO)

According to Sato et al. (2011), one particular function of the intracellular enzyme xanthine oxidase is the production of reactive oxygen species. The majority of its vascular tissue expression is due to its role as xanthine dehydrogenase/oxidase. It contributes to adenosine decomposition by transforming hypoxanthine into xanthine and uric acid, where superoxide and hydrogen peroxide are formed at the same time. It has been extensively studied in diseases associated with ischemia/thrombosis, which generate substantial levels of reactive oxygen species (ROS), despite the fact that it contributes less to superoxide formation than other enzymes under normal physiological conditions. According to Nasr-Esfahani and Johnson (1991) and Aitken et al. (2008), the xanthine/XO system is the principal ROS generator throughout early mouse embryo development in vitro. The release of purines and the XO-driven creation of ROS occur simultaneously with the end of maternal control and the breakdown of maternal RNA, suggesting a connection between the two. Embryonic arrest associated with assisted reproduction could be explained by possible cellular damage from these chemicals. Evidence from mouse studies shows that XO and other enzymes are active in trophoblast cells even after inhibition at later stages. Increased placental XO activity between certain gestational periods was seen in studies of antioxidant defenses in the rat placenta, indicating that XO-mediated ROS production is at its peak late in gestation. Both healthy and diseased states, such as preeclampsia, can cause the human placenta to exhibit variable XO activity (Many et al., 2000).

Furthermore, studies in mice shown that later in gestation, neither XO nor inhibitors of NADPH oxidase can completely reduce ROS generation in ectoplacental cone cells. This proves that trophoblast cells are actively using both ROS production systems (Gomes et al., 2012). Late in gestation, placental XO-driven ROS production increases, according to other research on antioxidant defenses in the rat placenta, which discovered enhanced XO activity in the placenta between gestational days 16 to 22. The fact that these species' placentas are so similar suggests that increased placental XO activity is a common features (Jones et al., 2010).

3.1.3. Cytochrome P450

The superfamily of enzymes known as cytochrome P450 catalyzes mono-oxygenation reactions with a wide variety of chemicals and activates dioxygen. These reactions catalyze critical processes in steroid biosynthesis and are linked to detoxification and oxidative inactivation of several drugs (Simpson and Davis, 2001). The transport of electrons from NADPH to FAD and an iron-sulfur protein is essential for mono-oxygenase processes. P450 enzymes have the ability to oxidize NADPH in the absence of a substrate, resulting in the production of superoxide and other reactive oxygen species (ROS) (Hanukoglu, 2006).

Take steroidogenesis in the ovary as an example; it occurs simultaneously with antioxidant enzyme activities including SOD, catalase, and GSH-Px (Sugino et al., 2000). Reason being, research has demonstrated that an overabundance of reactive oxygen species (ROS) produced by estrogen metabolism can directly damage DNA, leading to genotoxicity (Okoh et al., 2011). This process is important in the promotion and advancement of breast malignancies through epigenetic pathways involving redox-sensitive signaling molecules (Valko et al.,

2006). In placental microsomes, cytochrome P450 is essential for the conversion of androgens to estrogen (Milczrek et al., 2008). It is believed that deregulation of mitochondrial P450 and accelerated androgen production produce placental abnormalities and a heightened prevalence of maternal-fetal issues in pre-eclampsia (Satishkumar et al., 2011).

3.2. Exogenous sources of Free Radicals

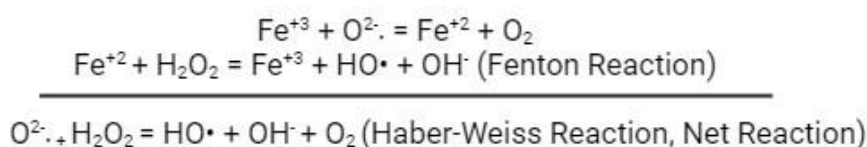
3.2.1. Oxygen Concentration

Some researchers found that low concentration (5%) oxygen tension had a positive effect on in vitro growth rates in buffalo (El-Sanea et al., 2021; Elamaram et al., 2012) and cattle (Ashibe et al., 2019; Leite et al., 2017; Yuan et al., 2003); some researchers (Corrêa et al., 2008) also stated that low concentration oxygen plays a stimulatory role but does not change blastocyst rates; some researchers (Yuan et al., 2003, Gaspar et al., 2015) state that high or low oxygen concentrations do not change in vitro growth rates.

Growth of reactive oxygen species (ROS) is enhanced by an increase in ambient oxygen content in cultured embryos of both mice (Goto et al., 1993) and bovines (Nagao et al., 1994). The opposite is true for ruminant and mouse embryos: lower oxygen concentrations promote better growth. According to Pabon et al. (1989), one way to get past the 2-cell block in mouse embryos is to reduce the oxygen content. Furthermore, cellular defense mechanisms against oxidative stress can be altered in aerobic settings. Spermatozoa incubated with ambient oxygen concentration have shown a decrease in the reduced glutathione pool (Bilodeau et al., 1999).

3.2.2. Metallic Cations

Through Fenton and Haber-Weiss reactions, even small concentrations of metallic cations like Fe and Cu can cause reactive oxygen species (ROS) to be produced. Further, when free hydroxyl radicals initiate iron's peroxidative destruction to lipids, it can be quite damaging. Toxic levels of these cations, which are present in many chemical products used to make water and other culture media, can harm embryonic development (Nasr-Esfahani et al., 1990a). However, as shown in vitro by Nasr-Esfahani and Johnson (1991). Metallic ion chelators such transferrin (Nasr-Esfahani et al. 1992a), or ethylenediamine tetraacetic acid (EDTA) (Nasr-Esfahani et al. 1992b) can prevent developmental stoppage.



3.2.3. Visible Light

The generation of base oxidation, DNA chain breaks, and oxidative damage in other biomolecules are all ways in which visible light triggers ROS production (Du Plessis et al., 2008). Visible light exposure leads to an overproduction of reactive oxygen species (ROS), which in turn affects sperm hyperactivation and motility (Shahar et al., 2011). Embryos briefly exposed to visible light in vitro also produced an excess of reactive oxygen species. Although filters mitigated some of the effects, the blastocyst apoptosis index showed that white fluorescent light—the most prevalent type used in labs—generated the highest levels of reactive oxygen species (ROS) in zygotes from mice and hamsters (Takenaka et al., 2007). Both natural light and artificial light, when exposed to pig embryos in various cultures for varying amounts of time, were found to diminish embryo quality and the proportion of parthenogenetic blastocysts, according to a study (Li et al., 2015). It appears that during in vitro production operations, it is necessary to shield culture media and embryos from light.

3.2.4. Amine Oxidase

There are polyamines in spermine and spermidine. Amino acid derivatives known as polyamines are key players in cellular maturation and expansion. The cellular process of oxidation degrades spermine and spermidine. This oxidative breakdown involves the enzyme amine oxidase, which is crucial. Spermine and spermidine are converted into aldehyde derivatives like 3-aminopropanal and 4-aminobutaldehyde by oxidizing them with amine oxidase (Parchment et al., 1990). Ammonia and other minor molecules are byproducts of the subsequent breakdown of these aldehydes. This ensures that polyamine levels are stable and that the cell's breakdown of spermine and spermidine is under control. The control of intracellular polyamine levels is thus largely dependent on amine oxidase (Quinn et al., 1998).

3.2.5. Spermatozoas

The harmful impact of ROS-producing spermatozoa during in-vitro fertilization of oocytes is another possible mechanism that could lead to ROS-induced embryo damage in IVF. Oxidative damage to the oocytes or pronucleate embryos could result by incubating them in vitro with a threshold amount of ROS-producing spermatozoa that are outside the oocyte (Alvarez et al., 1996).

4. Harmful effects of free radicals

4.1. Impact on embryonic lipids

Free radicals attack lipids' polyunsaturated chain. A peroxidation chain is initiated by oxidized lipid radicals. Membrane integrity is compromised by products like 4-HNE and malondialdehyde. The acronym for 4-hydroxynonenal is 4-HNE. The byproduct of lipid peroxidation is a crucial substance. The oxidation of lipids produces this reactive aldehyde. When lipids in a cell membrane undergo peroxidation, it is produced. Proteins, lipids, and nucleic acids in cells are vulnerable to its damaging reactions. The dangerous byproduct of lipid peroxidation known as 4-hydroxynonenal is thus described by the acronym 4-HNE. Endoplasmic reticulum and mitochondrial membranes are disrupted.

Lipid peroxidations are caused by reactive oxygen species (ROS), which have repercussions on cell division, transport of metabolites, and mitochondrial dysfunction. According to Nasr-Esfahani et al. (1990b) and Noda et al. (1991), an increase in lipid peroxides is linked to the 2-cell block seen in mouse embryos.

4.2. Impact on Embryonic Proteins

The embryonic proteins are affected by the targeting of sulfhydryl, imidazole, and phenol groups in the amino acid side chains. Changes in conformation, aggregation, and carbonylation take place. This impacts the enzymes' active areas. There is damage to structural proteins (Halliwell and Gutteridge, 2015).

4.3. Impact on Embryonic DNA

ROS degrade DNA by releasing electrons that are bound to individual bases. A purination/apyrimidination reaction and oxidative base residues takes place. Targeted areas include telomeres. As cells divide, their telomeres get shorter. Gene expressions are changing. The damage becomes persistent because DNA repair mechanisms are poor (Lopes et al. 1998).

4.4. Impact on Embryonic Mitochondrial Function

The generation of ATP is decreased when mitochondrial membranes undergo peroxidation. The chain of electron transport experiences disruptions. The opening transition of mitochondria is disturbed. The mtDNA of mitochondria is the target of reactive oxygen species (ROS) (Taanman, 1999).

4.5. Impact on Developmental Embryo Blocks

Alterations to developmental gene expression halt embryonic development. It stops the cell from dividing, implants itself, and moves on from the morula to the blastocyst stage. The presence of a 2-cell embryo block in mouse embryos is linked to an increase in reactive oxygen species (ROS) (Nasr-Esfahani et al., 1990b; Noda et al., 1991).

4.6. Impact on ATP Depletion

Mitochondrial malfunction decreases ATP production. Disruption of cellular metabolism occurs. Developmental processes cannot have their energy demands satisfied. There is a detrimental impact on cell signaling and cell division (Hyslop et al., 1988).

4.7. Impact on Apoptosis

Embryonic apoptosis pathways are impacted by reactive oxygen species (ROS). The potential across mitochondrial membranes changes. Blood chromophore C is secreted. The activation of caspases increases. Pro-apoptotic gene expression changes (Guerin et al., 2001).

4.8. Impact on Embryo Cryopreservation

Freezing and thawing heighten osmotic and oxidative stress. There is a decline in ATP levels and damage to the mitochondria. Damage has occurred to the cell membrane and its cytoplasmic contents. Preimplantation development encounters difficulties (Bilodeau et al., 1999; Alvarez and Storey, 1992).

5. Some Antioxidant Defences in the Mammalian Embryo and Its Surroundings

Embryos and their environments both have many defense mechanisms. In living organisms, oxygen scavengers found in oviductal and follicular fluids appear to shield embryos and oocytes from oxidative stress. When reactive oxygen species (ROS) are overproduced or when their clearance by scavenging systems is impaired, oxidative damage can occur. Interception (by antioxidants), repair, and prevention of ROS production are three ways to avoid oxidative stress. Metal chelation has a significant role in regulating DNA breakage and lipid peroxidation, and transferrin and other metal-binding proteins are crucial in regulating processes that could produce radicals. The amount of transferrin in tubal fluid is second only to albumin in terms of protein abundance. A chemical is considered an antioxidant if it "significantly delays or inhibits oxidation of that substrate" when present in low quantities relative to the substrate's oxidizability (Gutteridge and Halliwell, 1989). Both enzymatic and non-enzymatic substances are encompassed in this description.

5.1. Non-enzymatic Antioxidants

5.1.1. Glutathione (GSH)

Gardiner and Reed (1994, 1995a), Takahashi et al. (1993), and Gardiner et al. (1998) all point to GSH as the primary non-enzymatic defense mechanism against ROS in embryos. In addition to being the primary antioxidant enzyme glutathione peroxidase (GPX) substrate, GSH is involved in lowering environmental stress in oocytes and embryos. Particularly in pigs, oocytes have GSH concentrations that are out of the ordinary (Yoshida et al., 1993). While in hamsters GSH is greater in mature than immature oocytes, in bovines it is strongly associated with early development and viability upon freezing (Takahashi et al., 1993; Perreault et al., 1988). It has been documented that GSH is synthesized in the oocytes of various animals, including pigs, cattle, hamsters, and mice (Yoshida et al., 1993). Oocytes decondense the sperm nucleus and protect the embryo against ROS until blastocyst stage, thanks to this rise in GSH concentration (de Matos et al., 1995). While pig oocytes devoid of cumulus cells use cysteine directly for GSH synthesis, hamsters and pigs with cumulus cells produce enormous quantities of GSH (Funahashi, 1995). Inhibiting GSH synthesis has been found to cause DNA damage in embryos; in bovine embryos, GSH depletion increases H₂O₂ concentrations and produces DNA lesions (Takahashi et al., 1993). The opposite is true: supplementing bovine embryos with GSH improved their *in vitro* output (Luvoni et al., 1996).

Choe et al. (2010) added 0.5, 1, 5 and 10 mM GSH to the maturation medium of porcine oocytes and examined the blastocyst rates after fertilization. They obtained the highest (36/162) blastocyst rate in the group to which added 1 mM GSH.

5.1.2. Cysteamine

Zheng et al. (1988) found that CSH scavenges OH• and protects cells from ionizing radiation. Follicle fluid from several animals, including cows, swine, goats, and dogs, contains significant levels of CSH (Guérin et al., 1998). It is crucial for GPX function that oocytes maintain a high ratio of reduced glutathione to oxidized glutathione, and CSH may help with this. Grupen et al. (1995) found that CSH improves the *in vitro* growth of fertilized oocytes from pigs. According to de Matos et al. (1995), bovine oocytes can enhance their GSH synthesis when cysteine and CSH are added to the maturation medium. It has been suggested that cysteine has a role in oocyte endogenous GSH and CSH pool maintenance (Guérin et al., 1998). Both bovine and porcine (Grupen et al., 1995; de Matos et al., 1995) species showed a beneficial influence on future embryo development when CSH was added to the maturation medium. Nevertheless, according to our findings, adding the same concentrations of CSH to either the maturation or culture media did not enhance the development of bovine embryos in our *in-vitro* culture system (Guérin et al., 1998). Free radical scavenging has the potential to transform CSH into hypotaurine (Harvey et al., 1995).

5.1.3. Taurine and Hypotaurine

According to Guerin and Menezo (1995), all of the species that were studied had high concentrations of taurine and hypotaurine in their gametes and embryonic environments. Additionally, according to Guerin et al. (1995a), oviductal epithelial cells produce and release these substances. This antioxidant action of hypotaurine is efficient even at low concentrations (Aruoma et al., 1988), and it can neutralize hydroxyl radicals and prevent sperm lipid peroxidation (Alvarez and Storey, 1983). Hypotaurine in vaginal fluids may thus play a crucial antioxidant role in the development of gametes and embryos. Taurine is the major by-product of hypotaurine following free radical scavenging. Indirectly, taurine acts as an antioxidant by helping to reduce ROS damage by destroying cytotoxic aldehydes, which are byproducts of the peroxidation cascade reaction (Ogasawara et al., 1994). According to Van Winkle and Dickinson (1995), taurine can be taken in by mammalian embryos. Low concentrations of taurine (0.3 mmol/l) are sufficient to protect cells. It's possible that the effects of CSH, hypotaurine, and taurine on embryos and gametes are sequential and complementary. Embryo development in hamsters, mice, pigs, cows, and humans is positively impacted by the addition of hypotaurine to culture media (Guerin et al., 1997; Devreker and Hardy, 1997). When it comes to taurine, the findings are mixed. Some studies have shown that taking a taurine supplement can help with embryonic growth (Dumoulin et al., 1992; Li et al., 1993), while others have come to the opposite conclusion (Devreker and Hardy, 1997).

5.1.4. Ascorbate

A strong direct antioxidant, ascorbate makes lipoproteins more resistant to oxidation caused by metal ions by a stable alteration with dehydroascorbate. Fraga et al. (1991) found that ascorbic acid prevents DNA damage caused by endogenous oxidative stress. In addition, according to Guerin et al. (1995b), oviductal epithelial cells emit hypotaurine and taurine when exposed to ascorbate at physiological doses. During fertilization, a combination of tubal and follicular fluids is present in the ampullar region of the oviduct (Hansen et al., 1991). Follicle fluid contains high concentrations of ascorbate, which may cause the release of taurine and hypotaurine in oviductal fluid after ovulation (Paszowski and Clarke, 1999). But when transition metals are present, ascorbate becomes an oxidant (it facilitates the Fenton reaction by changing ferric ions into ferrous ions).

5.1.5. Lycopene

The main carotenoids found in tomatoes, lycopene, are powerful antioxidants that can neutralize free radicals (Islamian and Mehrali, 2015) and chemically neutralize singlet oxygen (Tamilselvan et al. 2014). Research has shown that lycopene is ten times more effective than α -tocopherol in scavenging singlet oxygen than β -carotene (Imran et al., 2020). Research has demonstrated that lycopene's antioxidant effects are around a hundred times more potent than those of vitamin E (Sachdeva and Chopra, 2015). Furthermore, lycopene has strong antioxidant properties that protect against DNA oxidation, lipid peroxidation, and protein degradation in both laboratory and living organism settings (Guerin et al., 2001).

5.1.6. Vitamin E

The amount of vitamin E in sperm seems to be related to the quality of the sperm; vitamin E is an antioxidant that occurs naturally (Cerolini et al., 1997). According to Schweigert and Zucker (1988), vitamins E and A are abundant in the follicular fluid and in the ovaries. Through its effect on NADPH oxidase, vitamin E blocks the synthesis of superoxide anions. These antioxidants, which protect the membranes from oxidative damage, are soluble in lipids (Pascoe et al., 1987).

