



# LIVESTOCK STUDIES

VOLUME 64 ISSUE 1 JUNE 2024 eISSN 2757-8240



Published by International Center for Livestock Research and Training, Ankara, TURKEY

**TJEM JOURNALS**



---

## *Aims and Scope*

Our Institute Lalahan International Center for Livestock, Research and Training has been operating in the field of Animal Science and Livestock since 1951. Among the livestock activities, our Institute continues its activities in the fields of cattle breeding, ovine breeding and poultry breeding. In addition Institute's Breeding, Animal Nutrition, Genetics, Artificial Insemination and Embryo laboratories actively serve. Numerous research projects have been completed or still continue to be carried out in these areas. Institute has a journal named "Lalahan Livestock Research Institute Journal" which has been publishing 2 issues per year since 1959. The journal has the status of a National Refereed Journal followed by ULAKBİM (Turkish Academic Network and Information Center) in the field of Livestock. The journal, which has a strong archive and knowledge in its field, will continue its publication in English in order to carry it to International Standards. The journal will continue its publishing life as its new name 'Livestock Studies'.

Livestock Studies covers all kind of studies related to farm animals from poultry and bees to cattle, sheep, goats, etc. as follows:

Livestock Studies has been monitored: ULAKBİM (Turkish National Academic Network and Information Center), FAO AGRIS, CAP Abstract, CABI Full Text, Animal Breeding Abstracts, Google Scholar.

Further information for "**Livestock Studies**" will be published biannually in July and December for the above mentioned subjects.

Livestock Studies is a fully open access journal, with free online access to all content. No page charges or publication fees are collected. There is no payment at any stage of the article publishing process.

E-ISSN: 2757-8240

Copyright© Livestock Studies 2024, All rights reserved Publishing Date: June 2024

---

## *Corresponding Address*

Address : International Center for Livestock Research and Training, (Uluslararası Hayvancılık Araştırma ve Eğitim Merkezi Müdürlüğü), Lalahan Mah. S. Sırrı İçöz Cad. Mamak - Ankara / Türkiye

Web : <http://arastirma.tarimorman.gov.tr/lalahanhmae>

E-mail : [lalahanhmae@tarimorman.gov.tr](mailto:lalahanhmae@tarimorman.gov.tr)

Phone : +90 312 865 14 18 - +90 312 865 11 96

Fax : +90 312 865 11 12

**Editor in Chief**

Sezer ÖZ, International Center for Livestock Research and Training, Ankara, Türkiye  
İlker ÜNAL, International Center for Livestock Research and Training, Ankara, Türkiye

**Advisory Board**

İbrahim Halil SÖZMEN, General Directorate of Agricultural Research and Policies, Ankara, Türkiye  
Abdul Shakoor CHAUDHRY, Newcastle University, Animal Science, Newcastle, UK  
Hayrettin OKUT, Kansas University, Medical, Kansas, USA  
Mustafa SAATÇI, Muğla Sıtkı Koçman University, Agricultural Faculty, Muğla, Türkiye  
Mehmet İhsan SOYSAL, Namık Kemal University, Agricultural Faculty, Tekirdağ, Türkiye

**Managing Editors**

Mehmet KIZILASLAN, International Center for Livestock Research and Training, Ankara, Türkiye  
İlke ÜNLÜSOY, International Center for Livestock Research and Training, Ankara, Türkiye  
Pınar ÖZDEMİR, International Center for Livestock Research and Training, Ankara, Türkiye

**Editorial Board**

**(Alphabetical Order by Surname)**

Ali Reha AĞAOĞLU, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye  
Rüveyda AKBAY, Ege University, Izmir, Türkiye  
Yılmaz ARAL, Ankara University, Ankara, Türkiye  
Selim ASLAN, Near East University, Nicosia, TRNC  
İbrahim CEMAL, Adnan Menderes University, Aydın, Türkiye  
Miyase ÇINAR, Kırıkkale University, Kırıkkale, Türkiye  
Bengi ÇINAR KUL, Ankara University, Ankara, Türkiye  
İbrahim ÇİFTÇİ, Ankara University, Ankara, Türkiye  
Gürsel DELLAL, Ankara University, Ankara, Türkiye  
İlkay DELLAL, Ankara University, Ankara, Türkiye  
Serkan ERAT, Kırıkkale University, Kırıkkale, Türkiye  
Samir Bachir Souheil GAOUAR, Abou Bekr Belkaid University of Tlemcen, Algeria  
Aytekin GÜNLÜ, Selçuk University, Konya, Türkiye  
İsmayil Safa GÜRCAN, Ankara University, Ankara, Türkiye  
Orhan KARACA, Adnan Menderes University, Aydın, Türkiye  
Mehmet Akif KARSILI, Kırıkkale University, Kırıkkale, Türkiye  
Mustafa KAYMAZ, Ankara University, Ankara, Türkiye  
Muhamed KATICA Sarajevo University, Veterinary Faculty Bosnia and Herzegovina  
Zahide KOCABAŞ, Ankara University, Ankara, Türkiye  
Ömür KOÇAK, Istanbul University, Istanbul, Türkiye  
Özgecan KORKMAZ AĞAOĞLU, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye  
Dariusz PIWCZYŃSKI, UTP University of Science and Technology, Poland  
Abdur RAHMAN, Lahore University, Pakistan  
Michael ROSE, University of Tasmania, Australia  
Behnam ROSTAMI, Zanjan University, Iran  
William SAIDEL, Rutgers University, New Jersey, USA  
Çağrı Melikşah SAKAR, International Center for Livestock Research and Training, Ankara, Türkiye  
Calogero STELLETTA, Padova University, Italy  
Çiğdem TAKMA, Ege University, Izmir, Türkiye  
Mustafa TEKERLİ, Afyon Kocatepe University, Afyonkarahisar, Türkiye  
Necmettin ÜNAL, Ankara University, Ankara, Türkiye  
Murat YILDIRIM, Kırıkkale University, Kırıkkale, Türkiye Onur YILMAZ,  
Adnan Menderes University, Aydın, Türkiye

**Technical Editors**

Gökçe ÜNAL, International Center for Livestock Research and Training, Ankara, Türkiye  
Aylin DEMİRAY, International Center for Livestock Research and Training, Ankara, Türkiye  
Esra KARADUMAN, International Center for Livestock Research and Training, Ankara, Türkiye  
Gülşah YARAN, International Center for Livestock Research and Training, Ankara, Türkiye  
Barış KILIÇ, International Center for Livestock Research and Training, Ankara, Türkiye  
Ecem HATİPOĞLU GÜRİSOY, International Center for Livestock Research and Training, Ankara, Türkiye  
Fatma Gül MIZRAK, International Center for Livestock Research and Training, Ankara, Türkiye



# LIVESTOCK STUDIES

Volume 64 Issue1 JUNE2024

---

## **CONTENTS**

- 1-7** Effects of the COVID-19 Pandemic on Red Meat Production in Turkey in 2020  
Osman Tufan ERTAN\*, Elif ÇELİK GÜRBULAK, Kaan İŞCAN
- 8-16** Carcass Characteristics, Meat Antioxidative State, and Gut Microbiota of Broilers Fed With a Mixture of Bitter Melon and Basil Leaves Powder  
Olugbenga David OLORUNTOLA\*, Simeon Olugbenga AYODELE, Samuel Adebawale ADEYEYE, Deborah Adebukola OLORUNTOLA, Ojurereoluwa Adebimpe AYODELE, Olufemi Emmanuel ADENIJI, Fehintola Stellamaris OLADEBEYE
- 17-23** Exploring the Economically Important Growth Traits and Environmental Influences on Akkaraman Lambs in Ankara Livestock Animals  
Mehmet KIZILASLAN, Yunus ARZİK\*, Sedat BEHREM
- 25-32** Investigation of Some Environmental Factors on Reproductive Characteristics, Milk Traits and Kleiber Ratios of Kilis Goats Reared in the Fertile Crescent of Türkiye  
Sabri GÜL \*, Mahmut KESKİN, Şerafettin KAYA, Mustafa DİKME
- 33-37** Evaluation of the Relationship of Some Environmental Factors and Number of Inseminations per Pregnancy with Milk Yield in Holstein Cows  
Onur ERZURUM\*, Tamer KAYAR
- 38-45** The Investigation of the Fermentative, Chemical and Microbial Effects of Grape and Tangerine Pomace Added to High Moisture Alfalfa Silage  
Erinç GÜMÜŞ\*, Yücel ÜNAL, Musa YAVUZ, Selim SIRAKAYA, Behlül SEVİM, Tugay AYAŞAN

RESEARCH PAPER

# Effects of the COVID-19 Pandemic on Red Meat Production in Turkey in 2020

Osman Tufan ERTAN<sup>1,\*</sup> , Elif ÇELİK GÜRBULAK<sup>2</sup> , Kaan İŞCAN<sup>3</sup> 

<sup>1</sup>Tarım ve Orman Bakanlığı, Kayseri İl Tarım ve Orman Müdürlüğü, Kayseri-TÜRKİYE

<sup>2</sup>Erciyes Üniversitesi, Veteriner Fakültesi, Biyometri Anabilim Dalı, Kayseri-TÜRKİYE

<sup>3</sup>Erciyes Üniversitesi, Veteriner Fakültesi, Zootekni Anabilim Dalı, Kayseri-TÜRKİYE

\*Corresponding Author

## Article History

Received: 30 May 2024

Accepted: 25 June 2024

First Online: 01 July 2024

## Corresponding Author

Tel.: 03523382144

E-mail: tufanertan@gmail.com

## Keywords

COVID-19 pandemic

Meat consumption

Meat production

Food security

## Abstract

This study aims to evaluate the amount of meat consumption during the COVID-19 pandemic and its aftermath on a monthly and seasonal basis for the years 2019 and 2020 in our country. The normality of the data distribution was analyzed using histogram graphs, Q-Q plots, and the Kolmogorov-Smirnov test. The significance of the difference between two independent groups in terms of carcass quantities was evaluated using the Mann-Whitney U test, while the significance of the difference between more than two groups was assessed using the Kruskal-Wallis test. Bonferroni tests were used for multiple group comparisons. Data are presented as Median (1st and 3rd Quartile). A significance level of  $P < 0.05$  was set. COVID-19 is a major pandemic that has spread worldwide and affected the lives of many people. From the beginning of the pandemic, many individuals and sectors have been impacted. Furthermore, it has been observed that the COVID-19 pandemic has not only affected the economy of people and countries but also significantly impacted many businesses operating in the food and health sectors. The COVID-19 pandemic has created a broad impact encompassing health, economy, psychology, socio-cultural, and political areas. In this process, it has become a critical necessity to develop short- and medium-term economic and technical solutions for problems in the agriculture and livestock sectors and to plan long-term agricultural and livestock policies. Additionally, to ensure the continuity of agricultural and livestock activities in Türkiye under challenging conditions such as pandemics, natural disasters, and wars, there is a need to review existing agricultural policies and create urgent action plans.

## Introduction

Covid 19, which started to be seen in the city of Wuhan in Hubei province of the People's Republic of China in mid-December 2019, showing a series of symptoms and is thought to be caused by the virüs. It has been reported as cases spreading rapidly and strongly among people worldwide (Carrasco et al., 2021; Kriaucioniene et al., 2020).

COVID-19 spread rapidly around the world, creating a global crisis, which was declared a pandemic by the World Health Organization (WHO) as of March 2020 (Yüce and Muz, 2021). The COVID-19 pandemic, which has been affecting the world for nearly two years,

has had a wide range of impacts both across countries and among individuals around the world. Assessing COVID-19 solely from a health perspective is insufficient, as the pandemic has evolved into a multifaceted crisis, profoundly affecting global unemployment and poverty, while also exerting a detrimental impact on the economy, leading to financial distress (Güler and Günaylı, 2021). While people spent their lives in quarantine due to COVID-19, the increase in the amount of ready-to-eat food consumption and high stress have negatively affected people's physical and mental health (Landaeta-Díaz et al., 2021). Türkiye became acquainted with the COVID-19 pandemic on 11 March 2020, and on March 15,

2020, the Minister of Health reported that an 89-year-old patient travel from China died. This was the first reported case of death in Türkiye. The Minister of Health announced on April 1, 2020 that coronavirus cases have spread all over the country (Güreşçi, 2020)

Throughout human history, food has remained the fundamental necessity for sustaining life. As this fundamental truth persists today, the ongoing COVID-19 pandemic crisis, spanning approximately four years, has starkly highlighted the paramount importance of food. (Arzik et al., 2022; FAO, 2009; Gul et al., 2023). Following the WHO's announcement on March 11, 2020 that it declared the COVID-19 outbreak as a pandemic, and panic-induced fluctuations in the purchase of food and various essentials have been observed worldwide. These behavioral responses are believed to stem from perceptions and fears of scarcity and hunger (Sim et al., 2020).