5.2. Enzymatic Systems

Oocytes and embryos are protected from peroxidative damage caused by SOD, catalase and GPX by a series of antioxidant enzymes (Li et al., 1993). Superoxide radical scavengers include manganese-superoxide dismutase (Mn-SOD) in the mitochondria and copper-zinc superoxide dismutase (Cu,Zn-SOD) in the cytosol. The two SODs are part of the initial enzymatic process that shields cells from harmful oxygen radicals. Catalase or GPX remove H₂O₂, a result of superoxide dismutase activity. The second enzyme acts as an antioxidant both directly and indirectly; although catalase is solely efficient against hydrogen peroxide, GPX reduces lipid hydroperoxides and H₂O₂. This data points to GPX's critical function in antioxidant defense of cells. It is possible to keep cellular GSH concentrations high through the action of glutathione reductase (GR) and glutamyl cysteine synthetase (GCS). Researchers found that 2-cell-stage and blastocyst-stage embryos could restore their GSH concentrations within 45 minutes after inducing a depletion of GSH in mouse embryos (Gardiner and Reed, 1995b). These findings point to GR's significant protective function in embryos.

Since oocytes contain Cu,Zn-SOD coding transcripts throughout maturation, especially in humans, this enzyme is crucial for avoiding oxygen toxicity in both experimental and living organisms. Additional evidence for their importance was provided, who discovered that they are highly expressed in the mouse blastocyst. In the GV and MII phases of metaphase II, GCS and Cu,Zn-SOD transcripts are detected in both human and mouse oocytes, as reported (El Mouatassim et al., 1999). Researchers have discovered that GPX and Mn SOD transcripts are present in the eggs of MII-afflicted mice and humans. Whether or not they are deadenylated determines whether they are present at the GV stage in people. Readenylation may play a role in regulating the expression of these transcripts, as suggested by Paynton and Bachvarova (1994). Cows and mice have been used to describe the last stages of altering mRNA polyadenylation to govern translation and expression (Brevini-Gandolfi et al., 1999). Deadenylation may explain why Mn SOD is either present or absent in cow oocytes and embryos; neither of these tissues contained Mn SOD (Harvey et al., 1995). Since catalase transcripts are present in mouse blastocysts but not in MII oocytes, it is possible that this protection does not take effect until after ZGA (El Mouatassim et al., 1999; Harvey et al., 1995). Catalase has been discovered in the oviductal fluid of pigs, cattle and humans. Antioxidant enzymes associated with gametes and embryos in the female reproductive system may also have a survival function, as suggested by the fact that catalase binds to sperm cells (Lapointe et al., 1998).

Because of the close relationship between messenger RNA, proteins, and enzyme activity levels, it seems that the pre-translational level is primarily responsible for controlling the levels of antioxidant enzymes (SOD, catalase, GPX) (Forsberg et al., 1996). So, it's safe to assume that the discovered transcripts are indeed translated into enzymes. Oogenesis preserves an endogenous pool of antioxidant enzymes as mRNA in the egg, according to Harvey et al. (1995). According to these findings, this pool is involved in embryo protection from reactive oxygen species. Piko and Clegg (1982) found that the total amount of maternal transcripts decreases until the ZGA is reached during embryonic development. Fluctuations in maternal mRNA synthesis or accumulation during oocyte maturation can influence the in vitro development of the embryo until ZGA is reached. Falling below a certain point can bring development to a halt. Gene expression can be up- or down-regulated by reactive oxygen species (ROS). The genes that code for antioxidant enzymes are upregulated in response to oxidative stress (Maitre et al., 1993).

Schultz (1993) states that reactive oxygen species (ROS) can modulate the activity of specific transcription factors, thereby activating genes involved in antioxidant defense. Embryonic cells may be more susceptible to reactive oxygen species (ROS) associated with an oxidative burst during the 2-cell block. Embryos in the oviduct have access to a number of radical scavengers while they are still alive (Guerin and Menezo, 1995). Regardless of the species, these protective systems are essential for the development of future embryos. El Mouatassim et al. (1999) observed that four of the five transcripts for antioxidant enzymes are present in human oocytes during the MII stage, whereas in mice they are identified during the GV/II stages.

In conclusion, the article points out that antioxidants play an important role in in vitro embryo production and have a positive effect on embryonic development. Antioxidants neutralize the harmful effects of free radicals and provide protection against cellular oxidative stress by supporting the healthy development of the embryo. Many studies have shown that antioxidants increase embryo viability and positively influence embryo morphology, cell proliferation and gene expression. It can therefore be concluded that antioxidants have great potential in the field of in vitro embryo production and can be used in future studies to achieve better results.

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Determination of Semen Characteristics of Locally Adapted Anatolian Black Bulls with CASA and Flow Cytometry

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The aim of this study is to determine the spermatological characteristics of Anatolian Black bulls, which have been taken under conservation within the scope of the "animal genetic resources conservation project" since their numbers are decreasing. A total of 72 semen samples collected from 6 Anatolian Black bulls were used in our study. After measuring the volume and pH of the collected ejaculates, fresh sperm quality assessment was made and semen with fresh motility $\geq 80\%$ were diluted with commercial extender (Optixcell®), frozen in 0.25 ml straws and stored in liquid nitrogen at -196°C until analysis. A computer-aided sperm analyzer (CASA) was used to determine sperm motility and other kinetic parameters, and Flow Cytometry was used to analyze plasma membrane and acrosome integrity (PMAI) and mitochondrial membrane potential (MMP, %). The mean of pH, volume and concentration values (mean $\pm$ SD) of fresh semen were found to be 6.73 ± 0.17 , 4.47 ± 1.02 (ml), 1263.96 ± 376.66 ($\times 10^6/\text{ml}$), respectively. The mean ($\pm$ SD) of pH, volume and concentration values of fresh semen were found to be 6.73 ± 0.17 , 4.47 ± 1.02 (ml), 1263.96 ± 376.66 ($\times 10^6/\text{ml}$), respectively. While the mean ($\pm$ SD) of total and progressive motility values of fresh semen were determined as $93.35 \pm 3.77\%$ and $45.01 \pm 6.37\%$ respectively, post-thaw total and progressive motility values (mean $\pm$ SD) were determined as $61.6 \pm 13.07\%$ and $24.4 \pm 7.93\%$, respectively. The mean ($\pm$ SD) post-thaw PMAI values was determined as $46.33 \pm 9.47\%$, and the high mitochondrial membrane potential (HMMP) was determined as $61.48 \pm 13.54\%$. In our knowledge, spermatological characteristics of Anatolian Black bulls were evaluated for the first time using CASA and flow cytometry in this study. In conclusion, it was evaluated that the post-thaw sperm quality parameters of locally adapted Anatolian Black bulls were well above the limits of total motility limits ($\geq 40\%$) determined by the "Turkish Ministry of Agriculture and Forestry" for artificial insemination and could be used for artificial insemination and embryo production.

Keywords: Acrosome integrity, Anatolian Black, Mitochondrial membrane potential, Motility

The Effects of Some Antioxidants Participating in Embryo Culture Media on *In vitro* Embryo Development

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This study was carried out to investigate the effects of erucic acid and arachidonic acid, which are essential fatty acids that are added to culture media of in vitro embryo production and have antioxidant properties, on embryo development and quality. Specifically, the effect of these antioxidants on the in vitro development capabilities of bovine embryos was examined. 1335 oocytes were obtained from 243 ovaries by aspiration method. 940 of these oocytes that were determined to be A or B quality were used in the study. Zygote division rates were found to be 49.65% for the control group, 55.71% (300 µM) for erucic acid and 52.14% (300 µM) for arachidonic acid groups. For the pairwise comparisons between the groups, it was observed that the 300 µM dose of erucic acid was more effective than the same dose of arachidonic acid and the control group ($P < 0.001$). The rates of zygote reaching to the morula-blastocyst stage were 10.95% for the control group, 15.35% (300 µM) for erucic acid and 12.50% (300 µM) for arachidonic acid. After the first 24 hours, the rate of reaching the morula-blastocyst stage after division was 22.22% in the control group, 27.56% for erucic acid and 23.97% for arachidonic acid, respectively. As a result, it was found that 300 µM dose of erucic acid increases the division rate and overall averages of the zygote, the rate and averages of reaching the morula-blastocyst stage and can be used as an antioxidant for in vitro embryo culture. Especially, comparisons between arachidonic acid and erucic acid led to the conclusion that erucic acid was more effective in terms of the mentioned parameters.

Keywords: Arachidonic acid, Embryo, Erucic acid, *In Vitro* embryo production, Bull

Some Reproductive Characteristics of Hair Goats and Survival Ability Rate of Their Kids at Breeders Conditions

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The aim of this study was to determine the reproductive characteristics of Hair Goats and survival ability rate of their kids at weaning in Hair Goats at Breeders Conditions flock in Karaman province. In this study, between 2019-2023 years totally 29.010 head of goats and 26.479 head of their kids' data obtained from 36 Hair Goats breeder flocks in Karaman province were used. The number of born and dead kids were recorded by breeders. Reproductive performance and Survival ability rate were determined by project crew. According to results for 2019-2023 years; The average kidding rate of the goats, single birth rate, twinning rate, litter size, fecundity and weaning survival ability rate of kids were found % 85.3, %92.9, %7.1, 1.07, 0.91, %87.4 respectively. While the effect of sex of kids on the survival ability was insignificant. Year, age of dam and type of birth were found to be statistically significant ($p<0.05$).

Keywords: Hair goats, Reproduction, Survival ability

Effects of Lysine and Organic Chromium Addition to Breeder Laying Hen Diets at Different Environmental Temperatures

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This study was carried out to determine the effects of dietary supplemental lysine and chromium-picolinate to breeder laying hen diets on performance, serum metabolic profile, oxidative status of breeder laying hens. The breeder laying hens reared under normal and high temperature. 35 weeks old Barred Rock-1 female (120) used in the experiment. Two temperature regimes were thermoneutral ($22 \pm 2^\circ\text{C}$ for 24 h) or recycling hot ($34 \pm 2^\circ\text{C}$ for 8 h and $22 \pm 2^\circ\text{C}$ for 16 h). A 2x2x2 factorial arrangement was employed with two levels of supplemental lysine (0 and 0.085% plus 20%) and two levels of supplemental chromium picolinate (0 or 800 ppb) under thermoneutral or high temperature regimes. In the study, effects of the chromium-picolinate and lysine on laying performance, blood parameters or antioxidative capacity were evaluated in breeder laying hens exposed to normal and high ambient temperature. Results showed that egg production and feed intake in laying hens were statistically affected. The findings obtained present study suggests that the lysine and chromium-picolinate supplementation positively affected some performance and antioxidative capacity of breeder laying hens exposed to high ambient temperature.

Keywords: Breeding layers performance, High environmental temperatures, Lysine, Organic chromium, Oxidative status of breeder laying hens

Effects of Pomegranate Peel Powder Added to The Feed of Laying Hens Exposed to Heat Stress on Egg Quality Characteristics

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In this study, it was aimed to determine the effects of pomegranate peel powder on egg quality characteristics in laying hens exposed to heat stress. For this purpose, a total of 320 Atabey hybrid white laying hens at the age of 24 weeks were used. The experiment was conducted for 4 weeks. Study groups: It was formed as a group of at 22°C; 1: control group, 2: 0.5% pomegranate peel powder (PPP) group, 3: 1% PPP group, 4: 2% PPP group. It was formed as a group of at 30°C; 5: control group, 6: 0.5% PPP group, 7: 1% PPP group, 8: 2% PPP. During the experiment, internal and external quality characteristics were determined in 30 eggs randomly selected from all eggs collected from subgroups of each group, once a week. In the groups that participated in PPP, there was improvement in egg quality characteristics such as yellow color index, haugh unit, egg shell thickness and egg break resistance. The yellow color index was found to be higher in the 4th and 8th groups. It would be beneficial to add pomegranate peel powder to poultry feeds in order to maintain egg shell thickness and breakage resistance in poultry houses with high ambient temperature. New studies are needed to better understand the bioavailability of pomegranate fruit and pomegranate by-products.

Keywords: Egg, Heat stress, Pomegranate

Pomegranate By-Products as an Alternative Feed Material in Ruminant

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By-product feed materials are becoming more important and striking in the feed industry due to its alternative feature. Pomegranate (*Punica granatum* L.) is a well known beneficial fruit which contains bioactive compounds such as polyphenols, flavonoids, anthocyanidins, tannins and ellagitannins. It is known that this fruit is a domestic mediterranean plant and been used in traditional medicine. It has antioxidant, antimicrobial and anticarcinogenic effects on ruminant health. Pomegranate by-products (PBP) can be used in various ways in ruminant nutrition which are its seed, peel and pulp. This is because its nutritional content can provide balanced nutrition in rations due to its high protein (up to 18 %) content as well as the presence of crude fiber, which is necessary for ruminants. Some studies found out that PBP such as seed pulp, increased the amount of fatty acids in meat and milk while it decreased the percentage amount of methane and total gas volume on rumen. Studies also found out increasing dosage of PBP in rations have shown decreased NDF digestibility. Because of its palatability; it is reported that total feed intake and average daily gain is increased in beef cattle. The purpose of this review is to understand the functioning of PBP by comparing both in vivo and in vitro studies for its future usability.

Keywords: Antioxidant, Bioactive compounds, Rumen fermentation

1. Introduction

Industry by-products are becoming increasingly important and remarkable in the feed industry due to their alternative properties. One of the most important reasons for this is the water scarcity experienced with the variable weather conditions in recent years and the resulting lack of feed raw materials. In this context, it is thought that by-products that are not used by humans can reduce the raw material deficiency in ruminant livestock (Ghoreishi *et al.*, 2021). When it comes to ruminant livestock, many factors need to be taken into consideration. Methane gas emitted by ruminants is one of them. Methane, which is 21 times more harmful than carbon dioxide, is responsible for 12% of energy loss in ruminants; it contributes to greenhouse gas emission, and the vast majority of it is supplied enterically (81%) (Jalal *et al.*, 2023). Current research also emphasizes the importance of antioxidants in ruminant livestock; It is aimed to improve metabolic profile and performance with its strengthened antioxidant level (Safari *et al.*, 2018; Abdel *et al.*, 2022).

Pomegranate (*Punica granatum* L.) is a beneficial fruit containing bioactive compounds for instance; polyphenols, flavonoids, anthocyanidins, tannins and ellagitannins. It is also known that this fruit is a native Mediterranean plant and is used in traditional medicine. (Abdel *et al.*, 2022). Pomegranate fruits can be consumed fresh and are also actively used commercially in the juice, jam and wine industry. While 78% of the edible part of the fruit consists of juice and 22% consists of by-products (seed, peel), by-products constitute approximately 52% of the total fruit weight (Bostami *et al.*, 2015). Pomegranate by-products (PBP), including seeds, peel, pulp and oil, can be used in various ways in ruminant livestock. The reason for this is the high protein content (up to 18%) it contains and its ability to provide balance in rations with the crude fiber required for ruminants (Jaberi *et al.*, 2023).

Ruminants have an important role in global food security and nutrition. Healing the rumen microflora can improve both milk and meat quality. In order to minimize the effects on greenhouse gas emissions due to their mechanisms, researchers are turning to alternative feed raw materials (Abarghuei *et al.*, 2013; Emami *et al.*, 2016; Ghoreishi *et al.*, 2021; Abdel *et al.*, 2022). The aim of this review is to understand the functioning of pomegranate fruit by comparing both in vivo and in vitro studies in terms of future usability of PBP.

2. Nutrient Content of PBP

Recently performed PS weende analysis from our department (Ankara University Faculty of Veterinary Medicine, Department of Animal Nutrition & Nutritional Diseases) is shown in **Table 1**.

Table 1. Analysed Nutrient Content of PS

Ingredient %	PS
DM	97.55
CA	1.65
CP	18.17
EE	25.39
CF	18.82
ADF	49.19
NDF	53.03

DM: Dry matter, CA: Crude ash, CP: Crude protein, EE: Ether extract, CF: Crude fiber, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, PS: Pomegranate seed

Many authors conducted researches about PBP in their studies. Its because the nutrient content and bioactive compound profile PBP has in it. Safari *et al.* (2018) compared the difference between pomegranate seed (PS) and pomegranate peel (PP). According to Safari *et al.*, PS was richer in crude protein (CP) content compared to PP but close in crude ash (CA). Another study from Mirzaei-Aghsaghali *et al.* (2011) confirms nutrient content of Safari *et al.* (2018) in addition, Jalal *et al.* (2023) investigated the nutrient contents of PP, PS and pomegranate peel (PGP); CP and neutral detergent fiber (NDF) content was highest in PS. As for bioactive compounds, Natalello *et al.* (2020) and Ghoreishi *et al.* (2021) demonstrated the richness of PP, PS and whole pomegranate by-products (WPB). The nutrient content of PBP from literature is shown in **Table 2** (Mirzaei-Aghsaghali *et al.* 2011; Safari *et al.* 2018, Natalello *et al.* 2020, Ghoreishi *et al.* 2021 and Jalal *et al.*, 2023).

Table 2. Nutrient Content of PBP From Literature

DM: Dry matter, CA: Crude ash, CP: Crude protein, EE: Ether extract, ADF: Acid Detergent Fiber, NDF: Neutral

Ingredient %	PS	PP	PGP	WPB
DM	88-95.1	81.3-96.2	91.16-91.2	96.7
CA	2.03-2.4	3.88-5.4	3.60-3.64	4.4
CP	12.6-15.4	3.02-3.6	6.9-7.8	7.51
EE	6-12.1	1.94-6.1	2.6-4.43	4.63
Starch	3.22	4.97	4.97	4.2
ADF	39.0-49.0	13.1-15.1	22.8	16.4
NDF	49.6-68	19.7-20.8	31.4-41.3	23.8
TP	0.9-1.61	21.5-25.5	9.53	17.0
TT	0.73-1.31	21.4-21.9	9.34	17.0

Detergent Fiber, TP: total phenols, TT: Total tannins, PS: Pomegranate seed, PP: Pomegranate peel, PGP: Pomegranate Pulp, WPB: Whole pomegranate by-products

3. Pomegranate By-Products in Ruminant Nutrition

Many researchers have conducted studies on this fruit to investigate its various effects on ruminants. Studies conducted on ruminants with the addition of PBS are shown in Table 3.

Author	Occasion	Conduction of PBP	Period PBP is given	Conclusion
<i>Mirzaei-Aghsaghali et al. 2011</i>	<i>In vitro</i> , steers	PS – 200 mg of 100 ml syringes PP – 200 mg of 100 ml syringes	Given in syringes – 96h	↑ Ruminal gas production In vitro OMD ↓ in PS
<i>Abarghuei et al., 2013</i>	<i>In vivo</i> , Holstein cows	PPE – 0 ml/d (Peel extract) PPE – 400 ml/day PPE – 800 ml/day PPE – 1200 ml/day	28 days	↑ Milk production ↑ Protein yield ↑ Milk efficiency (800) ↓ NH ₃ -N, total protozoa ↑ Microbial N pop.
<i>Emami et al., 2015</i>	<i>In vivo</i> , Mahabadi goats	PSP – 0%, 5%, 10% (P. seed peel)	84 days	× FCR, ADG, DMI PSP15 ↑ Antioxidant
<i>Emami et al., 2016</i>	<i>In vivo</i> , Mahabadi goats	PSO – 25 g/kg of Ration (Seed Oil)	Mid lactation period	↑ Milk fat ↑ FA in milk (Vaccenic) ✓ Paninic acid
<i>Maleki et al., 2016</i>	<i>In vitro</i> , Kacang crossbred goats	PSO – 5 mg of 100 ml syringes PSO – 10 mg of 100 ml syringes	Given in syringes – 24 h	PSO 10 ↓ methane × VFA except butyric
<i>Safari et al., 2018</i>	<i>In vivo</i> , dairy cows	PS – 400 g/day PP – 400 g/day PS+PP – 400 + 1200 g/day	25 d precalving 25 d postcalving	↑ Plasma antioxidant (total)
<i>Natelello et al., 2019</i>	<i>In vivo</i> , lambs	WPB – 200 g/kg DM	36 days	× DMI, Final and Carcass BW, ADG ↑ Total FA in liver and muscle ✓ Puninic acid
<i>Valenti et al., 2019</i>	<i>In vivo</i> , Comisana ewes	PP – 68% of Ration	Late lactation ±10 days in milk	↑ Somatic cells ↓ FA ✓ Puninic acid
<i>Natalello et al., 2020</i>	<i>In vitro</i> , Merino ewes	PS – 20% of Ration PPP – 20% of Ration WPB – 20% of Ration	Given in syringes – 24h	↓ Ruminal fermentation + ruminal biohydrogenation (tannins)
<i>Jaberi et al., 2023</i>	<i>In vitro</i> , camel and goat	PSP – 0%, 5%, 10%	Given in syringes – 120h	× Methanogen × Protozoa × Gas production ↓ Methane and VFA (10%) in camels

Table 3. PBP in Ruminant Nutrition

PS: Pomegranate seed, PP: Pomegranate peel, PPE: Pomegranate peel extract, PSP: Pomegranate seed pulp, PSO: Pomegranate seed oil, WPB: Whole pomegranate by-product, OMD: Organic matter digestibility, FCR: Feed conversion rate, ADG: Average daily gain, FA: Fatty acid, VFA: Volatile fatty acid, DMI: Dry matter intake, BW: Body weight.

4. Conclusion

As a result, with its rich nutritional content and deliciousness it is a suitable raw material for ruminants with its puniceic acid content, which improves milk yield, meat yield and plasma antioxidant effects.

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The Effects of Fumaric Acid on The Developmnet of digestive System and Growth Performance in Broilers

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This study has been carried out with a view to determine the usage activities of fumaric acid contribution of organic acids on fattened chickens rations. In the study asan animal material, one day year old 160 Ross 308 male fattened chicks have been used. In the experiment, while the group which is fed with basic ration, which is not included fumaric acid contribution (% 0), has formed the control group, the groups which the fumaric acid contribute to rations with 1, 2 and 4 per cent levels have formed the treatment groups. It has been taken care of testing groups“ having similar live weight average. Forage and water have been given freely. The study period is 42 days. As of the end of testing, the live weight profit of the fumaric acid contribution, which is added rations at different levels, has not had an important effect on the proportion of benefiting from forage, skeleton parameters, abdominal fat proportion, plasmacholesterol, triglyceride LDL (low-density lipoprotein), plasma total protein level, heart weight, andthe pH levels of adenoid (bezel) stomach. However, the difference, which has been observed between groups, has been considered something significant statistically when compared with average forage consumption that they have consumed during the testing of groups(P<0.05). The 2 percent fumaric acid contribution has increased the forage consumption significantly in comparison with testing group, has decreased the jejenum pH level significantly as well. The weightof cecum has increased 2 and 4 percent in the group that has fumaric contribution (P<0.0001). Consequently, in the nourishment of fattened chick, as a growth inducer by decreasing the jejenumpH of fumaric acid contribution, it has been determined that it tends to improving the utilization proportion of forage.

Keywords: Broiler, Digestive system, Feeding, Fumaric acid

Using Diverse Carbohydrate Source to Improve Legume Silage Quality

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Legumes are important feedstuffs and are mostly used in fresh and hay forms in both dairy and beef cattle nutrition. However, in periods when weather conditions are not favorable, legumes can also be utilized by silaging. The main problems in making legume silages include high moisture content, low sugar content for fermentation, and high buffering ability that prevents the pH value from decreasing. To prevent these problems, legumes are mostly silaged with different carbohydrate sources, which helps improve the quality of legume silage by providing additional sources of carbohydrates for fermentation. This review aimed to explore the effects of incorporating various carbohydrate sources on fermentation characteristics and nutritive quality. Different carbohydrate sources, including dried fruit powders, fruit pomaces, molasses, sucrose, inulin, whey, and grains, could be used as silage additives to improve legume silage quality. Adding carbohydrates to legume silages, such as alfalfa and lucerne, increases water-soluble carbohydrates, which also increases the lactic acid bacteria population in the silages and leads to lower pH and ammonia-N. Increasing lactic acid content in silage also prevents the proliferation of undesired bacteria such as Enterobacteriaceae and Clostridium spp. Additionally, using a carbohydrate source in legume silages also improves aerobic stability, total volatile acid profile, and in vitro rumen fermentation. In summary, the addition of carbohydrate sources improved the fermentation quality, aerobic stability, digestibility, and protein utilization of legume silages compared with untreated silages. The effects were dose-dependent, with higher inclusion rates resulting in better responses.

Keywords: Alfalfa, Carbohydrate, Lactic acid bacteria, Legume

1. Introduction

High-quality roughage production is crucial for dairy cattle production. Quality roughage, such as forage and silage, plays a critical role in meeting the fiber requirements of dairy cows and ensuring optimal health and productivity (Tatli Seven *et al.*, 2021). The rising cost of feedstuffs in global production poses a challenge for countries to produce and preserve adequate amounts of roughage to ensure sustainable milk production (Andrade *et al.*, 2021).

The cultivation of legume plants, including alfalfa, cowpea, clover, and soybean forage, presents numerous advantages for both the environment and livestock. These plants are noteworthy for their high protein content, essential amino acids, and other nutrients, which help fulfill the dietary needs of dairy cattle (Fulkerson *et al.*, 2007; Ni *et al.*, 2018). They are also considered important mineral sources (Tatli *et al.*, 2021). Legume forage is used for ruminant nutrition through direct grazing, feeding by mowing, or stored as hay or silage (Castro-Montoya and Dickhoefer, 2018). The use of ensiling to preserve forage has significantly increased globally, particularly in regions where rain patterns restrict the possibility of dependable hay production (Albrecht and Beauchemin, 2003). In addition, the preservation of legumes as silage provides a means of preventing deterioration owing to weather conditions and reduces leaf loss during the haymaking process (Wang *et al.*, 2019). There is a challenge in ensiling legumes, as they are abundant in minerals, possess a high pH-buffering capacity, and are deficient in water-soluble carbohydrates. (Canbolat *et al.*, 2013). To address these challenges, researchers have explored the use of different water-soluble carbohydrate sources, such as molasses, fruit pulps, grains, and sugars, as well as microbial inoculants, to improve the fermentation quality of legume silages (Canbolat *et al.*, 2013; Jahanzad *et al.*, 2016). This review systematically examines the impact of various carbohydrate sources, including molasses, fruit pulps, grains, sugars, and microbial inoculants, on the quality of legume silage and evaluates their effectiveness in addressing challenges and improving fermentation quality.

2. Nutritional Value of Legumes for Ruminant Nutrition

Legumes are highly valued for their nutritional significance in animal diets as they offer a rich source of essential nutrients and minerals (Kulkarni *et al.*, 2018). These leguminous forages are recognized for their high protein, essential amino acid, and mineral contents, making them a valuable component of animal nutrition

(Albrecht and Beauchemin, 2003). The chemical compositions of different fresh legume forages are listed in Table 1. The use of legumes as feed for livestock not only provides high-quality fodder and feed but also has a protein-rich nature that positively impacts nitrogen fixation, leading to a reduction in the need for inorganic nitrogen fertilizers. This makes legumes a valuable tool to improve animal nutrition and promote environmental sustainability (Kulkarni *et al.* 2018).

Table 1. The average chemical composition of different fresh legume forages (Feedipedia, 2023).

Parameters	Alfalfa	Cowpea	Common Vetch	Soybean
Dry matter (DM), %	19.9	20.9	19.3	24.0
Crude protein, % DM	20.6	18.1	23.0	15.7
Crude fibre, % DM	26.7	24.1	25.4	31.2
NDF, % DM	39.3	38.6	36.7	48.1
ADF, % DM	30.9	27.1	28.5	37.2
Lignin, % DM	7.6	4.6	6.1	5.8
Ether extract, % DM	2.9	2.8	2.5	4.4
Ash, % DM	11.5	11.3	9.8	9.3

More than 60 legume plant types have been farmed for use as animal forage and feed (Kulkarni *et al.*, 2018). Alfalfa, a cultivated legume plant with an economic life of seven years, is prominent among its peers. This plant is noteworthy for its substantial protein, vitamin, and mineral contents (Tatli Seven *et al.*, 2021). Alfalfa forage is typically conserved as hay following drying but is prone to experiencing a reduction in nutritional value as a result of aerobic degradation, particularly in damp environments and regions with abundant precipitation. Therefore, an effective preservation method is required (Gao *et al.*, 2021; Luo *et al.* 2021). In some regions of the world such as in sub-Saharan Africa (particularly in West Africa) and India, cowpea (*Vigna unguiculata* (L.) Walp.) has been an integral part of traditional cropping systems, where grains are used as food and haulms are fed to livestock as nutritious fodder (Kulkarni *et al.*, 2018). Vetches possess an upright growth pattern designed to optimize the gathering of legume foliage for conservation purposes. The common vetch is widely utilized in the Mediterranean and subtropical regions, whereas the hairy vetch, although less prevalent, is known for its resistance to frost (Hernández-Ortega *et al.*, 2011). In recent years, soybean, a leguminous annual plant known for its high protein and oil contents, has garnered considerable interest as a forage crop. Its diverse growth stages, capacity for effective reduction of soil erosion, and adaptability to a range of climatic zones have made it a valuable and sustainable crop for various agricultural applications (Kulkarni *et al.* 2018).

3. Using Different Carbohydrate Sources To Increase Legume Quality

Legume silage production is a critical component for ensuring the provision of high-quality roughage for the nutrition and well-being of dairy cattle (Castro-Montoya and Dickhoefer, 2018). Legumes, such as alfalfa, cowpea, clover, and soybean, are typically harvested and preserved through a process known as ensiling. However, challenges can arise owing to the high mineral content, high pH buffer capacity, and low levels of water-soluble carbohydrates present in these legumes. These factors can inhibit the fermentation process and adversely affect the quality of silage produced (Canbolat *et al.*, 2010). To address the challenges associated with the fermentation process, scientists have investigated the potential of various carbohydrate sources including molasses, fruit pulps, grains, sugars, and microbial inoculants. The aim of incorporating these additives is to improve the quality of the fermentation process by providing readily available sugars and stimulating the growth of beneficial microorganisms (Canbolat *et al.* 2013).

3.1. Alfalfa Silages

Alfalfa is a primary legume crop used in the cultivation of dairy cattle owing to its exceptional nutritional profile, which enhances the quality and quantity of milk produced (Kang *et al.*, 2021). In recent years, the focus of research on alfalfa silage has shifted towards enhancing its quality by incorporating homofermentative or heterofermentative bacteria or by introducing alternative sources of carbohydrates (Tatli Seven *et al.*, 2021).

In studies conducted on alfalfa silage, fruits or their pomaces are the most utilized carbohydrate sources. This is due to the fact that many of these items are not suitable for human consumption and may pose environmental concerns (Canbolat *et al.* 2013). In a study conducted by Ke *et al.* (2015), the effects of incorporating apple and grape pomaces into alfalfa silage on silage quality, aerobic stability, fermentation characteristics, and lipid composition were examined. The results indicated that the application of apple or grape pomace during the ensiling process not only allowed for the utilization of industrial waste as animal feed but also inhibited proteolysis and altered the fatty acid composition of the ensiled alfalfa. Besharati *et al.* (2021) undertaken an examination of the consequences of incorporating an essential oil containing ricinoleic acid, cardol, and cardanol, as well as apple

pomace, on the fermentation quality, chemical composition, and aerobic stability of alfalfa silage. In summary, this study shows that the utilization of apple pomace as a source of fermentable carbohydrates and/or the addition of essential oils can effectively alleviate the high buffering capacity and low carbohydrate content of alfalfa, thereby enhancing its ensiling properties. In another study, varying levels of grape pomace supplementation in alfalfa silage significantly increased *in vitro* gas production, aerobic stability, digestible organic matter, metabolizable energy, *in situ* dry matter degradability, and organic matter degradability (Canbolat *et al.*, 2010). Moreover, Canbolat *et al.* (2013) examined the potential of honey locust pods as silage additives for alfalfa silage. The findings revealed that incorporating honey locust pods at 80 and 100 g/kg enhanced silage quality.

Molasse is another significant carbohydrate source for alfalfa silage, which serves not only as a means to provide adequate substrates for the swift accumulation of LA and a marked decrease in pH value, but also enhances microbial protein synthesis and improves overall nutritional quality (Luo *et al.*, 2021). Luo *et al.* (2021) examined the effects of adding different levels of sugar cane molasses on the fermentation quality, microbial community, and taste profiles of alfalfa silage. In general, the addition of molasses had a beneficial effect on the fermentation process, reducing the activity of spoilage organisms and modifying certain flavor components. Specifically, the application of 3% molasses resulted in a more stable pH, enhanced nutrient retention, and desirable modifications to the taste of the alfalfa silage. In another study, the influence of various carbohydrate additives, including pectin, starch, molasses, and fructose, on the fermentation properties and chemical makeup of alfalfa silage over time was examined (Gao *et al.*, 2012). In this study, alfalfa was collected, wilted, and treated with additives prior to ensiling for 1, 3, 7, 15, and 30 d. These results demonstrated that the addition of fructose and pectin significantly improved the quality and nutritional value of alfalfa silage. The researchers propose that fructose, pectin, and other agricultural by-products may serve as effective alternative additives to enhance alfalfa silage fermentation.

In summary, according to different studies conducted on alfalfa silages, the inclusion of different carbohydrate sources in alfalfa silages enhanced alfalfa ensilability and increased lactic acid production and storage time. They inhibit microorganism growth, reduce protein breakdown during ensiling, and increase the total fatty acids in silage. These sources positively affected the digestibility and energy content of the silage. In contrast, certain ingredients, such as grape and apple pomace, can affect the aerobic stability of silage. Excessive amounts of additives can decrease protein content and fiber digestibility. The nutrient content of alfalfa grass may be reduced by the addition of certain carbohydrates. The availability of proteins may be limited by pectin-rich sources (Gao *et al.*, 2012). The fermentation process can be adversely affected by a rapid increase in sugar levels, leading to a reduction in fiber digestibility.

3.2. Cowpea Silages

Cowpea is a highly cultivated legume crop that offers numerous environmental and economic benefits while enhancing the diets and livelihoods of farming families in Africa, Asia, and South America (Andrade *et al.*, 2017). The available research on the inclusion of various carbohydrate sources as silage additives in cowpeas is rather scarce. Andrade *et al.* (2017) evaluated the nutritive value, fermentation characteristics, and aerobic stability of silage produced from a blend of discarded apples (85%) and cowpea stover (15%) that had been ensiled for 45 or 60 days. The findings indicated that the mixture of discarded apples and cowpea stover can create nutritious and stable silage through the ensiling process, thereby providing a novel feed source for livestock and utilizing agro-industrial byproducts. Previously, the same research team conducted another study that focused on evaluating cowpea straw (15% fresh) treated with *Pleurotus citrinopileatus* and discarded apples using a commercial inoculant. The findings revealed that utilization of the commercial inoculant resulted in a significant increase in the quantity of lactic acid bacteria, ultimately establishing a high ensilability potential (Andrade *et al.*, 2021). In conclusion, research on cowpea silage has demonstrated that incorporating discarded apples into cowpea stover improves its ensilability, enhances lactic acid production, and reduces pH. Additionally, the inclusion of an inoculant further promotes fermentation, increases lactic acid bacteria, and increases the levels of water-soluble carbohydrates and ethanol in silage.