During the COVID-19 pandemic, the importance of protein as a nutrient has been highlighted, alongside micronutrients such as vitamins D and C, zinc, and selenium. (Karaağaç, 2020). Adequate and balanced nutrition is very important for optimal immunity as it supports a strong immune system as well as prevents infection (Iddir et al., 2020). Malnutrition and poor-quality nutrition can lead to long-term adverse health effects (Tapan, 2021). A healthy diet is of great importance to be protected from infectious diseases and for the treatment of the disease to have a positive outcome (Çelikkaya, 2021). Furthermore, the importance of red meat has increased significantly during the pandemic due to its rich mineral and vitamin content, which not only supports a robust immune system against many diseases but also enhances overall health (Arzik et al., 2022; Özkorkut and Saka, 2022).

The change in the economic balance during the COVID-19 pandemic, the disruption of the supply chains led to increase in diesel and feed prices and decrease in animal product consumption. Moreover, the closure of food service establishments (restaurants, etc.) caused fluctuations in the demand for animal products and consumption habits (Demirhan and Şahin, 2022) reduction. Due to reduced tourism activity, closure of restaurants and communal kitchens, limited student mobility, and shutdown of dormitories and dining halls, the consumption of animal products in these sectors has significantly declined or even come to a halt. Consequently, businesses operating in the ready-to-eat food sector have experienced a notable decrease in revenues (Arzik et al., 2023; Yenişehirlioğlu, 2020).

The objective of this study is to comprehensively assess the impact of the sudden onset pandemic on the livestock sector. This was accomplished through the analysis of data gathered from slaughterhouses and records of imported red meat, spanning the entirety of 2019 and 2020. More

specifically, the study aimed to compare the overall consumption of red meat (encompassing cattle, sheep, goat, and buffalo) in Türkiye between the pre-pandemic period (2019) and the pandemic period (2020).

## Material and Methods

The study data were obtained from the Ministry of Agriculture and Forestry with official permission. A comparative analysis was conducted between the imported carcass and boneless meat data from the General Directorate of Food and Control of the Ministry of Agriculture and Forestry and the corresponding data released by TURKSTAT for 2019 and 2020. The study compared the total amount of red meat (cattle, sheep, goat, and buffalo) consumed in Türkiye during the pre-pandemic period (2019) and the pandemic period (2020).

The amount of red meat imported into the country was minimal and therefore not included in the analyses. Additionally, due to the unregistered slaughters during the Feast of Sacrifice, which affected both years, the exact quantity of red meat could not be estimated and was consequently excluded from our analyses. The data only encompasses the amount of red meat slaughtered under the Ministry of Agriculture and Forestry-approved slaughterhouses in Türkiye monthly for the 12 months of 2019 and 2020.

## Statistical Analysis

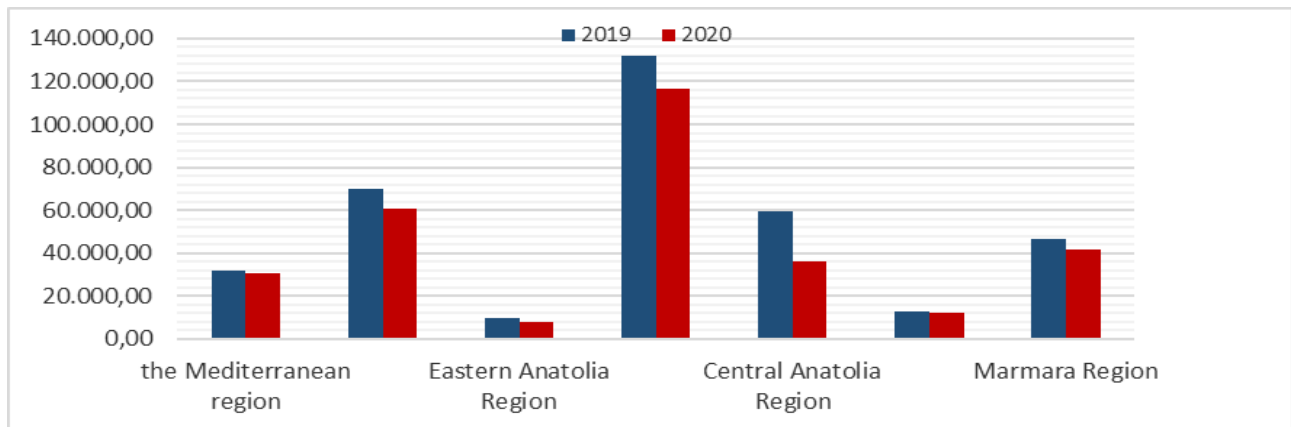
The conformity of the data to normal distribution was evaluated by histogram graph, Q-Q plot and Kolmogorov-Smirnov test. The Mann Whitney U test was used to check the significance of the difference between two independent groups in terms of carcass quantities and the Kruskal Wallis test was used to check the significance of the difference between more than two groups in terms of carcass quantities. Bonferroni test was used for multiple group comparisons. Data are given as Median (1st Quarter - 3rd Quarter). The significance level was determined as  $P < 0.05$ . R 4.0.4 ([www.cran.r-project.org](http://www.cran.r-project.org)) software was used to analyze the data.

## Results

The comparison of the amount of red meat carcasses slaughtered by slaughterhouses in Türkiye in 2019 and 2020 according to geographical regions is given in Table 1 and Figure 1. In terms of 2019 and 2020 red meat carcasses, it was determined that there was a significant difference between the Aegean and Central Anatolia regions ( $P = 0.006$ ,  $P < 0.001$ , respectively). In addition, it was determined that there was a significant difference ( $P < 0.001$ ) between the 2019 and 2020 in the total meat production of Türkiye (See Table 1 and Figure 1).

**Table 1:** Comparison of red meat carcass slaughtered by slaughterhouses in Türkiye in 2019 and 2020 by geographical regions (in thousand tons-carcass weight equivalent).

Region	2019 Median (1st Quartile - 3rd Quartile)	2020 Median (1st Quartile - 3rd Quartile)	P Value
Mediterranean	32,1 (10,4 – 99,9)	30,3 (9,8 – 79,1)	0.168
Aegean	69,6 (16,9 – 163,7)	60,8 (12,5- 138,5)	<b>0.006</b>
Eastern Anatolia	9,9 (2,9 – 36,6)	7,9 (2,5 – 30,4)	0.104
Southeastern Anatolia	131,8 (30,3 – 307,8)	116,5 (29,4 – 321,6)	0.476
Central Anatolia	59,2 (13,1 – 256,8)	35,9 (8,3 – 138,9)	<b>&lt;0.001</b>
Black Sea	12,7 (5,1 – 35,1)	12,2 (4,8 – 30,9)	0.486
Marmara	46,6 (13,4 – 125,4)	41,7 (10,9 – 141,2)	0.132
Türkiye in general	30,6 (7,9 – 112,1)	25,3 (6,6 – 90,2)	<b>&lt;0.001</b>



**Figure 1.** The amounts of red meat carcasses slaughtered by slaughterhouses in Türkiye in 2019 and 2020 across geographical regions (in thousand tons-carcass weight equivalent).

The analysis indicates that the amount of red meat slaughtered in all regions was lower in 2020 compared to 2019. Additionally, it was observed that the Black Sea region had a particularly low amount of red meat.

Table 2 presents a monthly comparison of red meat slaughtered in Türkiye in 2019 and 2020. Significant decreases were observed in March, May, and November 2020 compared to the same months in the previous year ( $P < 0.01$ ). Although the amount of red meat slaughtered in 2020 was consistently lower than in 2019 across other months, these differences were not statistically significant ( $P > 0.05$ ).

The analyses show that while the slaughter amounts in Türkiye in 2019 and 2020 vary across some months, the pattern in 2019 is more homogeneous. In 2019, the highest slaughter amount was recorded in May, approaching 45 tons. However, the greatest decrease between the corresponding months of both years occurred in May 2020. These decreases were followed by November and March 2020, respectively. When the

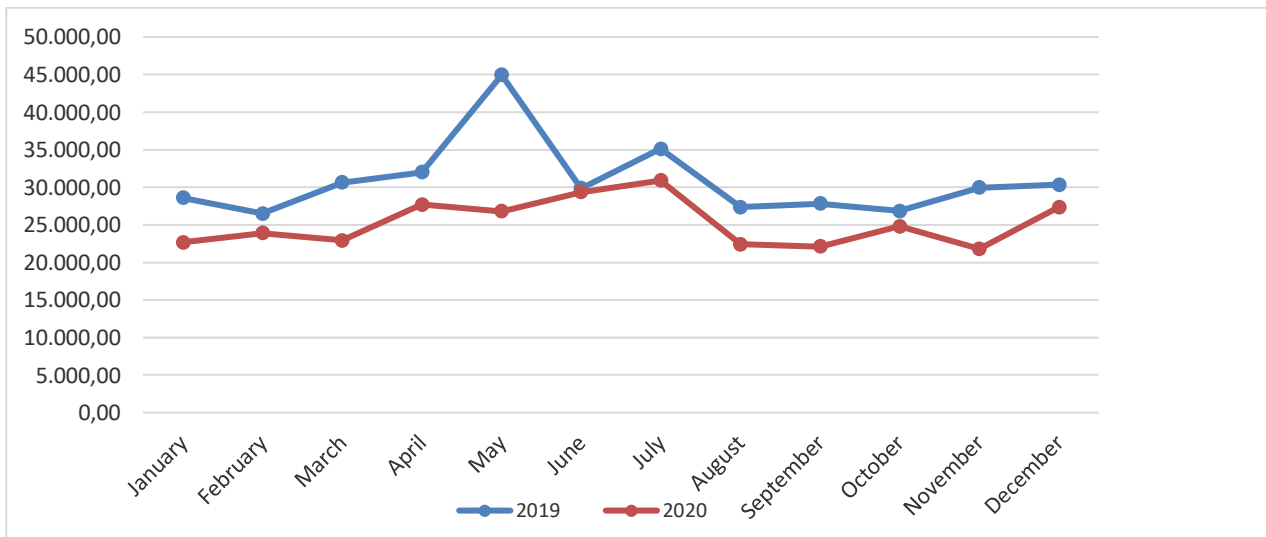
average monthly red meat carcass amounts for 2019 and 2020 were analyzed, the 2019 data was consistently higher than the 2020 data for all months except June (Figure 2).

The comparison of the average amount of red meat carcass production in Türkiye in 2019 and 2020 by season is given in Table 3. When we analyzed the differences in the amount of red meat by year and season, and also analyzed both years among themselves, the differences between some seasons were found to be significant (Table 3). In the analyses, it is seen that more slaughter was made in the spring and summer seasons of 2019 compared to the autumn and winter seasons significantly ( $P < 0.05$ ). Although there was no significant difference between the data of 2019 in summer and fall seasons, a significant decrease ( $P < 0.05$ ) occurred in the transition from summer to fall in 2020. Numerically, the slaughter amounts of all seasons in 2020 were lower than 2019. However, this decrease was significant ( $P < 0.01$ ) only in spring and fall.

**Table 2.** Monthly comparison of average red meat carcasses slaughtered by slaughterhouses in Türkiye in 2019 and 2020 (tons/month).

Aylar	2019	2020	P Değeri
January	28,5 (6,7-110,2) <sup>a</sup>	22,6 (60-84,8) <sup>a</sup>	0.107
February	26,5 (6,2-95,7) <sup>a</sup>	23,9 (5,1-81,9) <sup>a</sup>	0.262
March	30,6 (7,4-104,4) <sup>ab</sup>	22,9 (5,4-88,1) <sup>ad</sup>	<b>0.037</b>
April	32 (9,5-114,6) <sup>ab</sup>	27,7 (7,3-91,1) <sup>ab</sup>	0.061
May	44,9 (14,4-145,5) <sup>b</sup>	26,8 (7,9-94,5) <sup>ab</sup>	<b>&lt;0.001</b>
June	29,8 (8,7-105,1) <sup>ab</sup>	29,3 (8,9-104,7) <sup>bc</sup>	0.894
July	35,1 (10,2-126,8) <sup>ab</sup>	30,9 (9,6-106,6) <sup>b</sup>	0.180
August	27,3 (7,6-99,9) <sup>a</sup>	22,2 (6-85,5) <sup>a</sup>	0.071
September	27,8 (6,7-95,5) <sup>a</sup>	22,1 (5,9-78,7) <sup>ad</sup>	0.191
October	26,8 (6,9-106,8) <sup>a</sup>	24,8 (6,2-86) <sup>ace</sup>	0.254
November	29,9 (7,9-114,7) <sup>ab</sup>	21,8 (5,9-88,5) <sup>ad</sup>	<b>0.029</b>
December	30,3 (6,5-129,5) <sup>ab</sup>	27,3 (6,8-111,7) <sup>bde</sup>	0.765
<b>P Value</b>	<b>0.001</b>	<b>0.014</b>	

a, b, c, d, e: Different letters in the same column indicate the difference between groups.

**Figure 2.** Distribution of Türkiye's average red meat carcass by month in 2019 and 2020 (tons/month).**Table 3.** Comparison of Türkiye's average red meat carcass between seasons in 2019 and 2020 (tons/month).

Season	2019*	2020*	P Value
Spring	35,7 (9,9 - 124,1) <sup>a</sup>	25,7 (6,9 - 90,7) <sup>ab</sup>	<b>&lt;0.001</b>
Summer	30,4 (8,7 - 110,5) <sup>ab</sup>	27,2 (8,1 - 100,2) <sup>b</sup>	0.063
Autumn	28,3 (7,1 - 106,7) <sup>b</sup>	22,6 (6 - 83) <sup>a</sup>	<b>0.008</b>
Winter	28,1 (6,5 - 110,3) <sup>b</sup>	24,8 (6,1 - 88,5) <sup>ab</sup>	0.078
<b>P Value</b>	<b>0.003</b>	<b>0.043</b>	

a,b: Different letters in the same column indicate differences between groups.



## Discussion and Conclusion

In this study, the amount of meat consumption in Türkiye during and after the COVID-19 pandemic was compared by month and season. The COVID-19 pandemic is a major pandemic that has spread all over the world and affected the lives of many people all over the world. Since the beginning of the pandemic, many people and sectors have been affected by this pandemic. At the beginning of the pandemic, people in different countries looted markets due to concerns about access to food and tried to eat healthy food to strengthen their immune systems (Erdem, 2020). The reason why COVID-19 has reduced the purchase of animal products is due to supply chain disruption and panic buying (Akkartal, 2022). In addition to the sudden shift in demand from foodservice to retail due to the global pandemic, the shutdown of slaughterhouses and processing plants has exacerbated the economic hardship for animal farmers and led to the slaughter of animals (e.g. chickens) that were bred for meat production. As a result, meat shortages are now a concrete reality. Meat processing capacities have fallen 40% below 2019 levels during the Covid period (Luckstead et al., 2021; Walters et al., 2020). Beef processors surveyed in the United States and Brazil experienced a drop in production compared to January-March 2020 (21% in April and 19% in May). The production level in June-August 2020 was reported to be close to the highest level observed before the processing plants were shut down.

In Canada, 75% of beef processing plants were disrupted, especially due to the closure of meat plants (Hobbs, 2021). In Ghana, as the country is mainly dependent on livestock imports from the United States, Brazil and the European Union, COVID-19 brought supply problems and damaged the sector, with cattle, sheep and goat numbers falling by 57%, 61% and 64% respectively during the lockdown period (Obese et al., 2021).

Revenue from meat sales has reportedly fallen since the start of the pandemic, largely due to restaurant closures. When dining out, dishes made from meat and meat products are often preferred to vegetarian meals, and this dining out option has temporarily stopped for many. This has resulted in carcasses from animals slaughtered in more expensive slaughterhouses not being sold and storage facilities remaining at peak capacity (Sky News, 2020).

Except for June meat consumption in 2019 and 2020, there is a decrease in all months of 2020 compared to 2019. According to the statement made by ETBİR (Association of Red Meat Industrialists and Producers), while the annual per capita red meat consumption was 12 kilograms on average in 2019, this amount decreased to 7-8 kilograms in 2020. In the statement, it was emphasized that low-income people have the opportunity to eat red meat in their workplaces and that there has been a decline in consumption as they mostly

work from home during the pandemic period. In addition, it was stated that the inability of places such as restaurants, hotels and school cafeterias to do business due to the pandemic was also effective in the decline and that 40 percent of the total consumption in the domestic market was realized by individuals and 30 percent by restaurants and tourism enterprises (Cumhuriyet, 2022). They expect that the COVID-19 pandemic will re-emerge previous behavioral patterns and accelerate the transition to low-meat diets that we are starting to see in some high-income countries (Attwood and Hajat, 2020). In our research, when we look at the year 2019, it is seen that significantly more slaughter was done in the spring and summer seasons than in the fall and winter seasons. Although there was no significant difference between the 2019 data in the summer and fall seasons, there was a significant decrease in the transition from summer to autumn in 2020. Numerically, the slaughter amounts of all seasons in 2020 were lower than 2019. However, this decrease was significant ( $P < 0.01$ ) only in spring and fall. Due to uncertainty and drought in Türkiye, crises in the agriculture and livestock sector are predicted to continue in 2021. The rapidly evolving nature of the COVID-19 virus has created many problems for the meat industry. Restrictions on animal exports, logistical constraints and the closure of slaughterhouses, restaurants and food services have negatively impacted all stages of the meat supply chain. Farmers could not find suitable markets to sell their livestock. Meat processing capacity was also reduced due to the closure of processing plants. Factory closures and panic buying have also jeopardized the availability of meat and its products to consumers, causing price fluctuations. In addition to its devastating effects on human health, the COVID-19 pandemic has had unprecedented impacts on animal production and animal health worldwide. Restrictions on mobility and national and international trade have disrupted animal markets and access to consumers. This has caused a major crisis for livestock producers and a major disruption to the global economy. It has also raised concerns about food insecurity and hunger in different parts of the world. These changes have made it vital to develop and implement innovative strategies to mitigate, control and overcome the global impacts of COVID-19 on animal production and animal health. In addition, animal producers, animal health professionals, human health professionals, animal-related industries (such as meat, dairy and poultry), government agencies and non-governmental organizations need to coordinate and work together during this pandemic and ahead of any outbreak. Future pandemics that could affect global health. Therefore, in order to combat such serious situations in the future, some of the recommended measures

related to animals and animal production are included:

Although the subsidies are paid on time, the excessive debts of the farmers create a negative situation in terms of income and expenditure balance. Farmers who received support payments had problems in repaying their existing loan debts with this amount and damaged the continuity of animal husbandry. In addition to short- and medium-term economic and technical solutions, it would be useful to plan a long-term agricultural and livestock policy and review Türkiye's current agricultural policies to ensure continuity in agriculture and livestock production, especially in cases of epidemics, natural disasters and war. Farmers' access to animal feed and additives and the access of animals and animal products to markets and consumers need to be facilitated. Alternative systems for the storage of extra animals and animal products should be provided to help farmers in this outbreak and possible future outbreaks, and modern animal husbandry technologies should be introduced to the agriculture and livestock sector in order to maximize the efficiency of our country's livestock sector and activities. In this context, modern technological animal husbandry methods will contribute to the agriculture and animal husbandry sector by supporting agricultural activities that include smart agricultural application systems that minimize the need for labor force, especially in cases of epidemics and natural disasters, and the creation of interactive networks that enable producers to deliver their products to consumers by expanding this system. In addition, the development of new generation agricultural techniques such as precision agriculture and digital livestock practices involving satellite technology, drones, robotic systems and sensors will prevent crises that may occur in the agriculture and livestock sector during the pandemic.

## Conflicts

There is no conflict of interest between researchers.

## References








- Akkartal G. R. (2022). Covid 19'un Küresel Tedarik Zincirine Olan Etkisi Üzerine Bir Çalışma. *USBED*, 4 (6): 1-10.
- Arzik, Y., Behrem, S., & Kızılaslan, M. (2023). Economic evaluation of mohair production in Ankara province. *Black Sea Journal of Agriculture*, 6(1), 42-46.
- Arzik, Y., Kızılaslan, M., White, S. N., Piel, L. M., & Cinar, M. U. (2022). Estimates of genomic heritability and genome-wide association studies for blood parameters in Akkaraman sheep. *Scientific Reports*, 12(1), 18477.
- Arzik, Y., Kızılaslan, M., White, S. N., Piel, L. M., & Çınar, M. U. (2022). Genomic analysis of gastrointestinal

- parasite resistance in akkaraman sheep, *Genes*, 13(12), 2177.
- Attwood, S., Hajat, C. (2020). How will the COVID-19 pandemic shape the future of meat consumption?. *Public Health Nutr*, 23(17): 3116-3120.
- Carrasco, I., Muñoz, C. M., Vigil, V. S., Aguilera, A. D., Hernández, C., Sánchez-Sánchez, C., Oliver, C., Riaza, M., Pareja, M., Sanz, O., Pérez-Seoane, B., López, J., Márquez, E., Domínguez-Rodríguez, S., Hernanz-Lobo, A., León-Luis, J. A. D., Sánchez-Luna, M., & Navarro, M. L. (2020). SARS-COV-2 infection in pregnant women and newborns in a Spanish cohort (GESNEO-COVID) during the first wave. *BMC Pregnancy Childbirth*, 21(326): 1-10.
- Çelikkaya, H. (2021). Covid-19 salgını ile beslenme ilişkisi ve pandemi döneminde beslenme. *Göller Bölgesi Aylık Ekonomi ve Kültür Dergisi*, 8(97): 5-9
- Cumhuriyet. Pandemi dönemi makarna tüketimi yüzde 25 arttı, kırmızı et tüketimi yüzde 33 azaldı. <https://www.cumhuriyet.com.tr/haber/pandemi-doneminde-makarna-tuketimi-yuzde-25-artti-kirmizi-et-tuketimi-yuzde-33-azaldi-1861515>; Erişim tarihi: 04.04.2022
- Demirhan, S. A., & Şahinler, N. (2022). Küresel Salgın ve Türkiye Hayvancılığı Üzerine Etkileri. *Turkish Journal of Agriculture-Food Science and Technology*, 10, 2691-2695.
- Erdem, İ. (2020). Koronavirüse (Covid-19) Karşı Türkiye'nin Karantina ve Tedbir Politikaları. *e-Turkish Studies (elektronik)*, 15(4).
- Erdem, İ., Çetin, D., & Aral, N. (2022). Türkiye'de COVID-19 Pandemi Sürecinin Aile İçi İletişime Etkisi. *KTOKÜSB-D*, 3(2): 80-90.
- FAO. The State of Food and Agriculture 2009; <https://www.fao.org/3/i0680e/i0680e.pdf>; Erişim tarihi: 07.08.2023
- Grant, F., Scalvedi, M. L., Scognamiglio, U., Turrini, A., & Rossi, L. (2021). Eating Habits during the COVID-19 Lockdown in Italy: The Nutritional and Lifestyle Side Effects of the Pandemic. *Nutrients*, 13(7): 2279.
- Gul, S., Arzik, Y., Kızılaslan, M., Behrem, S., & Keskin, M. (2023). Heritability and environmental influence on pre-weaning traits in Kilis goats. *Tropical Animal Health and Production*, 55(2), 85.
- Güler, O., Günaylı, H., (2021). COVID-19 Sürecinde değişen beslenme alışkanlıkları: COVID-19 hastalığını geçiren aileler örneğinde nitel durum araştırması. *ATA Dergisi*, 2(2): 67-81.
- Güreşçi, M. (2020). COVID-19 COVID-19 Salgınında Türkiye'de Kriz Yönetimi İletişimi: T.C Sağlık Bakanlığı. *Avrasya Sosyal ve Ekonomi Araştırmaları Dergisi*, 7(5), 53-65.
- Hobbs, J. E. (2020). The Covid-19 pandemic and meat supply chains. *Meat science*, 181, 108459.
- Iddir, M., Brito, A., Dingeo G., Fernandez Del Campo, S. S., Samouda, H., La Frano M. R., & Bohn, T. (2020). Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis. *Nutrients*, 12, 1562
- Karaağaç, Y., Koyu, E. B. (2020). Viral Enfeksiyonlarda Vitamin ve Mineraller: COVID-19 Odağında Bir Derleme. *İKÇÜSBFD*, 5(2): 165-173.