3.3. Vetch Silages

Vetch, particularly the common vetch (*Vicia sativa* L.), is important in ruminant nutrition because of its potential as a valuable feedstuff. Common vetch is recognized as a multipurpose crop that can be utilized as green forage, dry forage, forage meal, silage, and haylage, making it a versatile resource for ruminants (Mikić *et al.*, 2014). Similar to other legume forages, studies have explored the effects of adding various carbohydrate sources to silage. Chen *et al.* (2015) investigated the impact of substituting whole-plant corn with oat and common vetch on the fermentation quality, chemical composition, and aerobic stability of total mixed ration (TMR) silage in Tibet. All TMR silages were well preserved, with high lactic acid content and low pH. Furthermore, increasing the proportion of oats and common vetch resulted in increased crude protein and ether extract, while reducing the fiber content in the silages. These findings suggest that oats and common vetch can serve as effective roughage sources

to produce high-quality TMR silage. Another study investigated the silage properties of Hungarian vetch and barley grass mixtures ensiled in various proportions. The treatments included 100% Hungarian vetch, 100% barley, 75% vetch + 25% barley, 50% vetch + 50% barley, and 25% vetch + 75% barley. The results showed that sole barley silage had the best physical properties, whereas 75% vetch + 25% barley had optimal chemical properties, with the highest crude protein and lowest fiber content (Bengisu, 2019). Turan (2020) evaluated the chemical composition and silage quality of mixtures of Hungarian vetch with wheat or barley at varying ratios, from 80% vetch + 20% cereal to 20% vetch + 80% cereal. The vetch-barley mixtures had higher crude protein, calcium, phosphorus, potassium, and relative feed values than the vetch-wheat mixtures. The fiber content was lower in the barley mixtures. Increasing the vetch ratio improved the protein content but increased the pH. Overall, the vetch-barley mixtures produced superior quality silage over vetch-wheat, with the best nutritional composition being 75% vetch + 25% barley. In summary, researchers found that cereal grains such as wheat, barley, and oats can enhance the dry matter and nutrient density of silage, improve protein quality, reduce fiber content, and improve aerobic stability in vetch silages. However, a higher cereal grain content can negatively impact rumen fermentation and decrease calcium, phosphorus, and potassium levels compared to pure vetch. This process can be partially replaced with corn owing to increased lactic acid bacteria.

3.4. Soybean Silages

The cultivation of soybeans is principally aimed at capitalizing on their substantial seed protein and oil content. However, recently, there has been a surge in interest in incorporating soybeans as forage crops (Kulkarni *et al.*, 2018). Lima *et al.* (2010) evaluated the effect of combined ensiling of two sorghum varieties with soybean, with or without molasses and lactic acid bacteria inoculant addition, on silage quality and *in vitro* rumen fermentation. Another study showed that mixing forage soybean with crop corn or sorghum lowered the pH of the silage to 3.5-3.8, below the pH of 4.5 for silage made solely from forage soybean mostly due to improvement of *Lactobacillus* population in the groups supplemented with crop corn and sorghum. Budakli Carpici (2016) examined the quality and properties of silages made from mixtures of maize and soybean ensiled at different ratios. The results showed that increasing the proportion of soybean in the mixtures decreased dry matter content but increased pH, crude protein content, and acid detergent fiber content of the silages. Silages with soybean proportions over 50% had higher pH values indicating inferior fermentation. The best quality silages with pleasant smell and color were obtained from mixtures with at least 50% maize content. Based on the determined silage characteristics, the study concluded that the optimal maize to soybean ratio for quality silage production is 50% soybean + 50% maize as this provides sufficiently high protein content while maintaining good fermentation qualities. Overall, the study concluded that ensiling forage soybean together with crop corn or sorghum could improve the quality of forage soybean silage (Ni *et al.*, 2018). In general, adding carbohydrate supplements like molasses, sorghum, or corn to soybean silages enhances the fermentation quality, aerobic stability, and nutritional value of the silages produced.

4. Conclusion

The most efficient and economical approach to preserving green legume forage is through ensilaging while simultaneously maintaining their nutritional content. The chemical composition and nutritional value of legumes present a challenging factor in terms of preservation, as ensilaging and supplementing legume forage silage with various carbohydrate sources is necessary. As per the research conducted on various legume forage silages, it has been found that incorporating grains, pomaces, or molasses into legumes enhances lactic acid production and preservation, while reducing fiber content and stimulating microbial activity. However, excessive dilution can lead to a decrease in protein and mineral content, negatively affect rumen function, and increase the risk of spoilage. The benefits are best realized at partial replacement rates of 20-40% for grains. Additionally, the impact of different carbohydrate sources on silage varies with varying levels of inclusion at optimal rates. It would be beneficial to investigate the impact of alternative carbohydrate sources other than alfalfa in legume silages in future research. This would provide useful insights and understanding.

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Determination of Calf Development Pasture and Intensively Fed Anatolian

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It was aimed to investigate the calf growth performance of pasture and intensively fed buffalos and preliminary data were evaluated. For this purpose, 40 Anatolian Buffaloes were divided into 2 groups in the enterprises included in the National Anatolian Buffalo Breeding Project carried out in Bartın Province. The pasture group was fed on pasture from May to October and 4 kg/day concentrate feed was given additional. The calves were fed mother's milk and growth fed ad-libitum for 4 months from birth, and after 4 months of age, they weaned and grazed on the pasture with their mothers. The pasture had an average fresh grass yield of 1,700 kg/da and 21.56% DM on May. When made in July, it yielded 1,155 kg/da and has 32% DM. It has a yield of 480 kg/da and 34.75% DM in September. Ash, NDF and ADF contents of pasture grass in May, July and September, respectively; 8.39 – 28.16 – 12.23%, 58.34 – 70.13 – 65.08% and 33.49 – 42.31 – 34.07%. The intensive group, fed with a mixture of forage and concentrated feed (50/50) under operating conditions. Calves were suckled milk and growth feed for 4 months and weaned at the age of 4 months. Buffaloes were fed totally 10 kg feed consisting of wheat straw, wheat bran and concentrate feed. As a result of the study, birth and 6-month live weights of calves in pasture and intensive groups, respectively; 32.57±0.37 - 34.57±1.26, 136.57±0.77 - 136.99±1.21 kg. There was no difference between the groups in terms of birth weight (P>0.05). Statistical analysis was not performed because the number n was insufficient in terms of six-month age. In the light of the data obtained from the study, there was no difference in terms of calf birth weight of the buffaloes fed on pasture and intensively.

Keywords: Anatolian Buffalo, Calf, Intensive, Pasture

Optimizing Poultry Production through Microencapsulated Feed Additives

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The intestinal microbiota plays a crucial role in nutrient digestion, absorption, and overall health of poultry. Strategies to modulate the intestinal microbiota have been explored to optimize nutrient utilization and improve performance in poultry production systems. The application of microencapsulated feed additives in poultry production has demonstrated favorable effects on nutrient utilization, growth performance, and environmental sustainability through improved nutrient absorption and reduced excretion of undigested nutrients. The aim of this review is to explain the process and purpose of microencapsulation in feed additives for poultry nutrition, and which feed additives effectiveness were improved after microencapsulation. Several feed additives could be microencapsulated such as essential oils, organic acids, organic acid salts, prebiotics, probiotics. Microencapsulation offers a significant benefit in enhancing the stability of the encapsulated bioactive compounds, thereby preserving their integrity and efficacy in the gastrointestinal tract. Controlled release is another significant advantage of micro-encapsulation. The encapsulation process provides a barrier that controls the release of active ingredients, allowing for sustained and prolonged effects. Furthermore, microencapsulation enables targeted delivery of bioactive compounds to specific sites in the gastrointestinal tract. Encapsulated additives can be designed to release their contents in specific regions of the intestine, where they can exert their beneficial effects. In conclusion, the use of microencapsulated feed additives in poultry nutrition offers a promising approach to enhance nutrient utilization, improve performance, and promote the overall health of poultry. The encapsulation process provides a protective barrier for bioactive compounds, ensuring their targeted delivery to the intestine. By modulating the intestinal microbiota and improving nutrient absorption, microencapsulated feed additives have the potential to optimize poultry production systems and contribute to sustainable and efficient poultry farming.

Keywords: Feed additives, Microencapsulation, Poultry nutrition

1. Introduction

The poultry sector plays a crucial role in meeting global demand for meat and ensuring food security because of its good nutritional quality and low production costs (Babot *et al.*, 2023). One of the important factors to increase meat or egg yield is to improve intestinal absorption of nutrients by improving microflora or enhancing microvillus development using feed additives, including antimicrobial agents (Stamilla *et al.*, 2020). On the other hand, increasing concerns about using antibiotics and anticoccidial agents as growth promoters in poultry nutrition have led researchers to find new natural alternatives (Galli *et al.*, 2021). The increasing attention towards phytochemicals in poultry nutrition research in recent years stems from a growing body of studies demonstrating various benefits associated with the consumption of these natural compounds (Moharreri *et al.*, 2022). It is estimated that different organic feed additives, such as plant extracts, essential oils, probiotics, prebiotics, symbiotics, exogenous enzymes, antimicrobial peptides, isolated plant components, and organic acids, might be potential alternatives to antibiotic growth promoters by replacing antibiotics in poultry production (Galli *et al.*, 2021; Gyawali *et al.*, 2022). However, these natural feed additives may lose their effectiveness during storage or transport when exposed to high temperatures, oxygen levels, humidity, or in the gastrointestinal system of poultry by lowering the pH of gastric secrets, enzyme activity, etc. (Gyawali *et al.* 2022).

Microencapsulation, which is widely used in the pharmaceutical and food industries, is an important process that involves the formation of very small particles with a coating wall to avoid chemical and physical reactions, while maintaining the properties and stability of the encapsulated compounds (Moharreri *et al.*, 2022; Poshadri and Kuna, 2010). Additionally, the use of encapsulated feed additives can improve feed efficiency, nutrient digestion, and immune enhancement in poultry (Moharreri *et al.* 2022). This technology increases the effectiveness of feed additives by delaying their rapid degradation in the upper gastrointestinal tract (Stamilla *et al.* 2020).

The objective of this review is to elucidate microencapsulation technology and techniques, as well as their significance in the poultry feed additive industry. Furthermore, the advantages of utilizing microencapsulation methods as feed additives in poultry nutrition will be examined and analyzed.

2. Microencapsulation Process in Feed Additives

Microencapsulation, a widely adopted technique in the food and feed additive industry, is a valuable method for preserving and stabilizing functional compounds. Within this process, the internal content is referred to as the core, whereas the outer layer is termed a wall or shell (Poshadri and Kuna, 2010). Notably, the core may contain one or more ingredients and exist in solid, liquid, or gaseous states (Bakry *et al.*, 2016; Poshadri and Kuna, 2010; Singh *et al.*, n.d.). The scale of encapsulation can vary, encompassing nanoencapsulation (200 nm), microencapsulation (200–5000 nm), and macroencapsulation (greater than 5000 nm) (Öztürk and Temiz, 2018). The parameters of microcapsules, including their form and dimensions, are generally determined by the coating substance and method of application utilized (Poshadri and Kuna, 2010). The choice of the coating material has paramount significance, exerting a substantial impact on the solidity of the droplets, core material stability, and the ability to control discharge under specific conditions. A diverse array of materials find applications in encapsulation, including proteins such as gelatin and albumin; carbohydrates such as starch, dextran, and sucrose; lipids, gums, and cellulose; and both non-biodegradable and biodegradable polymers (Trilokia *et al.*, 2022). The meticulous selection of these materials aligns with the desired characteristics of microcapsules, emphasizing the factors crucial for achieving optimal functionality and performance in various applications.

Microencapsulation methods have numerous advantages for use as food and feed additives. This technique could be effective in preserving bioactive compounds such as polyphenolic extracts and natural antimicrobials, which inhibit microbial growth (Calderón-Oliver and Ponce-Alquicira, 2022). Moreover, microencapsulation serves as a versatile tool that allows the conversion of liquid food components into more manageable solid forms. It plays a pivotal role in safeguarding sensitive food constituents, minimizing nutritional losses, and preserving flavors and fragrances, thereby contributing significantly to extending the shelf life of food products (Yuni Hendrawati *et al.*, 2019). In the context of feed additives, microencapsulation has been demonstrated to enhance the absorption of organic acids and other additives in poultry intestines (Gyawali *et al.*, 2022; Johnson *et al.*, 2023). Notably, this method acts as a protective shield, safeguarding the core material from the potentially corrosive effects of the gastrointestinal system, including variations in the pH, digestive acids, and enzymes (Ayoub *et al.*, 2019). The encapsulation of bioactive substances in microscopic particles enables their controlled release throughout the digestive process (Calderón-Oliver and Ponce-Alquicira, 2022). Microencapsulation enhances the taste and odor of the core substance (Hendrawati *et al.*, 2019).

Various methods can be used for microencapsulation, including spray drying, freeze drying, emulsification, coacervation, extrusion, fluidized-bed coating, and supercritical fluid technology (Bakry *et al.*, 2016). Spray drying is recognized for its efficiency, practicality, and speed. This method involves dissolving the core material, atomizing it into a heated chamber, and rapidly drying the sprayed droplets using a hot gas (Rajendran *et al.* 2022). Emulsification is a process by which oil droplets are dispersed in a polymer emulsifier, creating a mixture of two immiscible liquid phases. Droplets, typically 0.1 to 100µm in diameter, form layers in food products owing to their tendency to combine (Yuni Hendrawati *et al.*, 2019). Coacervation, also known as phase separation, is a microencapsulation technique characterized by complete entrapment of the core material within a matrix. This process is achieved by the precipitation of a colloidal phase from an aqueous phase (Poshadri and Kuna, 2010).

3. Using Microencapsulated Feed Additives On Poultry Nutrition

Several feed additives, such as prebiotics, herbs, and probiotics, have been shown to improve nutrient utilization, growth performance, and overall health of poultry (Agusetyaningsih *et al.*, 2022; Babot *et al.*, 2023; Stamilla *et al.*, 2020). Assessment of the efficacy of microencapsulation for feed additives was conducted on an individual basis in the following paragraphs.

3.1. Microencapsulated Organic Acids

Organic acids, including those with one to seven carbon atoms, such as formic, fumaric, propionic, lactic, and sorbic acids, exhibit diverse biological properties including antimicrobial, antioxidant, and immunostimulatory effects (Galli *et al.* 2021; Lee *et al.* 2020). The microencapsulation of organic acids in poultry nutrition is the preferred approach for several reasons.

The primary motivation for microencapsulating organic acids is to enhance their efficacy against pathogenic microorganisms, providing an alternative to antimicrobial and anticoccidial growth promoters. Galli *et al.* (2021) evaluated the effects of supplementing broiler chicken feed with a microencapsulated blend of organic acids (including formic, acetic, butyric, propionic, phosphoric, and lactic acid) as an alternative to antibiotic and coccidiostat growth promoters. Overall, the microencapsulated organic acid blend was able to maintain performance, control bacteria/coccidia, improve gut health, and positively affect meat quality when included as an

alternative to antibiotics and coccidiostat feed additives. Another study conducted in the broilers evaluated the effects of supplementing broiler chicken diets with a microencapsulated blend of organic acids (citric and sorbic) and essential oils (thymol and vanillin) on growth performance and gut health (Stamilla *et al.*, 2020). The authors concluded that supplementing broiler diets with a microencapsulated blend of organic acids and essential oils could offer favorable perspectives for maintaining performance and gut health as alternatives to antibiotic growth promoters. evaluated the effects of a microencapsulated blend of organic acids (citric and sorbic acids) and botanicals (thymol and vanillin) on cell signaling pathways in the jejunum and ileum of 15-day-old broiler chickens.

Another advantage of microencapsulating organic acids and their salts is that they improve their absorption from poultry intestines. Van Den Borne *et al.* (2015) found that uncoated butyric acid mostly absorbed in the upper parts of the gastrointestinal system. In contrast, fat coating significantly reduced proximal oxidation to 45%, indicating an extended release of coated butyrate throughout the intestinal tract. The findings of this study indicate that fat coating serves to safeguard butyrate from premature elimination, thereby facilitating its absorption throughout the entire length of the intestine in broilers. Another study evaluated uncoated and microencapsulated butyric acid, alone or in combination, as alternatives to antibiotics in broiler chickens challenged with *Eimeria* spp. (Ventura *et al.*, 2021). The results revealed that encapsulating butyric acid in microscopic form was more advantageous for its performance within the initial 3 weeks (1-21 days) in contrast to the uncoated butyric acid. While butyric acid improved some measures of intestinal morphology, it did not improve performance compared to that of antibiotic-fed birds. In summary, research has shown that encapsulated organic acid supplementation boosts the gut microbial populations, improves carcass yield and breast meat quality, and protects organic acids from dissociation. Encapsulation enhances its efficacy as an alternative antibiotic.

3.2. Microencapsulated Essential Oils

Essential oils are volatile compounds extracted from plants or plant parts via pressing and distillation. It is possible to utilize essential oils as an alternative to antioxidants in poultry nutrition, with the added benefit of reduced toxicity and the absence of residual traces (Stamilla *et al.*, 2020). Essential oils can also be used as natural alternatives to antimicrobial and anticoccidial agents in poultry nutrition (Lee *et al.*, 2020). Encapsulation of essential oils is particularly important as it helps to reduce volatilization and protects the oils from environmental factors such as radiation, humidity, and oxygen, thereby ensuring their effectiveness (Ribeiro *et al.*, 2015). It also improves the effectiveness of essential oils in the gastrointestinal systems of birds. Hafeez *et al.* (2016) reported that 100 mg/kg matrix-encapsulated essential oil blend (carvacrol, thymol and limonene) had better feed efficiency, and ileal absorption of crude protein than control and 150 mg/kg powdered form of menthol and anethole blend. Their findings suggested that the addition of these essential oils led to improved digestibility, which may be attributed to the increased secretion of digestive enzymes.

Microencapsulation further enhances the effectiveness of essential oils against microorganisms and coccidiosis. Lee *et al.* (2020) evaluated encapsulated essential oils containing thymol and carvacrol as alternatives to anticoccidials in broiler chickens challenged with a high dose of coccidiosis vaccine. These results indicate that encapsulated thymol/carvacrol essential oils can counteract coccidiosis vaccine-induced growth depression and improve physiological responses, demonstrating their potential as alternatives to anticoccidial agents. In another study, microencapsulated essential oils containing thyme, peppermint, savory, and black pepper extracts were evaluated as natural feed additives in broiler chickens experimentally infected with *Salmonella enteritidis*. Overall, the encapsulated essential oil mixture exhibited beneficial effects on the growth, gut health, and immunity of *Salmonella*-challenged broilers, thereby demonstrating its potential as a photobiotic alternative to antibiotics. Encapsulating essential oils in poultry improves growth performance, reduces bacterial counts, lowers blood lipid levels, and increases the number of beneficial bacteria. They also improve immune responses, leading to higher antibody titers against vaccines.