- Karakuş, K., Aygün, T., Alarşlan, E. (2008). "Gaziantep ili merkez ilçede kırmızı et tüketim alışkanlıkları". *YYÜ TAR BİL DERG*, 18 (2): 113-120.
- Kriaucioniene, V., Bagdonaviciene, L., Rodríguez, P. C. (2020). Petkeviciene J. Associations between changes in health behaviours and body weight during the COVID-19 quarantine in Lithuania: the Lithuanian COVIDiet Study. *Nutrients*, 12(10): 3119.
- Landaeta, D. L, González, M. G, Agüero, S. D. (2021). Anxiety, anhedonia and food consumption during the COVID-19 quarantine in Chile. *Appetite*, 164, 105259.
- Luckstead, J., Nayga J. R, R., M, Snell, H. A. (2021). Labor issues in the food supply chain amid the COVID-19 pandemic. *Applied Economic Perspectives and Policy*, 43(1): 382-400.
- Narin, M. (2021). COVID-19 Genel Salgın Sürecinin Tarım Sektörü Üzerine Etkileri. 4. Bölüm, COVID-19 Genel Salgın Sürecinin Sosyo ekonomik Etkileri, p.51-67.
- Obese, F. Y., Osei-Amponsah, R., Timpong-Jones, E., & Bekoe, E. (2021). Impact of COVID-19 on animal production in Ghana. *Animas*, 11(1), 43-46.
- Özçakmak, S., Var, I. (2020). Covid-19 salgınının yayılmasını önleyici hijyen uygulamaları. *Akademik Gıda®*, 18(4): 433-441.
- Özkorkut, İ., Saka, M. (2022). COVID-19 ve Mineraller. *BÜSBİD*, 7(3): 157-173.
- Sanchez-Sabate, R., Sabaté, J. (2019). Consumer attitudes towards environmental concerns of meat consumption: A systematic review. *Int. J. Environ. Res. Public Health*, 16(7): 1220.
- Saygın, Ö., Demirbaş, N. (2018). Türkiye'de kırmızı et tüketimi: Sorunlar ve öneriler. *Selcuk J Agr Food Sci*, 32(3): 567-574.
- Sim, K., Chan, Y. H., Chong, P. N, Chua, H., & Soon, S. W. (2010). Psychosocial and coping responses within the community health care setting towards a national outbreak of an infectious disease. *J. Psychosom. Res*, 68(2): 195–202.
- SkyNews. Coronavirus: People Urged to Eat More Steak, Chips and Cheese During Lock Sky News. <https://news.sky.com/story/coronaviruspeople-urged-to-eat-more-steak-chips-and-cheese-during-lockdown-11982121>; Erişim tarihi: 07.08.2023.
- Suryasa, I. W, Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *Int. J. Health Sci*, 5(2).
- Tapan, T. K. (2021). Covid-19 ve beslenme. *Başkent Üniversitesi Sağlık Bilimleri Fakültesi Dergisi*, 6 (COVID-19 Özel Sayısı), 38-55.
- USDA, Dünyada sığır varlığı ve sığır eti üretimi 2018; Available from: [http://www.etb.org.tr/media/raporlar/USDA\\_Rapor\\_Ekim\\_2017\\_TR.pdf](http://www.etb.org.tr/media/raporlar/USDA_Rapor_Ekim_2017_TR.pdf); Erişim tarihi: 02.09.2023
- Walters, L., Wade, T., & Suttles, S. (2020). Food and agricultural transportation challenges amid the COVID-19 pandemic. *Choices*, 35(3): 1-8.
- Wyatt, D. (2020) Retail sales of red meat fall short of replacing eating out losses. *AHDB*. 2020; <https://ahdb.org.uk/news/retail-sales-of-red-meat-fall-short-of-replacing-eating-out-losses>; Erişim tarihi: 04.05.2020
- Yasar, R. K., Aytakin, Ö. Ü. (2021). Covid-19 ve beslenme arasındaki ilişkiye güncel bir bakış. *Akademik Gıda®*, 19(1): 108-115.
- Yenişehirlioğlu, E., Salha, H. (2021) Covid 19 pandemisinin Türkiye iç turizmine yansımaları: Değişen talep üzerine bir araştırma. *İstanbul Ticaret Üniversitesi Sosyal Bilimler Dergisi*, 19(37): 355-368.
- Yüce, G. E., Muz, G. (2021) COVID-19 pandemisinin yetişkinlerin diyet davranışları, fiziksel aktivite ve stres düzeyleri üzerine etkisi *Çukurova med. J*, 46(1): 283-291.

RESEARCH PAPER

# Carcass Characteristics, Meat Antioxidative State, and Gut Microbiota of Broilers Fed With a Mixture of Bitter Melon and Basil Leaves Powder

Olugbenga David OLORUNTOLA<sup>1,\*</sup>, Simeon Olugbenga AYODELE<sup>2</sup>, Samuel Adebawale ADEYEYE<sup>3</sup>, Deborah Adebukola OLORUNTOLA<sup>4</sup>, Ojureroluwa Adebimpe AYODELE<sup>1</sup>, Olufemi Emmanuel ADENIJI<sup>1</sup>, Fehintola Stellamaris OLADEBEYE<sup>1</sup>

<sup>1</sup> Animal Science Department, Adekunle Ajasin University, Akungba Akoko, Nigeria.

<sup>2</sup> Agricultural Technology Department, the Federal Polytechnic, Ado Ekiti, Nigeria.

<sup>3</sup> Animal Health and Production Department, Federal College of Agriculture, Akure, Nigeria.

<sup>4</sup> Department of Medical Laboratory Science, University of Medical Sciences, Ondo City, Nigeria.

## Article History

Received: 09.02.2024

Accepted: 30.05.2024

First Online: 02.07.2024

## Corresponding Author

E-mail: olugbenga.oloruntola@aaua.edu.ng

Tel: +2348035841626

## Keywords

Basil leaves

Bitter melon leaf

Feed additives

Oxidative stress

Broiler

## Abstract

The impact of a mixture of bitter melon leaf and basil leaf powder (BBLPB) on the carcass characteristics, meat antioxidant state, and gut microbiota of broilers was investigated. Three hundred one-day-old Cobb 500 broiler chicks were allocated to five diets: diet 1 (negative control), diet 2 (positive control; 0.1% blend of probiotic, prebiotic, and acidifier (BPPA) supplementation), diets 3 (0.25% BBLPB), 4 (0.5% BBLPB), and 5 (0.75% BBLPB) randomly. The 0.25% BBLPB supplementation improved ( $P < 0.05$ ) slaughtered weight, dressed weight, dressing %, and growth rate of broilers, while organ weights remained unchanged ( $P > 0.05$ ). Slaughtered weight recorded in diets 2 and 3 was higher ( $P < 0.05$ ) than those in the rest diets. The dressing percentages in diets 2 (76.45%), 3 (75.6%), and 1 (73.96%) were statistically equivalent but higher ( $P < 0.05$ ) than diets 4 (72.12%) and 5 (70.34%). Dietary supplementation with BBLPB significantly increased ( $P < 0.05$ ) muscle glutathione peroxidase concentration while reducing meat lipid oxidation and cholesterol content in broilers compared to the control. Broilers fed BBLPB-supplemented diets exhibited higher ( $P < 0.05$ ) levels of lactic acid-producing bacteria in the gut compared to the control group. The 0.25% BBLPB supplementation enhanced broilers carcass characteristics, meat quality, and gut health.

## Introduction

In recent decades, the broiler industry has experienced remarkable progress compared to other livestock species, owing to extensive genetic selection and breeding programs aimed at achieving rapid growth rates, increased muscle yield, and enhanced nutritional content (Taverez and Solis, 2016). This surge in broiler production has been driven by consumer demand for leaner meat products with reduced fat and cholesterol levels, as well as the preference for products free from antibiotic residues (Mehdi *et al.*,

2018). Consequently, there has been a heightened focus on improving the quality of chicken meat (Mir *et al.*, 2017) and current global ban on the use of antibiotics in animal nutrition (Ma *et al.* 2021).

To meet these demands, there is a growing trend towards the use of natural alternatives such as medicinal plants and spices in poultry diets, as substitutes for conventional antibiotic feed supplements. Research by Adu *et al.* (2020) and Oloruntola *et al.* (2021) has demonstrated that supplementation of medicinal plants as feed additives in broiler diets could positively impact of the broiler performance and meat quality. These

studies have highlighted the beneficial effects of phytochemicals and other bioactive compounds present in medicinal plants, including improved growth performance, enhanced nutrient digestion, promotion of muscle development, elevation of endogenous antioxidant levels, and reduction of cholesterol levels in broiler meat (Adu *et al.*, 2020; Oloruntola *et al.*, 2021).

Bitter melon (*Momordica charantia*) and basil (*Ocimum gratissimum*) plants are noteworthy among medicinal plants used as feed supplements in poultry nutrition. Bitter melon and basil, which belong to the Cucurbitaceae and Lamiaceae (Labiatae) families respectively, are widely cultivated in tropical and subtropical regions of the world, including Africa, Asia and Australia. Rich in essential nutrients such as crude protein, crude fiber, ash, crude fat, and nitrogen-free extract, bitter melon and basil possess significant nutritional value. Furthermore, their phytochemical composition analysis reveals high levels of bioactive substances like tannins, flavonoids, phenols, saponins, alkaloids, and phytate, underscoring their potential health benefits (Oloruntola *et al.*, 2021).

Studies have also demonstrated the antioxidant properties of bitter melon and basil, showcasing their ability to scavenge free radicals and inhibit lipid peroxidation in biological systems (Oloruntola *et al.*, 2021). Despite these attributes, scant information exists regarding the use of bitter melon and basil plants as feed supplements to enhance broiler carcass yield and meat quality. While various studies have investigated the effects of other medicinal plants such as oregano, rosemary, sage, thyme, moringa, and basil leaf meal on carcass and meat quality (Manessis *et al.*, 2020), there is a dearth of research on composite blends of different medicinal plants (Puvača *et al.*, 2020). In addition, recent study reveals that the multifaceted action of phytogetic supplements encompasses probiotic-like promotion of beneficial bacteria, prebiotic-like support for their growth, and acidifying effects that create a favorable gut environment, collectively contributing to improved gut health, meat quality and oxidative status in animals (Murugesan *et al.*, 2015; Pandey *et al.*, 2023).

Studies by Oloruntola *et al.* (2018, 2020) have highlighted the potential synergistic and additive effects of composite meal mixtures containing various medicinal plants, suggesting that such blends may confer greater benefits on animal performance compared to individual plant additives. Using a blend of phytogetic supplements offers enhanced benefits for broiler chickens due to synergistic effects that amplify antioxidant, antimicrobial, and anti-inflammatory properties (Oso *et al.*, 2019; Oloruntola *et al.*, 2020). This combination provides a comprehensive nutritional profile, leading to improved nutrient absorption and balanced gut microbiota, which enhances digestion and reduces infection risks.

The mix of phytochemicals more effectively boosts the immune system, offering better disease protection and improved meat quality and oxidative status through more efficient metabolic processes (Santhiravel *et al.*, 2022). Overall, the blend maximizes positive effects on health and product quality by leveraging the complementary benefits of each supplement (Oso *et al.*, 2019; Oloruntola *et al.*, 2020; Santhiravel *et al.*, 2022). The hypothesis of the study is that dietary supplementation of broilers with a mixture of bitter melon leaf powder and basil leaf powder will improve their carcass characteristics, internal organs, meat composition, and gut microbiota, thereby enhancing poultry nutrition and meat quality. Thus, this study aims to explore how blends of bitter melon leaf powder and basil leaf powder impact broiler chicken carcass characteristics, internal organs, meat quality and oxidative status, and gut microbiota, contributing valuable insights to poultry nutrition and meat quality enhancement.

## Materials and Method

### Ethical clearance, collection, processing, and chemical analysis of bitter melon and basil leaves

The Research and Ethics Committee of the Agricultural Technology Department at The Federal Polytechnic, Ado Ekiti, Nigeria, approved the experimental protocols and animal handling procedures (Ethics Reference No: AAUA/FA/ANS/1/4766/2023). Freshly harvested bitter melon (*Momordica charantia*) and basil (*Ocimum gratissimum*) leaves were thoroughly washed with clean water, drained, and air-dried on polyethylene sheets in the shade for fourteen days. Subsequently, bitter melon leaf powder (BILP) and basil leaf powder (BALP) were obtained from the dried leaves. The proximate composition, phytochemical profile, antioxidant capacity, and mineral content of BILP and BALP were analyzed in a preliminary investigation, with the findings documented (Oloruntola *et al.*, 2021). The bitter melon leaf and basil leaf powder blend (BBLPB) were prepared by mixing equal proportions (1:1) of BILP and BALP.

### Blend of Probiotics, Prebiotics, and Acidifiers

The commercial blend of probiotic, prebiotic, and acidifier (BPPA) was obtained from a reputable feed mill in Akure. It was sourced from Xvet, GMBH, located at 22529 Hamburg, Germany. The composition of the BPPA includes: *Lactobacillus acidophilus* (5x4x10<sup>9</sup> CFU); *Bacillus licheniformis* + *Bacillus subtilis* (4x10<sup>9</sup> CFU); *Enterococcus faecium* (1x4x10<sup>9</sup> CFU); *Saccharomyces cerevisiae* (40.00%); Citric acid (2,000 mg); Formic acid (9,000 mg); Orthophosphoric acid (3,000 mg); Magnesium (5.00%); and Lactic acid (3,000 mg).



### The experimental site, diets and experimental layout

The feeding trial took place at the Teaching and Research Farm of the Federal Polytechnic in Ado Ekiti, Nigeria. A basal diet was formulated for both the starter phase (0 to 21 days) and the finisher phase (21 to 42 days) to meet the nutritional requirements of broilers as recommended by the National Research Council (NRC, 1994). The basal diet was processed for proximate analysis following the guidelines of the Association of Official Analytical Chemists (AOAC, 1995).

The experimental diets (Diets 1 to 5) were prepared by dividing the basal diet into equal parts for each phase and then supplemented as follows:

Diet 1: No supplementation (negative control)

Diet 2: 1% supplementation with BPPA

Diet 3: 0.25% supplementation with BBLPB

Diet 4: 0.50% supplementation with BBLPB

Diet 5: 0.75% supplementation with BBLPB

Using a completely randomized design, 300 one-day-old Cobb 500 broiler chicks (male and female) with an average weight of  $42.06 \pm 0.44$  g were randomly allocated to the five experimental diets. Each diet was replicated six times, with 10 birds per replication. The broiler chicks were housed in experimental pens

measuring 2m x 1m, with a 3cm thick layer of dried wood shavings serving as bedding material.

The temperature within the experimental facility was maintained at  $31 \pm 2$  degrees Celsius during the first week and gradually decreased by 2 degrees Celsius each subsequent week until it reached  $26 \pm 2$  degrees Celsius. Lighting conditions followed a schedule of 24 hours of light on the first day, followed by 23 hours of light on subsequent days. The feed and water were provided *ad libitum*.

### Viability and relative growth rate

Viability and relative growth rate were assessed following the methods described by Adebayo *et al.* (2020). Viability percentage (V%) was determined using the formula:

$$V\% = \left[ \frac{\text{Total number of live animals at the end}}{\text{Total number of animals at the start}} \right] * 100$$

The body weights of the broiler chicks were measured at the beginning and end of the feeding trial. Thereafter, the following formula was used to calculate the relative growth rate (RGR):

**Table 1.** The experimental basal diets' composition.

Ingredients	Starter feed	Finisher diet
Maize	52.35	59.35
Rice bran	0.00	6.00
Maize bran	7.00	0.00
Soybean meal	30.00	24.00
Fish meal	3.00	3.00
Soy oil	3.00	3.00
Salt	0.30	0.30
Bone meal	3.00	3.00
Limestone	0.50	0.50
Lysine	0.25	0.25
Methionine	0.30	0.30
*Premix	0.30	0.30
Analyzed composition (%)		
Crude fibre	3.52	3.61
Fat	4.21	2.32
Crude protein	22.18	20.03
Calculated composition (%)		
Metabolizable energy (Kcal/kg)	3018.89	3108.10
Available phosphorus	0.46	0.41
Calcium	1.01	0.99
Methionine	0.68	0.66
Lysine	1.36	1.24

\*Premix: Vitamin A (10,000 iu) D (2,000,000 iu), E (35, 000 iu); K (1,900mg); B12 (19mg); Riboflavin (7,000mg). Nicotinic acid (45,000mg) Folic acid (1,400mg); Pyridoxine (3800mg); Thiamine (2,200mg); Pantothenic acid (11,000mg); Biotin (113mg) and trace element such as Cu (8,000mg), Mn (64,000mg); Zn(40,000mg), Fe(32,000mg), Se(160mg), I(800mg); and other items as Ca (400mg); Chlorine (475,000mg) Methionine (50, 000mg); BHT (5,000mg) and Spiramycin (5,000mg) in 2.5kg of premix.

$$\text{RGR} = [(w_2 - w_1) / \frac{(w_1 + w_2)}{2}] * 100.$$

$W_1$ = Body weight at the start of the experiment;

$W_2$ = Bodyweight at the conclusion of the trial.

### Slaughtering techniques, blood sample collection, carcass analysis

On day 42 of the experiment, three birds per replication were randomly selected, weighed, and euthanized by severing the two jugular veins (Oloruntola *et al.*, 2018). Prior to euthanasia, the birds were deprived of feed overnight. Breast meat samples were aseptically collected from the slaughtered chickens, packed aerobically in oxygen-permeable bags, and stored frozen at  $-18^{\circ}\text{C}$  for 20 days. Simultaneously, the caecal contents of the slaughtered birds were collected for microbiological examination. Dressing procedures were performed following slaughtering, and the dressed weight and dressing percentage were calculated relative to the slaughter weight. Evaluation of internal organs including liver, heart, lungs, spleen, gall bladder, proventriculus, gizzard, and pancreas followed the method described by Oloruntola *et al.*, (2018). Breast meat samples were then analyzed for lipid oxidation, glutathione peroxidase, and cholesterol levels. The TBA reactive species technique was employed to assess meat lipid oxidation (Tokur *et al.*, 2006), while the method described by Cichoski *et al.*, (2012) and de Almeida (2006) was used to determine meat glutathione peroxidase and cholesterol concentrations respectively. Enumeration of aerobic bacteria, lactic acid-producing bacteria, and intestinal negative bacteria in the caecal contents was conducted. Serial

dilution examination of bacterial populations followed protocols outlined by Dibaji and Simoes (2014) and Seidavi and Simoes (2015).

### Statistical analysis

The model:  $P_{xy} = \mu + \alpha z + \beta zy$ , was employed in this study, where  $P_{zy}$  = any of the response factors;  $x$  = the average on the whole;  $\alpha z$  = the  $z$ th treatment's effect ( $P$ = diets 1, 2, 3, 4 and 5); and  $\beta zy$  = random error as a result of the study. Using SPSS version 20, One-way ANOVA was used to analyse all of the data. The SPSS Duncan multiple range tests were used to find discrepancies between the treatment means ( $P < 0.05$ ).

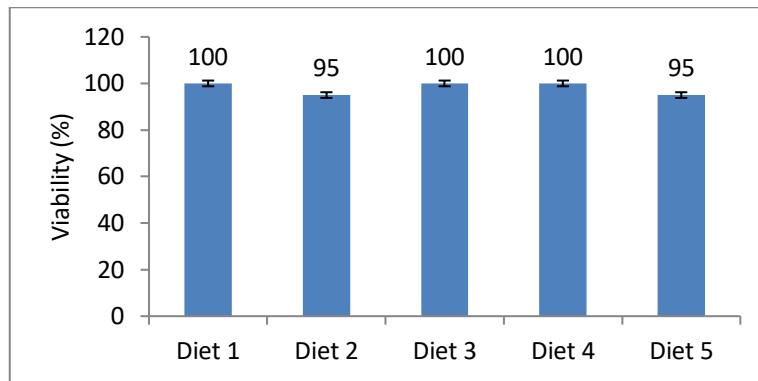
### Results

Figure 1 illustrates the viability percentage of broiler chickens fed BBLPB-supplemented diets. The dietary inclusion of BBLPB did not result in significant changes ( $P > 0.05$ ) in the viability percentage of broiler chickens across the experimental treatments. Furthermore, Figure 2 presents the relative growth rate of broiler chickens fed diets supplemented with BBLPB. The growth rates of broiler chickens fed diets supplemented with 0.25% BBLPB, 0.25% Bacflora, control, and 0.50% BBLPB were statistically similar ( $P > 0.05$ ), while the growth rate of those fed 0.75% BBLPB was notably lower ( $P > 0.05$ ) than the control group.

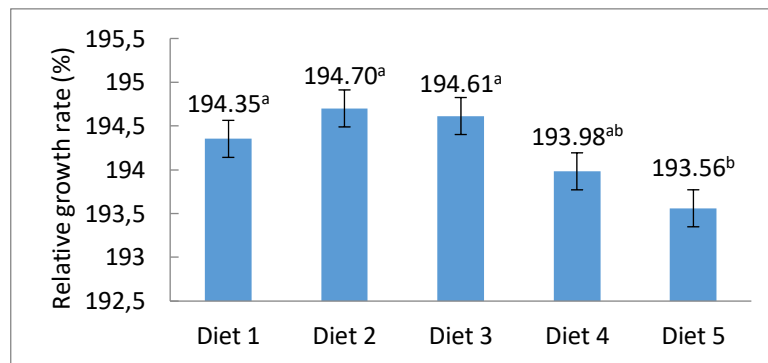
The impact of BBLPB on broiler chicken carcass and relative internal organ weights is presented in Table 2. Statistical analysis revealed a significant difference ( $P < 0.05$ ) in slaughtered weight, dressed weight and dressing percentage among treatments, while no significant ( $P > 0.05$ ) differences were observed in the

**Table 2.** Effects of BBLPB supplementation on broiler chicken carcass and relative internal organ weights (% slaughtered weight).

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Slaughter weigh (g/b)	2847.75 <sup>b</sup>	3168.06 <sup>a</sup>	2994.90 <sup>a</sup>	2659.52 <sup>bc</sup>	2523.07 <sup>c</sup>	115.04	0.02
Dressed weight (g/bird)	2106.21 <sup>b</sup>	2421.98 <sup>a</sup>	2256.95 <sup>a</sup>	1918.05 <sup>bc</sup>	1774.73 <sup>c</sup>	66.33	0.01
Dressing percentage (%)	73.96 <sup>b</sup>	76.45 <sup>a</sup>	75.36 <sup>ab</sup>	72.12 <sup>bc</sup>	70.34 <sup>c</sup>	0.66	0.01
Liver	2.15	1.84	2.19	2.02	2.03	0.05	0.25
Heart	0.45	0.47	0.46	0.45	0.49	0.01	0.89
Lung	0.71	0.12	0.17	0.14	0.11	0.03	0.49
Spleen	0.11	0.12	0.17	0.14	0.11	0.01	0.21
Gall bladder	0.23	0.12	0.16	0.08	0.15	0.01	0.20
Proventriculus	0.40	0.47	0.50	0.49	0.50	0.02	0.32
Gizzard	2.33	1.90	2.16	2.02	2.25	0.07	0.41



**Figure 1.** The effect of BBLPB supplementation on the viability percentage of broiler chicken. BBLPB: Bitter lemon and basil leaves blend; Diet1: control; Diet 2: 0.1% BPPA supplementation; Diet 3: 0.25% BBLPB supplementation; Diet 4: 0.50% BBLPB supplementation; Diet 5: 0.75% BBLPB supplementation;  $P>0.05$ .