3.3. Microencapsulated Probiotics

Probiotics are live microorganisms that, when administered in appropriate amounts, provide health benefits to the host in a safe and efficient manner. However, some probiotics may not work as intended because microorganisms may not tolerate gastrointestinal digestion well (Babot *et al.*, 2023; Dong *et al.*, 2019). Microencapsulation of probiotics could potentially be a highly effective solution for safeguarding these microorganisms so that they can successfully navigate the upper gastrointestinal system of poultry and ultimately reach the intestines without being adversely affected (Babot *et al.*, 2023; Zhang *et al.*, 2019). Zhang *et al.* (2019) compared the effectiveness of free, pre-encapsulated, and pro-encapsulated *Enterococcus faecalis* on growth performance, blood characteristics, and cecal microflora in broiler chickens. The authors found that pre-encapsulation enables better probiotic functionality and efficacy compared to other forms, improving growth, immunity, and gut health in broilers.

Probiotics can also be accepted as alternatives to antimicrobial growth promoters in broilers. Wang *et al.* (2018) found microencapsulated probiotic (*Enterococcus faecium*, *Lactobacillus plantarum* and *Bacillus subtilis*) and prebiotic (β -mannose and ructo-oligosaccharide) blend could significantly increase average daily gain, immunoglobulin A, interleukin-2 levels and total antioxidant capacity in blood serum and ceecal *Lactobacilli* counts of the birds at day 42. These results suggest that the microencapsulated probiotic and prebiotic blend can be used as an alternative to antibiotics. Similarly, another study conducted in broiler chickens challenged with *Escherichia coli* K88 to evaluate the effects of microencapsulated *Enterococcus faecalis* probiotics and *Camellia oleifera* seed extract supplementation showed that microencapsulated *E. faecalis* probiotics and *C. oleifera* seed extract can alleviate the negative impacts of *E. coli* challenge and enhance growth, immunity, and gut health in broilers. Moreover, Wu *et al.* (2023) investigated the effects of microencapsulated probiotics containing *Lactobacillus crispatus*, *L. johnsonii*, and *Pediococcus acidilactici* on growth performance, immune response, and gut health in *Salmonella typhimurium*-challenged broiler chickens. These results also suggest that microencapsulated probiotics have the potential to substitute antibiotics and enhance disease resistance in poultry production.

In summary, microencapsulated probiotics in poultry improved gut health by reducing pathogenic bacteria, increasing beneficial microflora, modulating cytokine levels, and improving antioxidant activity in tissues, leading to increased serum antibody levels and improved performance.

3.4. Microencapsulated Plant Extracts and Polyphenols

Plant extracts contain many phenolic compounds, such as antioxidants and growth promoters, that can affect metabolism (Agusetyaningsih *et al.* 2022). These extracts have been recognized for their potent antibacterial, antiseptic, anti-inflammatory, nematocidal, and immunomodulatory properties, which can help enhance the well-being and immune system of poultry (Meimandipour *et al.*, 2017). However, one of the primary difficulties associated with using plant extracts is maintaining their stability and preventing oxidation during storage (Agusetyaningsih *et al.* 2022). Microencapsulation shields plant extracts from the deleterious effects of the environment and light while simultaneously preserving their taste and aroma. This procedure not only enhances the bioaccessibility of plant extracts and polyphenols, but also safeguards them from biodegradation before reaching the intestines, thereby promoting their digestibility (Vasilopoulos *et al.*, 2022). According to studies on the use of microencapsulated plant extracts in poultry nutrition, these phenolic compounds can enhance poultry nutrition by improving meat quality, fatty acid composition, growth performance, immune function, gut microbial populations, intestinal morphology, stress reduction, and blood lipid levels. This resulted in improved growth performance, increased antibody production, and reduced pathogens. Additionally, it can improve intestinal morphology, reduce stress, and lower blood lipid levels.

4. Conclusion

According to studies conducted in broilers and other poultry, microencapsulation enhances the stability and controlled release of bioactive compounds, allowing targeted delivery to specific sites in the intestine. The use of microencapsulated feed additives offers a promising approach to modulate the intestinal microbiota and improve nutrient absorption, ultimately contributing to sustainable and efficient poultry farming and increasing the antioxidant activity of feed additives. These studies also demonstrated the potential of natural feed additives as alternatives to antibiotic growth promoters and the benefits of microencapsulation in preserving their effectiveness. More research is needed to evaluate the mechanisms of action of these additives in poultry include modulating intestinal microbiota, influencing nutrient digestion/absorption, and enhancing immunity. Shelf life stability and release kinetics of these additives can be evaluated over time.

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Determination of Lactation Parameters and Shape of Anatolian Buffaloes for Three Lactations

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This research was conducted to determine the lactation parameters and shape of three lactations in Anatolian Buffaloes. For this purpose, 3126 test day yield records of 521 head buffalos that birth in 2016 in Tokat province were used. Lactation curve parameters (a, b and c) were determined using the Statistica [16] package program, as a result of the Levenberg-Marquardt iteration process. During the iteration, 1.0E-8 was used as the convergence criterion. While the a parameter was determined as 3.92, 5.29 and 5.90 for the 1st, 2nd and 3rd lactations, the b parameter was determined as 1.10, 0.89 and 0.84, respectively. While the c parameter was determined as 0.335, 0.316 and 0.320 for the 1st, 2nd and 3rd lactations, the R² value was calculated as 0.966, 0.991, 0.982, respectively. As a result, using the parameters in this study estimated with the Wood model as criteria in breeding studies will contribute to success in this herd.

Keywords: Anatolian buffalo, Model, Parameter, Selection, Wood

Bio – Economic Modeling Approach in Livestock Enterprises

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Bioeconomy refers to the sustainable utilization of biological resources for food, energy, and commodity production. It involves the integration of technology, biology, and economics to create a more sustainable and efficient system. The bioeconomic approach in agriculture plays a significant role in addressing global issues like food security, climate change, and resource scarcity. It offers sustainable solutions that balance economic growth with environmental management. It's an approach aimed at analyzing and improving the economic value of farm animals. It integrates technologies such as precision agriculture, genetic enhancement, and waste management to create sustainable and profitable systems. Bioeconomic modeling is an analytical method that combines biological and economic factors for making economic decisions. Prominent models include WaNuLCAS, HyPAR, GRAZPLAN, and FLORES modeling systems. Through bioeconomic modeling, detailed results are obtained by simulating agricultural and ecological processes such as agriculture, livestock farming, crop production, forestry, and pasture management. The outcomes of bioeconomic modeling aid farm managers in making better decisions while enhancing the economic and environmental sustainability of livestock operations. It ensures more efficient use of resources like raw materials, energy, and water and prepares businesses for risks like natural disasters and economic fluctuations. In this study, the importance and methods of bioeconomic modeling, particularly in large livestock farming, have been analyzed in the light of current scientific literature, and examples from studies utilizing bioeconomic modeling have been compiled. As a result, it's evaluated that bioeconomic models have a significant impact on productivity, sustainability, and profitability in livestock operations and further research is deemed necessary for its wider dissemination.

Keywords: Bioeconomic model, Economic evaluation, Economic values, Livestock

Growth and Development Characteristics of Karakaş Breed Lambs

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This study was carried out between 2018 and 2023 in sheep farming enterprises in Çermik district of Diyarbakır province. The animal material of the research was examined within the scope of the Public Breeding of Karakaş Sheep project, which is being carried out in Diyarbakır province under the coordination of the The birth and weaning weights of lambs and their daily live weight increases were examined. With this study, it was aimed to increase the productivity of Karakaş lambs raised in Diyarbakır province in terms of growth and development characteristics. The least squares method was used to determine the effect of age, gender and birth type on birth and weaning weight of Karakaş lambs. The average birth weights of Karakaş Lambs for the years 2018, 2019, 2020, 2021, 2022 and 2023 are respectively; It was determined that the birth weights were 4.36, 4.35, 4.25, 4.21, 4.17 and 4.21 kg, female lambs were 4.14 kg, male lambs were 4.38 kg, and single lambs were 4.31 kg and twin lambs were 3.76 kg. The 90th day live weights are again averaged over the years, respectively; It was determined to be 19.15, 17.17, 18.31, 18.15, 16.21 and 21.21 kg, 18.44 kg for single-born lambs and 17.69 kg for twin lambs. As a result of the research, it was observed that there was wide variation in terms of growth and development both in the herds owned by the project breeders and within the herd. By taking advantage of this variation, genetic progress can be achieved in line with the data obtained in the herds through selection based on productivity characteristics.

Keywords: Birth, Karakaş, Lamb

Cattle Population in Eastern Anatolia and Southeastern Anatolia Regions

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Cattle Farming in Turkey is commonly practiced in various regions, with the Marmara, Aegean, Mediterranean, and Black Sea regions being among the most suitable areas for this activity. These regions offer vast grazing lands and favorable climate conditions. Furthermore, cattle farming holds a significant place in Turkey's agricultural sector and is economically important. According to the 2022 data from the Turkish Statistical Institute (TÜİK), in the fourteen provinces of the Eastern Anatolia Region, there are a total of 742,160 purebred cattle, 2,282,306 crossbred cattle, 248,907 native cattle, and a total of 3,273,373 cattle, in addition to 24,378 buffalo. In the nine provinces of the Southeastern Anatolia Region, there are a total of 313,980 purebred cattle, 842,817 crossbred cattle, 279,170 native cattle, and a total of 1,435,967 cattle, along with 17,415 buffalo. In the fourteen provinces of the Eastern Anatolia Region, the presence of these cattle is calculated as follows concerning the national livestock population: 8.95% of the national purebred cattle (742,160 out of 8,295,825), 31.16% of the national crossbred cattle (2,282,306 out of 7,324,866), and 23.79% of the national native cattle (248,907 out of 1,046,104). In conclusion, based on the 2022 data from the Turkish Statistical Institute (TÜİK), the Eastern Anatolia and Southeastern Anatolia regions in Turkey are significant contributors to the national livestock population. These regions host a substantial number of cattle, including purebred, crossbred, and native cattle, as well as buffalo. In the Eastern Anatolia Region, the contribution to the national livestock population is notable, with approximately 8.95% of purebred cattle, 31.16% of crossbred cattle, 23.79% of native cattle, and 14.19% of buffalo. Similarly, in the Southeastern Anatolia Region, there is a substantial presence of cattle, accounting for approximately 3.78% of purebred cattle, 11.51% of crossbred cattle, 26.69% of native cattle, and 10.13% of buffalo in the national livestock population.

Keywords: Animal existence, Cattle, Data, Eastern Anatolia, Southeastern Anatolia

Effects of Calving Age and Calf Sex on Colostrum Composition and Its Changes After Calving in Anatolian Buffaloes

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Colostrum is the first natural food for the newborn calf. Colostrum with high immunoglobulin (Ig) concentration provides passive immunity to the calf. The aim of this study was to investigate the changes in colostrum. Twenty-five newly calved Anatolian buffaloes raised under similar farm conditions composition which were breeding in the scope of the project of improvement of Anatolian buffalo in public hand supported by General Directorate of Agricultural Research and Policies in Amasya province of Türkiye were used in this study. Colostrum samples were collected at calving (first milk) (T0), 24 (T24), 48 (T48) and 72 h (T72) after calving in summer season. Post-partum time, calving age, and calf sex were assessed as non-genetic factors. It was found that the fat, solids-not-fat (SNF), protein and lactose were significantly higher in T0. Colostrum weight was the lowest in T0 and T24 and then increased gradually T48 and T72 ($P<0.05$). The effect of calving age and sex of calf on colostrum weight, fat, SNF, protein and lactose were not significant ($P>0.05$). In general, postpartum milking was major factors for alteration of chemical composition of buffalo colostrum. This is why it may be suggested that good quality colostrum should be provided for calf health as soon as possible.

Keywords: Anatolian buffaloes, Colostrum, Fat, Lactose, Protein

Growth of Awassi Sheep in Şanlıurfa Province

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The purpose of the study was to examine the growth of Awassi sheep under small-scale production systems in Şanlıurfa, Türkiye. Data were investigated on 17111 lambs from 2020 to 2023 in two sub-projects of Genetic Improvement of Awassi sheep that have been supported by the General Directorate of Agricultural Researches and Politics (TAGEM) in the scope of National Genetic Improvement Project of Small Ruminant Under Farmer Condition. Average live weight of lambs at birth (BW0), 2 (BW60) and 4 (BW120) months of age were 3.33 ± 0.016 , 15.95 ± 0.107 , 24.98 ± 0.051 kg, respectively. Weights in all ages were significantly ($P < 0.05$) affected by project, flock, year and season of birth, birth type, age of mother and sex of lamb. Therefore, large phenotypic variance was observed in terms of growth of lambs before and after weaning.

Keywords: Awassi sheep, Environment, Growth traits

Evaluation of Productivity Characteristics of Karacabey Merino in Balıkesir Province Between 2016 and 2020 (3rd Five Years) within The Scope of The National Sheep and Goat Breeding Project

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Improving the national meat yield aspect of Karacabey merino. The project was conducted with 5,813 heads of Karacabey Merino sheep in farm conditions, approximately 755 head of elite and 5,058 head of regular sheep were used. In the project, lambs born were subjected to performance tests according to their birth weight (1st weighing) and 90th day live weight (2nd weighing) According to the calculation of the performance tests' results, their breeding values were calculated. Weaning weight (day 90) was found to be 28.78 kg in a five-year average. On the general average, this shows an increase of more than 200 grams when the second five-year data of the project is compared with the data for the central districts

Keywords: Balıkesir, Breeding, Karacabey Merino, Productivity characteristics

Investigation of Some Parameters of Cocons Obtained from Commercial Hybrid (MxN) Silkworms Fed in Different Numbers

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Silkworm breeding in Anatolia started about 1500 years ago and continues today with a minor extent. Silkworm breeding, an agricultural activity, is very important because it supports rural development, does not require too much investment, and offers silk, which is a value-added product to economy. In addition, silkworm is an important gene source. This study was carried out under institute conditions within International Center for Livestock Research and Training. In the proposed project, the effects of different feeding program on silkworm larvae growth period and the effects of this program on cocoon quality was investigated. The research was designed according to the feeding program, with 3 groups and 3 replications for each group. The project was started with the preparation of feeding chambers in 2021 and the first feeding of silkworm larvae obtained from Kozabirlik. The research was designed according to the feeding program, with 3 groups and 3 replications for each group. Within the scope of the project, it was observed that the larvae in the 6 feeding groups completed their biological and physical development. In addition, they secreted silk earlier. The filament length and denier of the cocoons obtained within the scope of the study were for feeding groups 2, 4 and 6, respectively; 1500 ± 25.10 , 1379 ± 28.50 and 1474 ± 25.00 m; 2.49 ± 0.04 , 2.72 ± 0.05 and 2.64 ± 0.04 . As a result, it was determined that the feeding factor had an effect on the growth period and cocoon quality parameters of silkworm larvae.

Keywords: Cocoon yield and quality, Feed, Mulberry leaf, Sericulture

Investihgation of Growth, Birth Type and Sex Ratio Characteristics of Akkaraman – Şavak Lambs in Breeder Conditions

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This study was designed to investigate the growth, birth type and sex ratio characteristics of Akkaraman-Şavak lambs fed under breeder conditions. The research was carried out using a total of 14336 data in 7 residential areas of Tunceli city center and Pertek district between in 2018-220. Birth, 30th, 60th and 90th day live weight of lambs are statistically different from each other ($p < 0.0001$). The highest birth weight was 3.77 ± 0.76 kg in 2019, the highest 30th day live weight was 12.79 ± 2.72 kg in 2020, and the highest 60th and 90th day live weights were 22.72 ± 5.50 and 33.91 ± 8.25 kg in 2018, respectively. When the birth, 30th, 60th and 90th day live weights are examined according to the regions, there is a statistically significant difference between the birth and live weigh between the regions ($p < 0.0001$), the highest birth weight is 3.96 ± 0.87 kg, 30, 60 and 90 in Aşağıgölbahçe. On the other hand, it was determined that the live weights on the first day were 12.86 ± 2.36 , 22.75 ± 4.95 and 32.64 ± 7.72 kg in the Ayazpınar region, respectively. It was seen that the lowest sex ratio of Akkaraman-Şavak lambs was 50.3% in 2019 and the statistical difference ($p < 0.05$) was due to this low rate, while the sex ratio was similar (52.5%) in 2018 and 2020. It was determined that the twinning rate was significantly different from each other between the years ($p < 0.0001$), the highest twinning was 10.2% in 2019 and the lowest twinning was 4.2% in 2018. As a result; The fact that the live weights of Akkaraman-Şavak lambs on the 30th, 60th and 90th days were higher than those reported by the researchers showed that controlled breeding studies carried out under breeder conditions had a significant positive effect.

Keywords: Birth type, Breeder condition, Growing performance, Lamb sex, Şavak lamb

Growth Characteristics, Mohair Yield and Fineness of Angora Goats Raised in Ankara Province

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This study was carried out to investigate the growth characteristics of Angora goat II raised in farmer condition in Ankara province supported by the General Directorate of Agricultural Researches and Politics (TAGEM) in the scope of the National Small Ruminant Improvement Project Under Farmer Condition. In the current project, data are being followed in 12 existing flocks in the districts of G d l and Kızılcahamam in Ankara. This study was based on data obtained during 2022 from the Ankara goat II herds in Ankara within the the National Sheep and Goat Improvement Project. Within the scope of the project the goat mohair yield, birth weight, 90th day body weight, and lint fineness data are taken routinely in these flocks. Based on obtained data, the mean lint yield 1.86 kg (n=5078), lint fineness 27.65 μ (n=1138), average birth weight 2.36 kg (n=5294) and 90th day body weight 10.86 (n=2894) was determined. The results from the research show that the applied breeding program has a significant effect on the mohair fineness (35.58 μ) of Angora goat for 5 years (p <0.01).