**Figure 2.** The effect of BBLPB supplementation on the relative growth rate of broiler chicken. BBLPB: Bitter lemon and basil leaves blend; Diet1: control; Diet 2: 0.1% BPPA supplementation; Diet 3: 0.25% BBLPB supplementation; Diet 4: 0.50% BBLPB supplementation; Diet 5: 0.75% BBLPB supplementation;  $P<0.05$ .

weights of the liver, heart, lung, spleen, gall bladder, proventriculus, gizzard, and pancreas. The slaughtered weight recorded for birds fed 0.1% BPPA and 0.25% BBLPB (diets 2 and 3) were higher than those in the diets 1, 4 and 5. Broiler chickens fed a diet containing 0.25% BBLPB exhibited the highest dressed weight (2256.95g), which were statistically similar ( $P>0.05$ ) to birds on diet 2 (2421.98g), while those on diets 5 (0.75% BBLPB) and 4 (0.50% BBLPB) had the lowest dressed weight. The dressing percentages of birds fed diets 2 (76.45%), 3 (75.6%), and 1 (73.96%) were statistically equivalent but higher ( $P<0.05$ ) than diets 4 (72.12%) and 5 (70.34%).

The effects of BBLPB on lipid oxidation, glutathione peroxidase, and cholesterol content of broiler meat are presented in Table 3. Broilers fed a control diet (1.55 mgMDA/g) exhibited significantly higher meat lipid oxidation than those fed diets supplemented with 0.25% BBLPB (0.75 mgMDA/g), BPPA (0.83 mgMDA/100g), 0.50% BBLPB (0.91 mgMDA/g), and 0.75% BBLPB (0.90 mgMDA/g). Similarly, the meat cholesterol content of broilers given a control diet (123.71 mg/dl) was considerably ( $P<0.05$ ) greater than that of broilers fed diets supplemented with 0.25% BBLPB (30.35 mg/dl), BPPA (60.10 mg/dl), 0.50% BBLPB (17.25 mg/dl), and 0.75% BBLPB (22.47 mg/dl). Conversely, broiler chickens fed the control diet had the

lowest glutathione peroxidase content compared to those fed diets supplemented with 0.25% BBLPB (57.38 mg/ml), 0.1% BPPA (60.12 mg/ml), 0.50% BBLPB (59.74 mg/ml), and 0.75% BBLPB (60.27 mg/ml).

The results of the broiler chicken's intestinal microbiology are detailed in Table 4. The microbial analysis indicated that the counts of aerobic bacteria and intestinal negative bacteria did not exhibit significant differences ( $P > 0.05$ ) across the treatments. However, supplementation with BBLPB significantly ( $P < 0.05$ ) increased the population of lactic acid-producing bacteria in broiler chickens compared to the control group. The highest count of lactic acid-producing bacteria ( $\log_{10}$  CFU/g) was observed in broiler chickens fed a diet containing 0.75% BBLPB, followed by those on diets 2 (7.67), 4 (7.32), 3 (7.13), and the lowest count was recorded in the control group (6.71) ( $P=0.02$ ).

## Discussion

Antibiotic feed supplements and additives have exerted adverse effects on broiler productivity, prompting the quest for viable alternatives (Mehdi *et al.*, 2018). Medicinal herbs have emerged as promising candidates in numerous studies (Adu *et al.*, 2020; Oloruntola *et al.*, 2021), owing to their potential to enhance animal growth, bolster health, and improve



**Table 3.** Effects of BBLPB supplementation on the broiler chickens meat analysis.

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Lipid oxidation (mgMDA/g)	1.55 <sup>a</sup>	0.83 <sup>bc</sup>	0.75 <sup>c</sup>	0.91 <sup>b</sup>	0.90 <sup>b</sup>	0.08	0.00
Glutathione peroxidase (mg/ml)	31.07 <sup>b</sup>	60.12 <sup>a</sup>	57.38 <sup>a</sup>	59.74 <sup>a</sup>	60.27 <sup>a</sup>	3.09	0.00
Cholesterol (mg/dl)	123.71 <sup>a</sup>	60.10 <sup>b</sup>	30.35 <sup>c</sup>	17.25 <sup>c</sup>	22.47 <sup>c</sup>	10.81	0.00

\*Means within a row with different letters are significantly different ( $P < 0.05$ ); BPPA: Blend of probiotic, prebiotic and acidifier; BBLPB: Bitter melon and basil leave blend; SEM Standard error of the mean.

**Table 4.** Effects of the BBLPB supplementation on intestinal microbiology (log<sub>10</sub> CFU/g) of broiler chickens.

Parameters	Diet 1 Control	Diet 2 0.1% BPPA	Diet 3 0.25% BBLPB	Diet 4 0.50% BBLPB	Diet 5 0.75% BBLPB	SEM	P value
Aerobic bacteria	6.46	5.70	6.31	6.62	5.84	0.15	0.23
Lactic acid-producing bacteria	6.71 <sup>c</sup>	7.67 <sup>ab</sup>	7.13 <sup>bc</sup>	7.32 <sup>abc</sup>	7.83 <sup>a</sup>	0.13	0.02
Intestinal negative bacteria	6.32	6.39	6.47	6.56	6.44	0.06	0.87

\*Means within a row with different letters are significantly different ( $P < 0.05$ ); BPPA: Blend of probiotic, prebiotic and acidifier; BBLPB: Bitter melon and basil leave blend; SEM Standard error of the mean.

meat quality, thus yielding nutritious muscle food. The observed increase in slaughtered weight and dressed weight of broiler chickens receiving feed supplemented with 0.25% BBLPB in this trial, relative to the control treatment, suggests that BBLPB at this concentration has the capacity to enhance muscle yield in broiler chickens. However, it is noteworthy that when BBLPB is administered at concentrations exceeding 0.25%, the slaughtered weight, dressed weight and dressing percentage of broiler chickens decline compared to the control treatment. The presence of significant phytochemical concentrations such as tannin, saponin, alkaloid, and phytate in BBLPB could be attributed to this decline (Oloruntola *et al.*, 2021). Dietary intake of elevated levels of tannin, saponin, phytate, and alkaloid has been linked to the impairment of animal growth rate and nutrient absorption, thereby reducing carcass production (Sobayo *et al.*, 2012; Sugiharto *et al.*, 2019). In contrast, Adeyeye *et al.* (2020) observed that the nutritional inclusion of sunflower leaf meal and goat weed leaf meal blends had no significant impact on broiler chicken slaughter weight, dressed weight, or dressing percentage when compared to the control group. Similarly, Adegbenro *et al.* (2017) reported no significant difference in the dressed weight of broiler chickens fed diets supplemented with composite leaf meal.

The similarity in the relative internal organ values of broiler chickens across treatments suggests that the inclusion of BBLPB may promote the growth of internal organs without adversely affecting their integrity (Ayodele *et al.*, 2016). Adeyeye *et al.* (2020) similarly demonstrated no significant difference in the relative organ weight of broiler chickens fed a diet

supplemented with a blend of wild sunflower and goat weed leaf meal. However, this finding contrasts with the results of Adegbenro *et al.* (2017), who reported a significant difference in the relative organ weight of broiler chickens fed a diet containing composite leaf meal.

The ability of the inherent antioxidants, such as phenol, flavonoid, saponin, and phytate, present in the plants to scavenge free radicals and inhibit oxidation production in animals could account for the significant reduction in meat lipid oxidation observed in broiler chickens fed a BBLPB-inclusive diet. Previous studies have identified *Momordica charantia* and *Ocimum gratissimum* plant leaves as possessing high antioxidant scavenging abilities against free radicals and other reactive oxygen species that cause peroxidation in biological systems (Oloruntola *et al.*, 2021). Lipid oxidation has been identified as the primary cause of meat quality deterioration during storage (Falowo *et al.*, 2014), suggesting that BBLPB supplementation in the diet could serve as a preservative to extend the meat's shelf life. This finding is consistent with the results of Adu *et al.* (2020), who observed a reduction in lipid oxidation in the breast meat of broiler chickens fed a diet supplemented with *Syzygium aromaticum* leaf powder. Moreover, studies have demonstrated that adding phytochemicals to animal feed helps protect meat against lipid oxidation (Jiang *et al.*, 2007; Simitzis *et al.*, 2011; Valenzuela-Grijalva *et al.*, 2017).

Similarly, the significant decrease in cholesterol levels observed in broiler chickens supplemented

with BBLPB compared to the control suggests that these plants contain hypocholesterolemic compounds. Various studies have reported the potency of medicinal plants as hypocholesterolemic agents, reducing cholesterol levels in muscle food due to their inherent phytochemicals and high fiber content (Oloruntola *et al.*, 2021). Phytochemicals found in medicinal plants have been shown to reduce cholesterol by inhibiting cholesterol synthesis and fat storage in the carcass and other body areas, as well as decreasing fatty acid and triglyceride production (Santoso *et al.*, 2000). Alternatively, they may form insoluble complexes bonded with cholesterol from food in the gut, preventing cholesterol reabsorption by the intestine (Ueda, 2001; Dong *et al.*, 2007). This finding aligns with Adeyeye *et al.* (2020), who found that broiler chickens fed a diet inclusive of a blend of wild sunflower and goat weed leaf meal had significantly lower cholesterol levels than the control group. However, this result contrasts with the report of Adeyemi *et al.* (2021), who observed no discernible effect of mango leaf on broiler meat cholesterol levels.

Furthermore, the higher glutathione peroxidase recorded in broiler chickens fed a BBLPB-inclusive diet reveals the ability of BBLPB to enhance the animal's endogenous antioxidant capacity when used as an additive at this level. Endogenous antioxidant enzymes play a crucial role in protecting cells from the harmful effects of free radicals and reactive oxygen species. Specifically, glutathione peroxidase is involved in oxidizing glutathione and catalyzing the degradation of various peroxides to protect cells against oxidative damage (Jomova *et al.*, 2024). This could also explain why broiler chickens fed a BBLPB-enriched diet showed decreased lipid oxidation. Adu *et al.* (2020) previously reported reduced meat glutathione peroxidase levels in broiler chickens fed a diet enriched with *Syzygium aromaticum* leaf meal.

It was observed in this study that supplementation with BBLPB significantly increased the production of lactic acid-producing bacteria (LAB) in the gut of broiler chickens compared to the control group. This indicates the ability of BBLPB to enhance the growth of LAB in the gut. The combined phytochemicals from both plants may modify the broilers' gut environment in favor of LAB proliferation by strengthening the gut barrier, preventing harmful bacteria from colonizing, reducing inflammation, and promoting a healthier microbial balance, thereby creating a favorable environment for beneficial bacteria like LAB (Kikusato, 2021; Santhiravel *et al.*, 2022). LABs play a crucial role in broiler production due to their ability to inhibit the growth or adhesion of harmful microorganisms, improve nutrient acquisition, and stimulate the immune system to enhance feed intake and growth rate (Vieco-Saiz *et al.*, 2019). This result is consistent with the findings of Sjöfjan *et al.* (2019), who reported higher levels of lactic acid-producing bacteria in broiler chickens fed diets

containing bay leaf meal at various inclusion rates compared to the control.

The observed similarities in viability percentages between broiler chickens fed BBLPB and the control diet indicate that the medicinal plant is not detrimental to the health and livability of the broiler chickens. Similarly, the similar relative growth rates observed at 0.25% BBLPB and 0.50% BBLPB dietary inclusion rates in broiler chickens compared to the control suggest the potential of the plant to maintain or support normal growth performance when used as an additive at relatively high dietary levels in broiler production. The utilization of medicinal plants as feed additives at relatively low concentrations has been reported to enhance growth performance in broiler chickens (Adeyeye *et al.*, 2020).

## Conclusion

In conclusion, supplementation of broiler chicken diets with 0.25% bitter melon and basil leaves powder blend (BBLPB) had notable effects on carcass characteristics, meat antioxidative state, and gut microbiota. BBLPB inclusion at 0.25% positively influenced dressed weight and antioxidative parameters in meat, while also enhancing the population of beneficial lactic acid-producing bacteria in the intestine. However, excessive supplementation, particularly at 0.75% BBLPB, may have adverse effects on growth performance.

## Conflict of Interest

The authors declare there is no conflict of interest.

## References

- Adebayo, F. A., Adu, O.A., Chineke, C.A., Oloruntola, O.D., Omoleye, O.S., Adeyeye, S.A., & Ayodele, S.O. (2021). The performance and hematological indices of broiler chickens fed Chromium Picolinate, and vitamin C supplemented diets. *Asian Journal of Research in Animal and Veterinary Sciences* 6(4): 54-61.
- Adegbenro, M., Ayeni, A. O., Agbede, J. O., Onibi, G. E., & Aletor, V. A. (2017). Performance characteristics of broiler chickens fed composite leaf meal as alternative premix. *Animal Research International* 14(3): 2883 – 2891.
- Adeyemi, K. D, Olanrewaju, S. M, Adewunmi, P. S, & Abdulqadir, Z. (2021). Mango leaf and synthetic additives in broiler diet: impact on growth, caecal microbiota, immunity, blood chemistry, and meat quality. *Journal of the Saudi Society of Agricultural Sciences* 20: 482-489. doi: 10.1016/j.jssas.2021.05.005
- Adeyeye, S. A, Oloruntola, O. D, Ayodele, S. O, Falowo, A. B., & Agbede, J. O. (2020). Wild sunflower and goat weed leaf meals composite-mix supplementation in broiler

- chickens: effects on performance, health status and meat. *Acta fytotechn zootecn* 23(4): 205-212
- Adu, O. A., Gbore, F. A., Oloruntola, O. D., Falowo, A. B., & Olorotimi, O. J. (2020). The effects of *Myristica fragrans* seed meal and *Syzygium aromaticum* leaf meal dietary supplementation on growth performance and oxidative status of broiler chicken. *Bulletin of the National Research Centre* 44: 149 (2020). <https://doi.org/10.1186/s42269-020-00396-8>
- AOAC (1995) Official Methods of Analysis 16th ed. Association of Official Analytical Chemistry, Washington DC.
- Ayodele, S. O., Oloruntola, O. D., & Agbede, J. O. (2016). Effect of *Alchornea cordifolia* leaf meal inclusion and enzyme supplementation on performance and digestibility of Weaner rabbits. *World Rabbit Science*, 24:201-206.
- Cichoski, A. J., Rotta, R. B., Scheuermann, G., Junior, A. C., & Barin, J. S. (2012). Investigation of glutathione peroxidase activity in chicken meat under different experimental conditions. *Food Science and Technology*, 32(4): 661-667.
- de Almeida, J.C., Perassolo, M. S., Camargo, J. L., Bragagnolo, N., & Gross, J. L. (2006). Fatty acid composition and cholesterol content of beef and chicken meat in Southern Brazil. *Brazilian Journal of Pharmaceutical Sciences* 42(1): 109-117.
- Dibaji, S. M., Seidavi, A., Asadpour, L., & Mareira da Silva, F. (2014). Effect of a symbiotic on the intestinal microflora of chickens. *Journal of Applied Poultry Research* 23: 1-6.
- Dong, X. F., Gao, W. W., Tong, J. M., Jia, H. Q., Sa, R. N., & Zhang, Q. (2007). Effect of polysavone (alfalfa extract) on abdominal fat deposition and immunity in broiler chickens. *Poultry Science*, 86(9): 1955-1959. doi: 10.1093/ps/86.9.1955.
- Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Research International* 64 (2014): 171–181
- Jiang, Z. Y., Jiang, S. Q., Lin, Y. C., Xi, P. B., Yu, D. Q., & Wu, T. X. (2007). Effects of soybean isoflavone on growth performance, meat quality, and antioxidation in male broilers. *Poultry Science* 86:1356–1362.
- Jomova, K., Alomar, S.Y., Alwasel, S.H., Nepovimova E., Kuca K and Valko M (2024). Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol* 98, 1323–1367 (2024). <https://doi.org/10.1007/s00204-024-03696-4>
- Kikusato M. (2021). Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. *Animal bioscience*, 34(3), 345–353. <https://doi.org/10.5713/ab.20.0842>
- Ma, F., Xu, S., Tang, Z., Li, Z., & Zhang, L. (2021). Use of antimicrobials in food animals and impact of transmission of antimicrobial resistance on humans. *Biosafety and Health*, 3(1), 32-38. <https://doi.org/10.1016/j.bsheat.2020.09.004>.
- Manassis, G., Kalogianni, A. I., Lazou, T., Moschovas, M., Bossis, I., & Gelasakis, A. I. (2020). Plant-Derived Natural Antioxidants in Meat and Meat Products. *Antioxidants* (Basel, Switzerland), 9(12), 1215. <https://doi.org/10.3390/antiox9121215>
- Mehdi, Y., Létourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S. K., Côté, C., Ramirez, A. A., & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal nutrition* (Zhongguo xu mu shou yi xue hui), 4(2),170–178. <https://doi.org/10.1016/j.aninu.2018.03.002>
- Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of food science and technology*, 54(10), 2997–3009. <https://doi.org/10.1007/s13197-017-2789-z>
- Murugesan GR, Syed B, Halder S and Pender C (2015) Phytogetic feed additives as an alternative to antibiotic growth promoters in broiler chickens. *Front. Vet. Sci.* 2:21. doi: 10.3389/fvets.2015.00021
- NRC. (1994). Nutrient requirements of poultry (9th Revised Ed.). Washington, DC: *National Academy Press*.
- Oloruntola, O. D., Agbede, J. O., Ayodele, S. O., & Oloruntola, D.A. (2018). Neem, pawpaw, and bamboo leaf meal dietary supplementation in broiler chickens: Effect on performance and health status. *Journal of Food Biochemistry*.e12723. <https://doi.org/10.1111/jfbc.12723>
- Oloruntola, O. D., Ayodele, S. O., Adeyeye, S. A., Jimoh, A. O., Oloruntola, D.A., & Omoniyi, S.I. (2020). Pawpaw leaf and seed meals composite mix dietary supplementation: effects on broiler chicken's performance, caecum microflora, and blood analysis. *Agroforestry Systems* 94, 555–564. <https://doi.org/10.1007/s10457-019-00424-1>
- Oloruntola, O. D., Ayodele, S. O., Olowu, O. P. A., Falowo, A. B., Adeyeye, S. A., Omoniyi, I. S., & Osowe, C. O. 2021. The Proximate Analysis, Phytochemical Screening, Antioxidant Activity and Mineral Composition of *Momordica charantia* and *Ocimum gratissimum* Leaf Powder. *Asian Journal of Research in Biochemistry* 8(4): 30-39.
- Oso, A. O., Suganthi, R. U., Manjunatha Reddy, G. B., Malik, P. K., Thirumalaisamy, G., Awachat, V. B., Selvaraju, S., Arangasamy, A., & Bhatta, R. (2019). Effect of dietary supplementation with phytogetic blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. *Poultry Science*, 98(10), 4755-4766. <https://doi.org/10.3382/ps/pez191>
- Pandey S, Kim ES, Cho JH, Song M, Doo H, Kim S, Keum GB, Kwak J, Ryu S, Choi Y, Kang J, Choe J and Kim HB (2023) Cutting-edge knowledge on the roles of phytobiotics and their proposed modes of action in swine. *Frontiers in Veterinary Science*.10:1265689. doi: 10.3389/fvets.2023.1265689
- Puvača, N., Brkić, I., Jahić, M., Roljević, Nikolić, S., Radović, G., Ivanišević, D., Đokić, M., Bošković, D., Ilić, D., Brkanlić, S., & Prodanović, R. (2020). The Effect of Using Natural or Biotic Dietary Supplements in Poultry Nutrition on the Effectiveness of Meat Production. *Sustainability*2(11):4373; <https://doi.org/10.3390/su12114373>
- Santhiravel, S., Bekhit, A. E. A., Mendis, E., Jacobs, J. L., Dunshea, F. R., Rajapakse, N., & Ponnampalam, E. N. (2022). The Impact of Plant Phytochemicals on the Gut

- Microbiota of Humans for a Balanced Life. *International journal of molecular sciences*, 23(15), 8124. <https://doi.org/10.3390/ijms23158124>
- Santhiravel, S., Bekhit, A. E. A., Mendis, E., Jacobs, J. L., Dunshea, F. R., Rajapakse, N., & Ponnampalam, E. N. (2022). The Impact of Plant Phytochemicals on the Gut Microbiota of Humans for a Balanced Life. *International journal of molecular sciences*, 23(15), 8124. <https://doi.org/10.3390/ijms23158124>
- Santoso, U., Ohtani, S., & Tanaka, K. (2000). Tu-Chung leaf meal supplementation reduced an increase in lipid accumulation of chickens stimulated by dietary cholesterol. *Asian-Australian Journal of Animal Science* 13:1758-1763. <https://doi.org/10.5713/ajas.2000.1758>
- Seidavi, A., & Simoes, J. (2015). Evaluation of dietary fish oil plus green tea supplementation on the gizzard, ileum and cecum microflora in broiler chickens. *Archos de Zootecnia*, 64, 397-402.
- Simitzis, P. E., Symeon, G. K, Charismiadou, M. A, Ayoutanti, A. G., & Deligeorgis, S. G. (2011). The effects of dietary hesperidin supplementation on broiler performance and chicken meat characteristics. *Canadian Veterinary Journal*, 91,275–282.
- Sjofjan O., Adli D. N., Hanani P. K., & Sulistiyaningrum D. (2019). The utilization of bay leaf (*syzygium polyanthum* walp) flour in feed on carcass quality, microflora intestine of broiler. *International Journal of Engineering Technologies and Management Research*, 6(11). <https://doi.org/10.29121/ijetmr.v6.i11.2019.458>
- Sobayo, R. A., Adeyemi, O. A., Sodipe, O. G., Oso, A. O., Fafiolu, A. O., Ogunade, I. M., Iyasere, O. S., & Omoniyi, L.A. (2012). Growth response of broiler birds fed *Asystasia gangetica* leaf meal in hot humid environment. *Journal of Agricultural Science and Environment*, 12(1),53-59.
- Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I., & Sartono, T.A. (2019). Recent advances in the incorporation of leaf meals in broiler diets. *Livestock Research for Rural Development* 31(7), 2019.
- Taverez, M. A., & Solis, F. (2016). Impact of genetics and breeding on broiler production performance: A look into fast present and future industry. *Animal Frontiers*, 6 (4). DOI:10.2527/af.2016-0042
- Tokur, B., Korkmaz, K., & Ayas, D. (2006). Comparison of Two Thiobarbituric Acid (TBA) Method for Monitoring Lipid Oxidation in Fish. *EU Journal of Fisheries and Aquatic Science* 23(3-4): 331–334.
- Ueda, H. (2001). Short-term feeding response in chicks to tea saponin. *Journal of Poultry Science*, 38, 282-288.
- Valenzuela-Grijalva, N. V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Dominguez-Diaz, D., & Gonzalez-Rios, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. *Journal of Animal Science and Technology*, 59, 8. DOI 10.1186/s40781-017-0133-9
- Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., & Drider, D. (2019). Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Frontier. Microbiology* 2019 | <https://doi.org/10.3389/fmicb.2019.00057>

# Exploring the Economically Important Growth Traits and Environmental Influences on Akkaraman Lambs in Ankara

Mehmet Kizilaslan<sup>1</sup> , Yunus Arzik<sup>1\*</sup> , Sedat Behrem<sup>2</sup> 

<sup>1</sup> International Center for Livestock Research and Training, 06852, Ankara, Türkiye

<sup>2</sup> Faculty of Veterinary Medicine, Aksaray University, Aksaray, Türkiye

\*Corresponding Author

## Article History

Received:19.02.2024

Accepted:13.05.2024

First Online: 03.07.2024

## Corresponding Author\*

Tel.: +905342044360

E-mail: yunusarzik@hotmail.com

## Keywords

Pre-weaning growth traits

Kleiber ratio

Environmental factors

Akkaraman Sheep

## Abstract

This study focused on the Akkaraman sheep breed, specifically examining the pre-weaning growth characteristics and Kleiber ratio, within the framework of Türkiye's National Community-Based Small Ruminant Breeding Program. The research involved Akkaraman lambs born between 2017 and 2021 across 20 farms in the Ankara province. The dataset comprised 19,119 observations, covering key attributes such as birth weight, weaning weight, average daily weight gain, and the Kleiber ratio. Statistical analyses were conducted to identify outliers, assess normality, and develop linear models to explore the impact of environmental factors on the traits. Birth weight was significantly influenced by sex, birth type, birth season, birth year, and flock size. Weaning weight exhibited significant variations based on the same factors, emphasizing the importance of gender, birth type, birth season, birth year, and flock size. Average daily weight gain was notably affected by gender, birth type, birth season, birth year, flock size, and environmental factors, emphasizing their impact on growth. The Kleiber ratio demonstrated significant variations influenced by gender, birth type, birth season, birth year, and flock size. The results highlighted the intricate interplay between environmental factors and pre-weaning growth traits in the Akkaraman sheep breed. The study contributes valuable insights to enhance productivity and underscores the potential of the Akkaraman breed in Türkiye's overall agricultural development, considering its adaptability to arid climates and challenging pasture conditions.