Keywords: Angora goat, Growth characteristics, Breeding, fineness

Lactation Characteristics of Awassi Sheep in Şanlıurfa Province

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The purpose of the study was to examine the growth of Awassi sheep under small-scale production systems in Şanlıurfa, Türkiye. Data were investigated on 17111 lambs from 2020 to 2023 in two sub-projects of Genetic Improvement of Awassi sheep that have been supported by the General Directorate of Agricultural Researches and Politics (TAGEM) in the scope of National Genetic Improvement Project of Small Ruminant Under Farmer Condition. Average live weight of lambs at birth (BW0), 2 (BW60) and 4 (BW120) months of age were 3.33 ± 0.016 , 15.95 ± 0.107 , 24.98 ± 0.051 kg, respectively. Weights in all ages were significantly ($P < 0.05$) affected by project, flock, year and season of birth, birth type, age of mother and sex of lamb. Therefore, large phenotypic variance was observed in terms of growth of lambs before and after weaning.

Keywords: Awassi sheep, Environment, Lactation characteristics

Investigation of the Structural Characteristics of Central Anatolian Merino Sheep Farms and Effectiveness of the breeding Project in Ankara Province

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The aim of this research is to determine the general characteristics and care and feeding practices of 33 Central Anatolian Merino sheep farms in Ankara within the scope of the "Public Breeding National Project" coordinated by TAGEM. Within the scope of this purpose, a survey consisting of a total of 78 questions was conducted with the business owners. The questionnaire consists of questions about general information about the farmers and farms, herd management, determination of the care and feeding methods of the animals and the effectiveness of the breeding project. At the end of the study, it was determined that 60% of the producers were between the ages of 41-50, 90.91% of them were primary and secondary school graduates, and all of them kept regular records for herd management. Sheep breeders interviewed that they do supplemental feeding (approximately 34%) before ramming and that they are milking by hand. Sheep breeders stated that they gained the habit of keeping records thanks to the breeding project, lamb rearing and breeding selection were made more effectively, so they benefited positively from the project. In addition, it was determined that the breeders wanted to stay in the project and wanted the project to continue.

Keywords: Breeding project, Sheep farms, Structural features

Growth and Development Characteristic of Mahalli Goats Cubs Raised in Diyarbakır Rural Area

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It is aimed to increase the yield amounts of some of the species within the scope of the project through selection and breeding. In this study, the growth, development and fertility information of goat cub born on the 60th and 90th days of birth, within the scope of the Local Goat Breeding project carried out in Diyarbakır province, Çınar district, under the coordination of the General Directorate of Agricultural Research and Policies, were examined. In the findings obtained regarding the local goat, the average and standard error of the birth weights of the kids according to the years 2022 and 2023 are respectively; 3.09 ± 0.01 , 3.07 ± 0.01 kg, 3.01 ± 0.01 kg in female kids and 3.15 ± 0.01 kg in male kids, 3.1 ± 0.01 kg in single-born lambs and 3.1 ± 0.01 kg in twin lambs in terms of birth type 2.96 ± 0.01 kg was determined. The 90th day live weights are again averaged over the years, respectively; When the 90th day weight was examined in terms of birth type, it was determined as 14.43 ± 0.06 in single-born lambs and 13.96 ± 0.09 kg in twin lambs. When the findings obtained from the studies carried out within the scope of this project are examined; It has been determined that the herds owned by breeders contain highly productive goats. It has been demonstrated that improvements in farm-based productivity can be achieved by keeping these goats within the herd.

Keywords: Birth weight, Growth cub, Mahalli goat, Twin

Determination of Some Fertility and Growth Characteristics of Akkaraman Breed Sheep Raised in Konya Province: The Example of Yağlıbayat Neighborhood

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This study was performed between 2019 and 2023 to investigate reproductive efficiency and growth traits of lambs in Akkaraman breed flocks in the Community Animal Breeding project in Yağlıbayat neighborhood of Karatay district of Konya province. Birth rate, number of lambs born per ewe under ram, number of lambs born per ewe under ram, number of lambs per ewe giving birth, twinning rate and survivability at weaning and birth weight and GCAA, 60th, 120th day live weights and survivability ratio among growth traits were analyzed according to birth type, sex and age of mother. Analysis of variance was performed to determine the effect of year, maternal age, birth type and sex factors on the live weight traits. Tukey multiple comparison test was applied to compare the subgroup averages of the factors with significant effects. Alpha (α) level was taken as 5%. Fertility traits of ewes; average birth rates in 2019, 2020, 2021, 2022 and 2023 were 96.66%, 97.89%, 93.49%, 94.17% and 90.34%; number of lambs born per ewe under ram were 1.009, 1.096, 1.013, 0.999 and 0.971; number of lambs per ewe giving birth were 1.044, 1.120, 1.084, 1.061 and 0.979; twinning rates were 4.37%, 11.91, 8.82%, 4.37%, 11.91%, 8.82% and 0.979%. 971; number of lambs born per ewe, 1.044, 1.120, 1.084, 1.061 and 0.979; twinning rates were 4.37, 11.91, 8.82, 5.92 and 7.35 %; survival rates of lambs at weaning were 93.04, 92.10, 91.16, 90.61 and 91.06 %. The average birth weight of the lambs obtained from the 5-year data set was 4.34 kg, GCAA 0.207g, 60th day live weight 16.51 and 120th day weights 29.31 kg, survival was 91%. Birth weights of single and twin lambs were 4.69 and 3.98 kg, and of male and female lambs were 4.46 and 4.21 kg.

Keywords: Akkaraman, Growth, Reproductive efficiency, Sheep, Survival rate

Estimation of Breeding Value and Genetic Trends for Milk Yield Traits of Simmental Cattle

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This research was conducted to determine the breeding values and genetic trend of Simmental cattle regarding 305-day milk yield. In the study, the 305-day milk yield of Simmental cattle that started lactation between 2007 and 2014 in Gökhöyük State Farm conditions were evaluated. The data were analysed using Minitab [12] statistical program. MTDFREML program was used to estimate the breeding values of Simmental cattle. Genetic trend was determined by regression of values related to 305-day milk yield according to birth years. The genetic trend for 305-day of Simmental cattle were 30.3 kg/year, respectively. While the genetic trend calculated for the years 2008, 2009, 2010 and 2011 was found to be positive, the genetic trend in the other years was found to be negative. Consequently, In the research is using the estimated breeding values in the selection of breeding simmental cattle will increase the success of the selection to be applied in this Simmental cattle herd.

Keywords: Breeding values, Cattle, Genetic trend, Herd, Simmental

Bottleneck Analysis of Brown Stud Bulls Used in Turkey

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Brown Swiss cattle, which is originated from Switzerland, is widely bred all over the world. It is one of the largest cattle breeds in the world, used for milk and beef purposes. In Türkiye, Brown Swiss is also called Esmer or Montofon and it's imported semen is commonly used for artificial insemination. In this study, it was investigated whether the Brown Swiss stud bulls used to inseminate Brown Swiss cow populations in Türkiye were under the bottleneck effect. For this purpose, the material of the study was consisted of semen from 36 Brown Swiss stud bulls that are domestically produced or imported. Genetic evaluation was performed based on microsatellite data of ten loci. The data was cleaned and prepared via R 3.6.3 (R Development Core Team, 2019) statistical software and bottleneck assesment were performed using the Bottleneck v.1.2.02 (Cornuet ve Luikart, 1996) programme based on ten microsatellite loci. First of all, the heterozygosity of the loci were calculated under SMM (Stepwise Mutation Model) and TPM (Two-Phase Mutation Model) mutation models and compared with the results of the current population under Hardy-Weinberg equilibrium. According to these evaluations, excesses of heterozygosity were observed in 7 loci when compared to the estimation under SMM, and in 8 loci when compared to the estimation under TPM. TGLA126 was determined as a monomorphic locus. Four different evaluations were performed for bottleneck analysis, including 2 tests and 2 mutation models. Sign-Test under TPM ($p < 0.05$), Wilcoxon Test under TPM ($p < 0.01$), Wilcoxon Test under SMM ($p < 0.05$) showed statistical significance for the differences, while Sign Test under SMM ($p > 0.5$) showed insignificance. Additionally, according to the Mode-Shift analysis, a deviation from the normal L-shaped distribution was observed. As a conclusion, these results showed that the Brown Swiss stud bulls evaluated were under the bottleneck effect.

Keywords: Bottleneck, Brown Swiss cattle, Microsatellite, Stud bull

Morphological Characterization of Cyprus Fat – tailed Sheep Breed Using Quantitative and Qualitative Trait Analysis

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The loss of genetic diversity in farm animal genetic resources is a growing concern, with many local breeds facing extinction. Local sheep breeds are vital for adapting to local climates, sustainable land management, preserving cultural heritage, supporting research and genetic improvement, and building resilience to climate change. In Cyprus, milk obtained from Cyprus fat-tailed sheep is mainly used to produce one of the most exported dairy products of Cyprus, Halloumi. However, the breed is facing the risk of extinction, mainly due to uncontrolled crossbreeding. This study aims to identify physical and morphological traits of the indigenous Cyprus fat-tailed sheep breed to support its accurate identification and conservation. In the present study, 24 Cyprus fat-tailed sheep in different categories of age were used. About 8 qualitative traits (presence of horn, horn shape, presence of wattle, beard, head shape, coat color, fleece color, and tail type) and 10 quantitative morphological traits (Table 1) like body weight, ear length, head length, withers height, heart girth, chest depth, chest width, body length, hip height, and tail length and width were recorded by the same group of people in order to minimize human errors while collecting. The study found that the majority of female Cyprus fat-tailed sheep (75%) had a white coat color pattern, followed by white-black and brown coat colors at the same rate (12.5%). Males had higher values ($P < 0.05$) for wither height, heart girth, chest width, hip height, foreleg length, compact index, area index, and body weight than females. Morphometric traits were significantly positively ($P < 0.01$) correlated with body weight. In conclusion, the study provides important information for the conservation and breeding programs of the Cyprus fat-tailed sheep breed. It is crucial to preserve the breed's genetic diversity and maintain its unique traits to ensure the sustainability of the sheep production industry in Cyprus.

Keywords: Biodiversity, Cyprus fat-tailed sheep, Indices, Morphological body measurements

An Investigation of {OLFML3} Gene Polymorphism Related to Meat Quality in Berrichon Du Cher Sheep

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The OLFML3 gene, located on chromosome 1 in sheep and 1343 bp in length, encodes an extracellular matrix glycoprotein involved in embryo development, protein-cell interaction, and cell adhesion processes. It was observed that the genetic and epigenetic studies on the OLFML3 gene in farm animals are limited and insufficient. In sheep, there is only one study in the literature related to g.90317673 C>T polymorphism and its contribution to meat yield which was performed Javanese Fat-tailed (JFT), Javanese Thin-tailed (JTT), Garut Composite (GCS), Barbados Cross (BCS), Kompas Agrinak (CAS), Garut (GS) and Jonggol (JS) sheep breeds. Moreover, it has not been studied in Berrichon Du Cher sheep, which is one of the meat breeds and widely bred. This study aims to determine the allele and genotype frequencies of OLFML3 gene polymorphism in Berrichon Du Cher sheep. After DNA isolation by phenol-chloroform method from blood samples (n=48), the flock was genotyped for OLFML3 gene g.90317673 C>T polymorphism by PCR-RFLP. The PCR product was cut with MspI restriction enzyme, and the restriction products obtained were visualized after agarose gel electrophoresis. As a result, the investigated flock was found polymorphic for g.90317673C>T. The most frequent genotype was determined as CC in Berrichon sheep. In addition, only one animal has a TT genotype in the flock. As a result, the effects of the polymorphism of the OLFML3 gene should be qualified for a larger population in further studies.

Keywords: OLFML3, PCR-RFLP, Sheep, SNP

1. Introduction

Especially sheep breeding is one of the first breeding types that come to mind when evaluated socially, economically, and geographically in animal breeding, which is one of the most important branches of agricultural development in Turkey. The sheep species, which serves profitability targets in profit and efficiency-based studies in animal husbandry, provides benefits to humanity in various sectors (nutrition, covering, medicine). Meat yield is an essential factor in livestock breeding for economic purposes. Growth performance has a linear relationship with this important yield trait. This factor, which affects productivity in sheep breeding, is influenced by many genetic and environmental factors such as genotype, nutrition, maternal age, and type of birth [1]. Molecular techniques developed in recent years have been used effectively in evaluating meat yield, which is one of the crucial factors in livestock. Some sheep breeds are prioritized in terms of meat or meat/leaf type yield. Berrichon Du Cher breed is among the sheep breeds that are often preferred in this direction. Berrichon Du Cher sheep, which is breeding for high meat yield and quality, is a French meat sheep breed that emerged as a result of the crossbreeding of Merino and Berrichon de l'indre French sheep breeds in the late 18th century. The breed has white fleece, a deep and wide body, a round curved chest, and strong, wide hind legs [2]. Sheep breeds such as Berrichon Du Cher and Ile De France have recently been in high demand in our country, especially in crossbreeding studies or pure breeding due to the demand for their yield characteristics.

The *OLFML3* gene, which is thought to play an essential role in meat yield, is a member of the olfactomedin-like gene family that contains an olfactomedin (OLF) domain in the C-terminal region and a coiled-coiled domain in the N-terminal region [3]. The *OLFML3* gene (Gene ID:101121985) encodes an extracellular matrix glycoprotein involved in different physiological processes such as embryo development, protein-protein interactions, cell adhesion, and cell interaction [4]. Therefore, it is thought that the *OLFML3* gene contributes to the development of muscle tissue and maturation of skeletal muscles in the embryonic process and plays a vital role in myogenesis by taking part in tissue homeostasis [4]. The functional properties of the *OLFML3* gene, which is located on chromosome 1 in sheep and has three exons and is 1343 bp long, remain unclear in this species (Primary_assembly 1: 96,121,872-96,124,453).

A genome-wide association study (GWAS) for daily average body weight gain in Italian Great White pigs identified 23, 42, 30, 34, 16, 23, 42, 30, 34, 16 SNPs for pig chromosomes (SSC) 4, 5, 6, 7 and 16, respectively [5]. One of the expressed SNPs, M1GA0006343, located in SSC4, was negatively correlated with back fat

thickness in GWAS studies conducted in the same pig population, but it was reported to be close to olfactomedin-like 3 gene that affects prenatal skeletal muscle [6].

In another study conducted in pigs, the relationship between MIR-155, a multifunctional miRNA member, and *OLFML3* was examined, and it was shown that *OLFML3* was regulated by miR-155. It is thought that miR-155 may accelerate the cell cycle, [7] cell proliferation [8] and myogenic differentiation [9].

The *OLFML3* gene was cloned, and its expression was examined in Landrace (lean type) and Tongcheng (obese type) pigs for skeletal muscle development. *OLFML3* was found to be abundantly expressed in the liver and pancreas, moderately expressed in the lung, small intestine, placenta, weakly expressed in other tissues and postnatal muscle. These findings will help to understand the biological function and regulation of the *OLFML3* gene in mammals [10].

In another study in another species (Hanwoo cattle breed-Korea), an artificial selection was performed using whole genome SNP data to improve meat production traits such as marbling appearance and carcass weight. It was reported that *OLFML3* was effective in improving meat production in the studied cattle population and could be used in selection [11].

In another study performed to determine meat tenderness in Nelore cattle, 37 candidate genes were identified. Among these candidate genes, the *OLFML3* gene was reported to be involved in myogenesis, which is responsible for muscle tissue formation during embryonic development, regeneration of mature skeletal musculature, and maintenance of tissue homeostasis [12].

In a study conducted by Listyarini *et al.* (2022), the relationship between *OLFML3* gene polymorphism and meat quality, carcass traits, retail meat cut and fatty acid composition traits were examined in Indonesian sheep breeds. The polymorphism examined in the study was found to be associated with quality traits such as tenderness and loss of doneness in meat. As a result, it was predicted that polymorphism in the *OLFML3* gene could be a potential marker for meat quality, carcass characteristics, retail meat slaughter, and fatty acid composition in sheep [4].

In a study to identify candidate genes associated with tenderness, which is an important measure of retail meat cuts, meat acceptance, and eating satisfaction, the transcriptome of five high and five low lamb meat tenderness samples were analyzed, and the results showed potential candidate hepatic genes and polymorphisms affecting lamb meat tenderness. In this study, selected polymorphism in the *OLFML3* gene (C > T, g.90317673) was found to be associated with meat quality traits. Polymorphism in the *OLFML3* gene was significantly associated with tenderness and cooking loss [13]. Although some studies have been carried out in different breeds, it was determined that the studies for sheep breeds were limited and insufficient. It was concluded that the yield characteristics of the gene expressed in various sheep populations should also be studied.

For this reason, it was thought that studying the yield characteristics of the *OLFML3* gene in Berrichon du Cher breed sheep, which is known to have high meat yield, would contribute to the literature and provide guidance for future studies. In this context, it was aimed to reveal the genotypic structure in terms of g.90317673 C>T SNP in the *OLFML3* gene, which is thought to be related to meat yield in Berrichon Du Cher sheep. Thus, it is anticipated that it will be beneficial for the development of breeding strategies and to benefit the livestock sector.

2. Material and Methods

Genomic DNA was pre-isolated by phenol-chloroform method from blood samples of Berrichon Du Cher sheep (n=48) (4ml) (Et.No: 2021-01/03) collected in EDTA tubes. DNA samples stored at -20 °C were used to analyze the targeted SNP. PCR-RFLP method was used to determine the polymorphisms related to *OLFML3* gene, and the details of the method are presented in Table 1. The products obtained after PCR and RFLP procedures were evaluated by agarose gel electrophoresis. For this purpose, 1.6% agarose gel electrophoresis at 90 volts for 80 minutes was applied to the PCR products. Then, the restriction products were run in proportion to the product size in 2% agarose gel electrophoresis at 90 volts for 90 minutes. All products were visualized and evaluated on DNR's MiniLumi bio-imaging system.

Table 1. The primer sequences, PCR conditions, and product size.

GENE	ENZYME	FORWARD (PRIMER) REVERSE (PRIMER)	PRODUCT SIZE	PCR MIX	PCR CONDITIONS	RFLP
<i>OLFML3</i> (Listyarini <i>et al.</i> ,2022) (Listyarini <i>et al.</i> ,2023)	<i>MspI</i>	F: 5'- ATGATGGCTACCAGATTGTC- 3' R: 5'- AGTCTGCAGTACAGAAGGA G-3'	498 bp	dH2O: 5,5 One- taq:15 µl F:1µl R:1µl	94°C 1 dk (94°C 10 sn 59°C 15sn 72°C 15 sn) x35 72°C 1 dk	CC: 195,303 bp CT: 195,303,498 TT: 498 bp

				+2.5 μl DNA		
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3. Results

In this study, the *OLFML3* gene, located on chromosome 1 in sheep and thought to be of functional importance for meat quality, muscle development, and cell interactions, was studied by PCR-RFLP in Berrichon Du Cher breed sheep. The target primer sequences were diluted and prepared first as a master stock and then as an intermediate stock; PCR was performed using the appropriate protocol. A 1.6% agarose gel was prepared to observe DNA amplification and gel progression was monitored at 90 V, 80 min. The expected 498 base pair long PCR bands were observed (Figure 1).

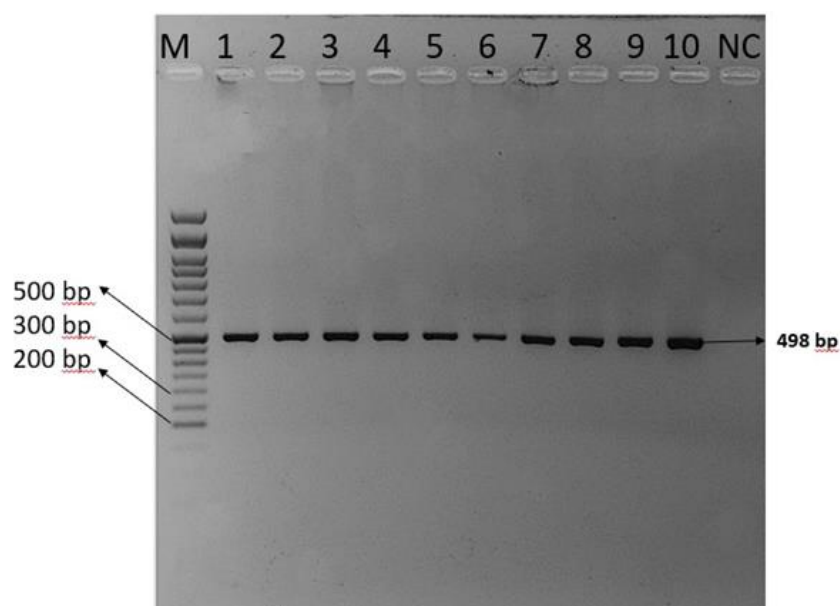


Figure 1. Agarose gel electrophoresis image of PCR products of the *OLFML3* gene. (M: Marker, Column 1-10: 498bp PCR product, NC: Negative Control)

The PCR bands obtained were reacted with *MspI* restriction enzyme at 37°C for 16 hours. After incubation, a 2% agarose gel was prepared for the electrophoresis step, and the samples were run at 90V for 90 min. Enzyme cut products after agarose gel electrophoresis are shown in Figure 2.

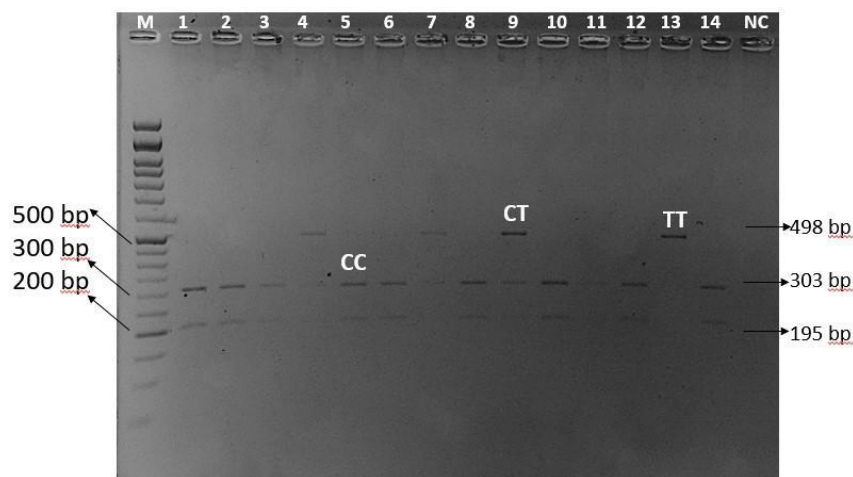


Figure 2. Agarose gel electrophoresis image of enzyme digestion products of the *OLFML3* gene. (M: Marker, Columns 1-3, 6, 8, 10-12, 14: CT, Column 5: CC, Column 13: TT, NC: Negative Control)

As a result of the genotyping performed for the targeted gene region, three different genotypes, CC (n=39), CT (n=8), and TT (n=1), were detected in the herd. Thus, it was determined that the Berrichon Du Cher herd examined within the scope of the study showed a polymorphic structure for this gene region of *OLFML3*. Allele and genotype frequencies and population genetic parameters such as expected heterozygosity (He), observed heterozygosity (Ho), effective allele number (Ne), or polymorphism information content (PIC) are presented in Table 2.

Table 2. Allele and genotype frequencies of polymorphisms in the *OLFML3* gene, population genetic diversities (He, Ho, Ne, PIC), and compatibility with the Hardy-Weinberg equilibrium.

GENOTYPE	CC	CT	TT
N	39	8	1
FREQUENCIES (%)	81,25	16,67	2,08
ALLEL FREQUENCIES	C	T	
	0,896	0,104	
HE	0,1866		
HO	0,8333		
NE	1,2295		
PIC	0,7705		
X ² (HWE)	0,714394		
P	0,397989		

4. Discussion and Conclusion

In the present study, the g.90317673 C>T polymorphism of the *OLFML3* gene, which is predicted as a potential candidate gene for meat quality and yield, was evaluated in the Berrichon Du Cher sheep breed, which is known to have qualitative characteristics in terms of meat/leaf yield. This polymorphism was reported by Listyarini *et al.* (2022, 2023) in Javanese Fat-tailed, Javanese Thin-tailed, Garut Composite, Barbados Cross, Kompas Agrinak, Garut, and Jonggol sheep breeds for its effect on meat quality, carcass characteristics, retail meat cuts and fatty acid composition.

Listyarini *et al.* (2022), the frequency of CT genotype was found to be high in JFT, JTT, GS, and JS breeds, while the highest frequency of CC for g.90317673 C>T polymorphism was notified in BCS and CAS breeds. Similar to BCS and CAS breeds, the genotype CC (81.25%) was found to have the highest frequency in Berrichon breed sheep in the present study. Listyarini *et al.* (2022) showed that the highest genotype frequency belonged to individuals with TT genotype in GCS breed sheep. Unlike Listyarini *et al.* (2022), in the Berrichon flock studied, the lowest genotype frequency belonged to TT. In another study by Listyarini *et al.* (2023), it was reported that the lowest frequency belonged to the TT (15%) genotype in GCS breed sheep, and the highest genotype frequency of 44% belonged to the heterozygous structure.

In the present study, unlike the study of Listyari *et al.* (2023), the rate of a heterozygous genotype was determined as 16.67%. Regarding allele frequencies, the highest frequency belonged to allele C (0.896). It was found that the result obtained was very similar to that reported by Listyarini *et al.* (2022) in BCS and CAS breed sheep and different from GCS (0.26) sheep.

Table 3. The comparison of the current study and the literature.

	n	GENOTYPE FREQUENCIES (%)			ALLEL FREQUENCIES		REFERENCES
		CC	CT	TT	C	T	
JFT	20	45	50	5	0.70	0.30	Listyarini <i>et.al.</i> (2022)
JTT	76	36	47	17	0.59	0.41	
GCS	45	9	33	58	0.26	0.74	
BCS	36	61	39	0	0.81	0.19	
CAS	35	77	23	0	0.89	0.11	
GS	20	35	55	10	0.63	0.37	

JS	96	40	47	14	0.63	0.37	
GCS	140	41	44	15	0.63	0.37	Listyarini et.al. (2023)
BERRICHON DU CHER	48	81.25	16.67	2.08	0.896	0.104	Present study
<i>JFT; Javanese Fat Tailed, JTT; Javanese Thin Tailed, GCS; Garut Composite Sheep, BCS; Barbados Cross Sheep, CAS; Compass Agrinak Sheep, GS; Garut Sheep, JS; Jonggol Sheep</i>							

These variations observed in both genotype and allele frequencies are thought to be due to breed differences. The fact that no study was found in the literature on the Berrichon Du Cher sheep breed caused the present study's results to not be compared with the targeted breed, and the discussion was limited to other breeds.

In terms of population parameters, expected and observed heterozygosity values were calculated as 0.1866 and 0.8333, Ne value 1.2295, PIC value 0.7705, and Hardy-Weinberg equilibrium (HWE) P value 0.3979, respectively. Higher than the expected heterozygosity is known to indicate that the population is homogeneous. In the present study, the Ho value was higher than He, suggesting that the herd was homogeneous. When the herd was analyzed regarding Hardy-Weinberg compatibility, the P value was greater than 0.05, indicating that the flock was in equilibrium. According to Botstein (1980), PIC values for SNP markers are closely related to determining how informative the diversity observed among animals in a population is, and PIC>0.50 is expressed as a highly informative marker. The high PIC value (0.7705) of the studied population indicates that the *OLFML3* gene g.90317673 C>T polymorphism is a high marker.

In conclusion, three different genotypes were detected in Berrichon Du Cher sheep, which were analyzed in terms of *OLFML3* gene g.90317673 C>T polymorphism, and it was determined that the flock showed a polymorphic structure. The data obtained contributed to the literature on this subject and constituted a source for future studies is thought. The fact that the genotypic structure of the flock differed in terms of this polymorphism revealed the necessity of investigating this SNP in Berrichon Du Cher sheep regarding meat yield and quality. In this context, it was predicted that it would be crucial to study the targeted polymorphism in terms of genotype and phenotype relationship in a larger Berrichon Du Cher sheep population known to have high meat yield capacity.

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Examination of Some Environmental Factors Affecting Birth Weight in Awassi Lambs Using Exhaustive Chaid Determination Tree Algoritm

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The Ivesi breed, which is bred in the southern regions of Turkey, is also bred in different climates (continental climate, Mediterranean climate) and at different altitudes. This situation causes differences in the data obtained from the Ivesi breed. This research was carried out with the aim of determining the factors affecting the birth weight of lambs born in 15 Awassi sheep farms, which were bred in Mersin and whose pedigree and feeds records were kept within the scope of the "National Small Ruminants BreedingProject in Public", by using the Exhaustive CHAID algorithm. The results of regression trees showed that birth weight was statistically significant according to birth type ($P=0.001$), maternal nutrition ($P=0.001$), paternal age ($P=0.001$), altitude ($P=0.001$), maternal age ($P=0.02$) and sex ($P=0.03$). The highest birth weight of $4\,574 \pm 470$ g was obtained from lambs born as singletons, born to mothers who were fed crushed barley and grass hay during pregnancy and whose fathers were 2, 3 and 4 years old. In the regression tree analysed in terms of altitude, it was determined that sugar beet + grass hay in the lowland (200-600 m) and crushed barley + grass hay in the hillside (600-1000 m) and plateau (+1000 m) regions contributed positively to birth weight. For higher birth weight, crushed barley or sugar beetand grass hay should be added to the rations of Awassi ewes in the last two months of pregnancy. Awassi breeding is recommended to be carried out in areas below 1000 m above sea level.

Keywords: Awassi, Birth weight, CHAID, Mersin, Regression tree

Effect of Coat on the Physiological Response of Heat – Stressed Late – lactation Dairy Cows

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Global climate change is one of the major threats to the sustainability of animal production. Livestock respond to heat stress through genotypic and phenotypic adaptations. Coat color is described as an important morphological feature, which confers adaptability to animals in case of exposure to heat stress. Dairy cows have a coat color of white and/or black. When compared to a lighter coat color, a dark coat color absorbs more solar radiation, and thus, serves as a predisposing factor for heat stress in cattle. This study was aimed at determining the effect of coat color on the physiological response of heat-stressed late-lactation dairy cows. The respiration rate (RR) and rectal temperature (RT) of 15 white-colored (WC) and 15 black-colored (BC) dairy cows were measured at 14.00 pm for three consecutive days between 22-24 July. The study groups were compared based on the means of the values measured during the 3-day period. The degree of heat stress was determined by calculating the temperature-humidity index. The study data were tested for normality of distribution with the Shapiro-Wilk test. The significance of the differences between the study groups for the RR and RT values was analyzed with Student's t test. The days in milk (DIM) in Group WC ranged from 211 to 625 d with a mean $\pm$ standard deviation and median of 427.5 ± 139.1 and 421.0, respectively. The DIM in Group BC ranged from 270 to 528 d with a mean $\pm$ standard deviation and median of 363.1 ± 71.5 and 362.0, respectively. No statistically significant difference was determined between Groups WC and BC for RR (78.5 ± 13.9 and 74.0 ± 7.6 , respectively) and RT (39.6 ± 0.4 and 39.7 ± 0.4 , respectively). In conclusion, this study demonstrated that coat color had no effect on the physiological response of late-lactation dairy cows to chronic heat stress exposure.

Keywords: Coat color, Dairy cow, Heat stress, Rectal temperature, Respiration rate

Growth and Development Characteristics of Karakaş Breed Lambs

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This study was carried out between 2018 and 2023 in sheep farming enterprises in Çermik district of Diyarbakır province. The animal material of the research was examined within the scope of the Public Breeding of Karakaş Sheep project, which is being carried out in Diyarbakır province under the coordination of the The birth and weaning weights of lambs and their daily live weight increases were examined. With this study, it was aimed to increase the productivity of Karakaş lambs raised in Diyarbakır province in terms of growth and development characteristics. The least squares method was used to determine the effect of age, gender and birth type on birth and weaning weight of Karakaş lambs. The average birth weights of Karakaş Lambs for the years 2018, 2019, 2020, 2021, 2022 and 2023 are respectively; It was determined that the birth weights were 4.36, 4.35, 4.25, 4.21, 4.17 and 4.21 kg, female lambs were 4.14 kg, male lambs were 4.38 kg, and single lambs were 4.31 kg and twin lambs were 3.76 kg. The 90th day live weights are again averaged over the years, respectively; It was determined to be 19.15, 17.17, 18.31, 18.15, 16.21 and 21.21 kg, 18.44 kg for single-born lambs and 17.69 kg for twin lambs. As a result of the research, it was observed that there was wide variation in terms of growth and development both in the herds owned by the project breeders and within the herd. By taking advantage of this variation, genetic progress can be achieved in line with the data obtained in the herds through selection based on productivity characteristics.

Keywords: Birth, Karakaş, Lamb

Investigation of Some Maternal Effects on Lamb Birth Weight in Akkaraman and Lalahan Sheep (Kıvrıcık x Akkaraman G1)

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In this study, 100 lambs born from Akkaraman and Lalahan (Kıvrıcık x Akkaraman G1) sheep at International Center for Livestock Research and Training in February- May 2021 were used. The aim of the study was to examine the effects of live weight during matingperiod and after birth in sheep and the effects of Body Condition Score (BKS) at birth on birth weightof lambs born from these sheep. For this purpose, 100 lambs, 29 Akkaraman and 71 Lalahan, born from sheep, which have different ages (2-7 years old), BCS (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0) and live weight (light-medium-heavy) were used. The effects of dam's breed, age, live weight during mating period, live weight after birth and VKS values during birth period on birth weights of the lambs were examined. In the study, the average of Akkaraman and Lalahan sheep live weights in mating period and birth period and BCS values were found to be 59.17 and 54.07 kg ($P=0.001$); 61.58 and 59.05 kg ($P=0.142$); 2.69 and 2.82 ($P=0.230$), respectively. Birth weights of Akkaraman and Lalahan lambs were found to be 4.95 and 4.75 kg, respectively ($P = 0.025$). Among the parameters examined, a positive correlation was found between vii lamb live birth weight and maternal factors at various levels. There were medium positive correlations between maternal mating period live weight and lamb live birth weight ($r=0.48$, $P=0.008$), between maternal weight at lambing and lamb live birth weight ($r=0.40$, $P=0.031$), and between maternal BCS values and lamblive birth weight ($r=0.54$, $P=0.003$). Since Lalahan genotype, which was bred for the purpose of lamb meat production, gave higher values than literature studies, it was another output of the study, and it can be said that the breeding study achieved its purpose.

Keywords: Akkaraman, Body condition score, Lalahan sheep, Live weight

Examination of Some Breeding and Welfare Proctise in Akkaraman Sheep Farms

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In this study, it was aimed to reveal basic information about some breeding and animal welfare practices in Akkaraman sheep breed raised within the scope of "National Small Ruminant Improvement Project in Farmer Condition" in Çankırı, Çorum and Kırşehir Provinces. In this regard, 71 farms were visited in March 2022 and information was collected by meeting with breeders face to face. While rams are kept in herd throughout year in 15farms, ram recruitment is applied freely in 66 farms. Mating period starts in August in 25 farms and in September in 23 farms. Supplementary feed is given to sheep in summer only in 3 farms. Flushing is fed to sheep in 9 farms and to rams in 28 farms. Farms are divided into 4 groups according to their pen size (less than 100m², between 100-200m², between 200-300m² and larger than 300 m²). According to this;the highest rates of barn height, window area and manger length were found in large farms (6.05m,16.0m² and 75.3m, respectively). When compared between provinces, barn height was found to be at the highest value in Çankırı (4.66m), window area was at the highest value in Çorum (10.3m²) and manger length was found at the highest value in Kırşehir (73.6m). There was no statistical difference in all values of barn climate parameters (temperature, humidity, HNI, CO₂, NH₃) between the groups according to barn area size. When differences between cities were examined,the THI value was found to be the highest in Çorum, CO₂ value was found to be the highest in Kırşehir, while NH₃ values were found to be close to each other. As a result, it was observed that allowing animals to go out to yard and pasture had a positive effect on animal welfare.

Keywords: Akkaraman, Animal production, Animal welfare

3rd International Livestock Science Congress
POSTER PRESENTATIONS

Presence of Small Ruminants in the Provinces in the GAP International Agricultural Research and Training Center (GAPUTAEM) Study Area

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In this review, the presence of small ruminants in eleven provinces (Diyarbakır, Mardin, Şırnak, Siirt, Batman, Elazığ, Adıyaman, Malatya, Şanlıurfa, Bitlis and Hakkari) that are included in the GAPUTAEM study area, their change over the years and the effects of these provinces on Türkiye's average were mentioned. According to TUIK data in 2021, there are 9,266,248 sheep, 3,336,781 goats, a total of 12,603,029 sheep and goats in 11 provinces within the GAPUTAEM study area. The total number of sheep in Türkiye is 45,177,690 heads. The ratio of sheep presence in eleven provinces within the study area of GAPUTAEM is 20.51%. The total number of goats in Türkiye is 12,341,514 heads. The ratio of goats in the eleven provinces within the study area of GAPUTAEM is 27.04% in total. While the sheep population of the eleven provinces included in the GAPUTAEM study area was 4,728,997 head and 18.77% in 2004, its ratio to Turkey's total increased to 20.51% with 9,266,448 head in 2021. The increase in both numbers and rates is remarkable. Again, while the goat population of eleven provinces within the scope of GAPUTAEM was 20.83% in 2004 with 1,376,533 heads, it increased to 27.04% with 3,336,781 heads in 2021. The increase in the presence of goats is much higher than that of sheep.

Keywords: Diyarbakır, GAPUTAEM, Goat presence, Sheep presence

Freezing Possibilities of Rooster Sperm

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Cryopreservation causes serious damage to spermatozoa through osmotic stress and intracellular ice crystal formation, negatively affecting motility and fertilization. During the freezing and thawing process, semen is exposed to oxidative, osmotic and thermal damage. These damages reduce sperm quality and fertilization ability and cause damage to spermatozoon DNA. The avian spermatozoa membrane contains high levels of polyunsaturated fatty acids. In the presence of reactive oxygen species, polyunsaturated fatty acids easily undergo lipid peroxidation and produce lipid peroxyl radicals that damage cell membranes. Semen contains endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), but semen metabolism reduces the antioxidative effect and in this case, an auxiliary antioxidant must be added to the storage medium. For this purpose, in recent years, there have been studies using many antioxidants to increase semen quality in freezing and thawing. 9 articles were examined to reveal the freezing possibilities of rooster semen. Such studies contribute to the literature and enable the production of quality semen for artificial insemination. Antioxidant additions to semen extenders appear to be a suitable strategy to preserve spermatozoa viability during cryopreservation or storage in males. More studies are needed on sperm freezing.

Keywords: Cryopreservation, Oxidative stress, Rooster

The Use of Non-Protein Nitrogenous Compounds in Ruminant Feding

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Most of the protein in feed plays an important role in ruminant nutrition because it is a source of amino acids and nitrogen for microbial protein synthesized in the rumen. But, the high cost of protein sources leads to the reduction of feed costs in a way that does not adversely affect the efficiency characteristics of the animal. The use of NPN compounds in ruminant nutrition allows an increase in feed intake and feed utilization, as well as the substitution of protein sources in the feed. This allows for more cost-effective ration preparation. However, excessive or sudden administration of NPN compounds to the animal may adversely affect microbial protein synthesis and may cause poisoning in the animal. In this study, the possibilities of using urea, slow-release urea and ammonium sulfate, which are non-protein nitrogenous compounds, in ruminant nutrition were reviewed.

Keywords: Nonprotein nitrogen, Protein, Ruminant nutrition

Some Growth Traits of the Kids in Maltese Breed

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Growth characteristics of 84 kids born from 47 Maltese does in Yakacık village of Fethiye have been observed from birth to 120 days. Birth weight, periodic live weights and 90. day body measurements were taken and the effects of gender, birth type and dam age on these characteristics were investigated. Statistically significant effects of gender were observed on birth, 30., 60., 90. and 120. day weights ($P < 0.05$). The weights of male and female kids from birth to 120. day were found respectively 3.26 kg and 3.00 kg, 9.37 kg and 8.31 kg, 13.33 kg and 11.86 kg, 19.25 kg and 16.76 kg, 35.47 kg and 29.13 kg. Birth type has significant effects on birth and 30. day weight and respectively listed as 3.51 kg and 2.76 kg, 10.07 kg and 7.61 kg for single and multiple born kids ($P < 0.05$). Dam age has made significant effect on birth and 30. day weights ($P < 0.05$). Although all the body measurements were significantly affected by investigated factors, they did not show a constant interaction ($P < 0.05$). While gender affected all the body measurement traits, chest circumference, rump height and rump width were affected by birth type. Dam age has only effect on rump height. Kids of Maltese goats in a certain location were examined within a small population and an essential contribution was made to the gathering of data on the growth characteristics of this breed. The study also has an importance to indicate the existence and usage of Malta goat breed in the region.

Keywords : Body measurements, Live weights, Maltese goat, Yakacık-Fethiye.

Investigation of Some Breeding and Welfare Practices in Farmer Condition Angora Goat Farms

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In this study, it is aimed to reveal basic information about some breeding and welfare practices in Angora goats raised within scope of "National Small Ruminant Improvement Project in Farmer Condition" in Ankara. In this direction, 28 farms were visited in May 2023 and information was collected by meeting with breeders face-to-face. The farms were determined as 11 large (>500) farms, 11 medium (250-500) farms and 6 small (≤ 250) farms according to the number of goats. 7 farms do not have any shepherds. The departure dates of farms to plateau take place in May (6), June (12) and July (10). Daily grazing period of goats around pastures, plateaus and villages varies between 12-15 hours in summer and 5-7 hours in winter. While goats are kept in herd throughout year in 21 farms, in 25 farms method of mating bucks is applied as free. In 16 farms, mating time starts between 15 October and 1 November. While all farms do not give additional feed to goats in summer period, additional feeding is done in winter period in 27 farms. In addition, only male animals are given additional feed at mating time in 6 farms. While there is no bedding material in all of farms, floors of 27 farms consist of natural material. Bedding area was sufficient in 23 farms and it was observed that bedding quality of only 6 farms was clean and dry. While animal hoof care is not done in 21 farms, it is done once a year in 6 farms and twice a year in 1 farm. As a result, it was observed that animals going to courtyard and pasture had a positive effect on animal welfare, but insufficient ventilation of farms, wetness of ground and density of animals in closed areas were observed as negative factors.

Keywords: Angora goat, Animal production, Animal welfare

Some Characteristics Obtained from Denizli and Gerze Chicken Breed Conservation Flocks

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The program for the conservation of Turkish domestic chicken breeds as genetic resources was initiated in 1997 at the International Livestock Training and Research Center Directorate (UHAEM) under the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies as research project no TAGEM/HAYSÜD/95/A01/P02/01-033. The project was later included within the scope of the Turkish animal genetic resources conservation main project and was accepted as a sub-project named "Denizli and Gerze Chickens ex-situ in vivo conservation project". The conservation project of these two chicken breeds, which are among our local genetic resources, is currently carried out within UHAEM. In addition to conserve genetic resources, the project also carries out research to determine the characteristics of these breeds. Since 1997, data has been obtained by making a research plan in these conserved breeds, and these data have been published in national and international journals and presented orally and as posters at congresses. In this review, sample data from scientific publications related to the project mentioned within UHAEM are briefly presented. As a result, this poster aimed to increase the knowledge and awareness of our native breeds for researchers and breeders.

Keywords: Denizli breed, Genetic resources, Gerze breed, Preservation

Evaluation of Pelemir (*Cephalaria syriaca*) as a Feed Source

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The importance of promising alternative forage plants that can adapted climatic conditions and extreme environmental is more increasing than ever with global warming. Pelemir (*Cephalaria syriaca*), which is resistant to cold, salinity and drought, is a potential alternative oil crop for the future. Pelemir was previously known as a weed but is now considered a cultivated plant. Currently, there are two varieties of pelemir named Karahan and Ziya. Biofuel obtained from pelemir oil is a renewable, environmentally friendly energy source that is an alternative to liquid fuels used today. In addition, the meal remaining after the oil of the Pelemir seed is extracted is a source of concentrated feed that can be used in animal feeding (Sezgin et al., 2017). Researches on the pelemir plant were scanned and 13 studies were mentioned in this review. Pelemir has the opportunity to make a significant contribution to the country's economy by using it as an alternative feed source for animal feeding.

Keywords: Animal nutrition, Biofuel, Pelemir, The national list

A Case of Atresia Ani in a KivircikxAkkaraman Crossbred Male Lamb

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In this case report, the operative treatment of a one-day old male KivircikxAkkaraman crossbred lamb with a closed anus showing symptoms of no defecation, swollen abdomen and abdominal pain was aimed. As a result of the clinical examination, atresia ani was diagnosed. Following local anesthesia, a local incision was made on the anal region skin. With the incision, the closed anus was opened to the outside. After the anus was opened to the outside, the subcutaneous connective tissue and skin around the anus were fixed by suturing. Postoperative intramuscular antibiotic treatment and wound care with antiseptic warmed water were performed for 7 days. Skin sutures were removed after 10 days. Following the operation, the lamb was observed for 3 months (i.e., until weaning) and no complications were observed. The lamb with a birth weight of 4.2 kg, was weaned at a live weight of 19.2 kg and joined the herd.

Keywords: Atresia ani, Lamb, Surgery

1. Introduction

The cause of congenital anomalies is not known for certain, but; it is stated that it may develop due to genetic factors such as mutations and chromosomal disorders and also infectious diseases that develop in the fetal period and feed-borne toxicities. (Magda and Youssef, 2007; Chauhan et al. 2011). In addition, various environmental factors and nutritional disorders, stress factors, incorrect breeder selection, the use of various drugs that have negative effects on embryo development and teratogenic viruses are among the causes of congenital anomalies (Belge et al. 2000).

Gastrointestinal system anomalies are among the most common anomalies in domestic animals. The case of atresia ani is commonly encountered in domestic animals (Chauhan et al. 2011, Özeydin et al. 2006). It is stated that anorectal anomalies are the most common anomalies among gastrointestinal system anomalies (Belge et al., 2000). In this case, it was aimed to describe diagnose, surgically treat and evaluate the operation results of atresia ani in a KivircikxAkkaraman crossbred male lamb.

2. Case Description

The case material consisted of a one-day-old KivircikxAkkaraman hybrid who could not defecate normally, had swelling and severe pain. According to the anamnesis, it was learned that the lamb had had no appetite for a day and had swelling in the abdomen (Figure 1A). After the anamnesis and clinical examination, it was determined that the anus of the lamb was not shaped and the closed end of the rectum could be felt in the anal region during abdominal palpation, and as a result of the findings, an operation was decided. In this case (Figure 1B), the lamb was diagnosed with atresia sudden type 2 (Table 1).

The operation was performed with local anesthesia. Local infiltration anesthesia was applied to the anal area where the operation would be performed with 5 ml Lidocaine hydrochloride (Adokain, Sanovel, 20 mg/ml). After anesthesia, an incision was made on the skin of the anal area (Figure 1C). With the incision made, the existing but closed anus was opened and the large amount of meconium accumulated inside was drained. After all the meconium was evacuated, the subcutaneous connective tissue and skin around the anus were stitched closed with number 2/0 silk thread (Figure 1D). Following the operation, 0.5 ml Penicillin G potassium, Penicillin G procaine, Streptomycin sulfate (Vethymicin, Vetaş, 33.333 IU/ml, 100.000 IU/ml, 166.67 mg/ml), 0.5 ml Vitamin B₁+Vitamin B₆ (Nervit, Vetaş, 100 mg/ml, 10 mg/ml) intramuscularly; 0.5 ml meloxicam (Maxicam, Sanovel 5 mg/ml) was injected subcutaneously.

Postoperatively, 0.5 ml Penicillin G potassium, Penicillin G procaine, Streptomycin sulfate (Vetaş, Vetimisin) was injected intramuscularly for 7 days. The incision made in the anal area was cleaned with 0.1% povidone-iodine solution for 10 days. On the 10th day following the operation, the lamb was examined and its stitches were removed. During periodic checks performed for three months, it was determined that the lamb could defecate normally and there were no complications after the operation.

Table 1: Classification of atresia ani (Purohit et al., 2000)

Atresia Ani	Degree of Agenesis or Dysgenesis of the Anus and Rectum
Type 1	The rectum is shaped. The anus is open but narrow.
Type 2	The rectum is generally normal and the anus is covered with a thin skin membrane. Sometimes the rectum is in the form of a blind sac and the anus is not developed.
Type 3	The proximal rectum terminates cranially in the form of a blind sac. The anus is not shaped.
Type 4	The proximal rectum terminates cranially in the form of a blind sac. The anus is shaped normally.

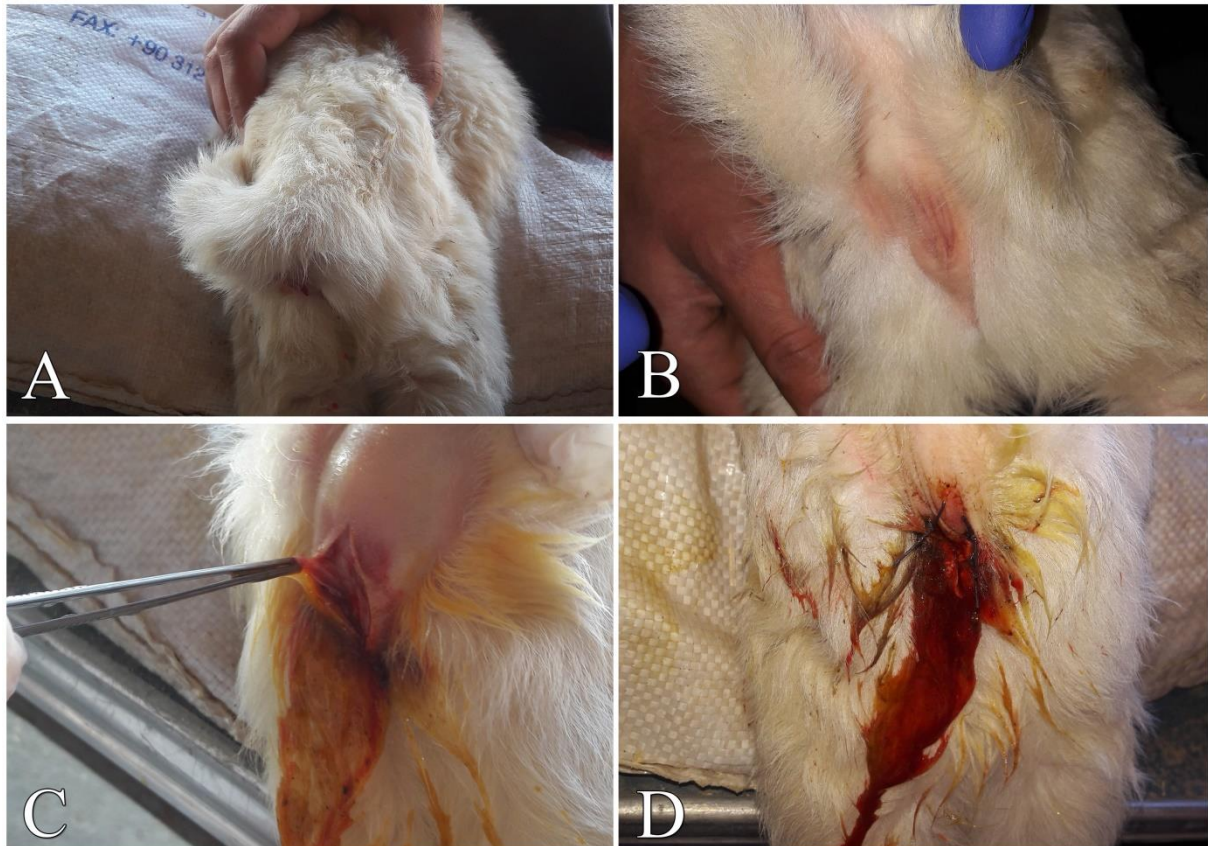


Figure 1. **A:** Clinical examination of the lamb and the swelling observed in the abdominal area. **B:** Anal region and atresia ani. **C:** Incision in the anal area and opening of the anus. **D:** Postoperative view.

3. Discussion

Atresia ani; it is defined as the anus being closed and the rectum ending blindly under the skin of the anal area (Çeçen, 2012). Type 1 was detected in a two-day-old male calf (Chauhan et. al 2011) and a female Simmental calf (Durmuş and Çınar, 2011), in the male calf of an Anatolian buffalo (Varol et al., 2018) and in a female lamb (Chaudhary et al. 2016) There are reported cases of Type 2 and Type 3 atresia ani in a Simmental male calf (Durmuş and Polat, 2019). In this case report, it was determined that the anus was closed by a thin membrane and the rectum ended just beyond the anus in the form of a blind sac (Type 2 atresia ani).

In the present case report, a surgical technique known for the treatment of atresia ani was applied, and the positive results obtained were found to be compatible with previous results (Chaudhary et al., 2016).

Surgery: Anesthesia was applied to the area where the rectum should be located for sedation (0.2 mg/kg dose of xylazine HCl, Rompun®, Bayer) and local infiltration (2 ml, 2% lidocaine HCl, Adokain®, Sanovel). After an oval incision was made on the skin in this area, the blind rectal sac was reached by blunt dissection of the subcutaneous connective tissue. After the rectum wall was sutured to the subcutaneous connective tissue with simple separate sutures all around, the middle part was incised and the contents were emptied. After the incision,

the edges of the rectum were sutured to the skin edges with simple separate stitches and the operation was completed.

As a result, it was concluded that Type 2 atresia ani can be commonly observed in lambs and that the lamb can survive normally by applying surgical methods in such cases.

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