## Introduction

Securing food resources for small ruminants holds paramount importance for several compelling reasons. In many regions, particularly in developing countries where alternative protein sources are mostly limited, small ruminants like sheep and goats play a crucial role in providing essential protein for numerous people. Smallholder farmers, whose livelihoods and income are closely tied to these animals, heavily rely on sheep and goats (Selçuk et al., 2023). Thus, ensuring and contributing to food security for small ruminants becomes a pivotal factor in supporting the economic well-being of these farmers and their communities. Furthermore, the inexistent advantages of sheep and goats, requiring less feed, water, and labor compared to

larger ruminants, make them adaptable to a diverse range of ecosystems (Joy et al., 2020).

Türkiye with a population of 58 million sheep and goats (comprising 79.4% sheep and 20.6% goats), boasts diverse genotypes well-adapted to its varied geographic and climatic conditions (TUIK, 2023). Among these, the Akkaraman as fat-tailed sheep has the most ratio the small ruminant population in the country (Şirin et al., 2017; Unlusoy et al., 2016). It is well known that Akkaraman is an important breed and play a fundamental role the meeting the needs of people (Behrem & Gül, 2022; Gül et al., 2022; Mondal & Reddy, 2017). However, to enhance the meat production per individual, ensure a sustainable food supply, and address food security concerns (Yardımcı & Özbeyaz, 2001), it becomes imperative to focus on



improving the Akkaraman sheep. (Ünal, 2002; Yalcin, 1986).

Sheep breeders practice in employing conventional production methods, and the persistently low predicted yield can be attributed primarily to deficiencies in record-keeping, suboptimal feeding practices, and inadequate herd health and management. Traditionally, breeders have based their selections on the morphological traits of lambs (Ceyhan et al., 2019). However, advancing sheep and goat breeding in the nation necessitates the adoption of suitable breeding strategies. The initial step in a systematic selection method involves characterizing and mitigating the effects of environmental factors, with genetic selection for cumulative gain following subsequently (Sönmez et al., 2009). In sheep selection applications, it is imperative to consider lamb birth weight, weaning weight, feed efficiency, and average daily gain as key productivity indicators. Another vital trait employed for assessing feed efficiency is the Kleiber ratio, a metric that has been utilized extensively in sheep selection practices (Eskandarinasab et al., 2010; Mahala et al., 2020; Supakorn & Pralomkarn, 2012).

Many studies conducted within the scope of the national small ruminant breeding program revealed the developmental characteristics of many indigenous sheep and goat breeds such as İvesi, Hair goat, Akkaraman, and Pırlak (Bağkesen & Koçak, 2018; Biçer et al., 2019; Gül et al., 2019; Güngör et al., 2021; Kutlu et al., 2022). Since the beginning of the Akkaraman community-based sheep breeding initiative in 2011, it has had significant success in Ankara. It is carried out in conjunction with numerous research institutions, universities, associations, and breeders of sheep and goats as a key stakeholder of small ruminant breeding. Lambs' average daily gain, weaning weight, and birth weight are all regularly reported as part of the study. Therefore, the purpose of this study was to characterize the distributions of birth weight, weaning weight, and average daily gain in Akkaraman sheep raised in the province of Ankara, as well as to estimate the influence of various environmental factors on these traits.

## Materials and Methods

### Animals and Phenotype

The research was conducted on Akkaraman lambs born in 2017 and 2021, encompassing 20 farms participating in Türkiye's National Community-Based Small Ruminant Breeding Program within the Ankara region. Situated in the northwest of the Central Anatolian Region, Ankara province (39° 55' north latitude and 32° 50' east longitude). The climate in Ankara exhibits a steppe climate characterized by dry steppe conditions with hot, arid summers and cold, snowy winters. During the study, lambs typically stayed with their dams until the 90-day weaning stage. The ewes were nourished with roughage, including alfalfa, wheat straw, and vetch, supplemented with an average of 0.6 kg/day of concentrated feed, and housed in barns with a daily allowance of 1.5 kg per animal during the winter. In the spring and summer, they grazed on challenging pastures together with their lambs. Post-weaning, males underwent fattening, while females continued to graze on pasture.

A comprehensive dataset comprising approximately 19,119 observations was utilized to analyze key attributes such as birth weight (BW), weaning weight (WW), average daily weight gain (ADWG), and the Kleiber ratio at weaning (KR). Additionally, routine records encompassed information on birth and weaning dates, sex, birth type (singletons/twins), and the season of birth (Winter/Spring). Birth weight and weaning weight data for each animal were meticulously assessed and interpolated to the 90th-day weight. The calculation of average daily weight gain (ADWG) involved linear statistical methods utilizing BW and WW. Furthermore, the Kleiber ratio at weaning (KR) was derived from the ADWG and WW using the formula  $(ADWG/WW)^{0.75}$ . A detailed breakdown of the data structure and sample size is provided in Table 1, following the removal of outliers.

**Table 1.** Descriptive statistics of the growth traits.

Trait	BW (kg)	WW (kg)	ADWG (g)	KR
Number of observations	19119	19119	19119	19119
Mean	4.44	27.14	252.18	20.99
Standard error	0.01	0.04	0.47	0.02
Minimum	1.40	11.09	68.23	11.00
Maximum	6.99	44.94	457.79	27.01
Coefficient of Variation	19.51	21.71	25.61	10.11

BW: birth weight. WW: weaning weight. ADWG: average Daily weight gain. KR: Kleibe

## Statistical Analyses

The identification and examination of outliers, defined as values deviating more than three standard deviations from the mean, were carried out on the observations and removed. Shapiro-Wilk tests were employed to assess the normality of the responses. Furthermore, a plot depicting the residuals versus fitted values of the responses was generated to visually assess the homogeneity of variance. In the development of the final linear mixed models, the impact of environmental factors (including sex, birth type, birth season, herd size, and birth year) was initially explored. Basic packages such as "lme4," "lmerTest," and various other foundational tools within the R statistical environment were utilized for data management and all statistical analyses (R Core Team, 2020).

Following the fitting of the ultimate models for the traits, linear mixed models were employed to quantify the influence of environmental factors. These mixed models generated the least square means for the respective factors. To account for random variability, herd, and maternal persistent environmental effects were incorporated into the models. Subsequently, distinctions between groups about the significant factors were scrutinized using Duncan's Test. The ensuing paragraphs provide detailed descriptions of the final linear mixed models for the individual traits:

$$Y = \mu + X\beta + Z\alpha + \epsilon$$

Where **Y** are the variables that affect the values (BW, WW, ADWG, and KR);  **$\mu$**  is the intercept;  **$\beta$**  is the fixed effects of sex, birth type (2 levels), birth season (2 levels), birth year (5 levels), herd size (3 levels);  **$\alpha$**  is the random herd and maternal persistent environmental effects and  **$\epsilon$**  is the residual error of observations in the models. **X** and **Z** are the design matrix for fixed and random effects respectively.

## Results and Discussion

### Birth weight

As observed in Table 2, when examining the impact of fixed factors on birth weight, it was found that, except for the season, the effects of other factors were significant. Analyzing the results obtained based on least-square means, the influence of sex on birth weight was noteworthy, with male lambs weighing  $4.31 \pm 0.01$  kg, while female lambs weighing  $4.09 \pm 0.01$  kg (Table 2). Furthermore, a significant difference in birth weight between male and female groups was identified ( $P < 0.001$ ). Comparing these findings with a study conducted on Akkaraman breed lambs in Niğde province, the results align closely (Ceyhan et al., 2019). However, in contrast to this study, the birth weights of male and female lambs in Çankırı province Akkaraman

lambs were higher (Behrem, 2021). This consistency in results across various studies reaffirms the tendency for males to have higher birth weights than females. It is reported that the higher birth weight in males compared to females can be attributed to the greater effectiveness of endocrine factors in promoting growth in males and the limiting impact of estrogen hormone on bone growth in females. (Assan, 2020).

In the study, the birth weights of singleton and twin-born lambs were reported as  $4.46 \pm 0.01$  kg and  $3.94 \pm 0.02$  kg, respectively, as indicated in Table 2. The difference in birth weight between singleton and twin-born lambs was found to be significant ( $P < 0.001$ ). Similar to this study, they have also determined that singleton lambs have a higher birth weight compared to twin-born lambs (Çolakoğlu & Özbeyaz, 1999; Ünal, 2002). It has been reported that the variability in birth weight is attributed to the effects of pregnancy care and nutrition during the gestation period of ewes (Koyuncu & Duymaz, 2017).

The least-square means for the birth season were determined to be  $4.19 \pm 0.01$  kg in winter and  $4.21 \pm 0.02$  kg in spring-born lambs (Table 2). The difference in birth weights between lambs born in winter and spring was found to be insignificant. Studies on Angora goats (Güngör et al., 2021) and Ivesi sheep (Gül et al., 2020) have identified the influence of season and birth month on birth weight. This observation underscores the variability in the impact of season on birth weight across different regions and breeds. However, it is noteworthy that the feeding system during pregnancy plays a critical role in regulating fetal and placental development in sheep, potentially affecting both short- and long-term health outcomes (Behrem et al., 2022; Heasman et al., 1999).

In the study, birth weights based on the birth year, namely 2017, 2018, 2019, 2020, and 2021, were observed to be  $4.14 \pm 0.01$ ,  $4.06 \pm 0.02$ ,  $4.15 \pm 0.02$ ,  $4.31 \pm 0.02$ , and  $4.34 \pm 0.02$  kg, respectively, as depicted in Table 2. The highest birth weight was observed in 2021, while the lowest birth weight was recorded in 2018. When evaluating the groups by years, the difference in birth weights among lambs born in different years was found to be significant ( $P < 0.001$ ). Similar to this study, previous research has reported variations in birth weights across years (Ceyhan et al., 2019; Ünal, 2002). Factors influencing birth weight across years can be attributed to care, feeding, and flock management.

According to the results obtained in the study, as shown in Table 2, birth weights in farms categorized by flock size as 0-150, 150-300, and 300 or more heads were  $4.04 \pm 0.02$  kg,  $4.06 \pm 0.01$  kg, and  $4.50 \pm 0.01$  kg, respectively. Farms with 300 or more heads exhibited the highest birth weights, while those with 0-150 heads had the smallest weights. The impact of flock size on birth weight was found to be significant in our study ( $P < 0.001$ ). When considering the influence of flock size,

**Table 2.** The least mean squares (LSM) of the traits.

Fixed Effects	BW (kg)			WW (kg)			ADWG (g)			KR		
	n	LSM ± SE	P-value	n	LSM ± SE	P-value	n	LSM ± SE	P-value	n	LSM ± SE	P-value
<b>Sex</b>			***			***			***			***
Male	9574	4.31±0.01 <sup>a</sup>		9574	28.52±0.07 <sup>a</sup>		9574	268.97±0.81 <sup>a</sup>		9574	21.57±0.03 <sup>a</sup>	
Female	9545	4.09±0.01 <sup>b</sup>		9545	26.51±0.07 <sup>b</sup>		9545	249.08±0.81 <sup>b</sup>		9545	21.04±0.03 <sup>b</sup>	
<b>Birth type</b>			***			***			***			***
Single	15759	4.46±0.01 <sup>a</sup>		15759	28.23±0.05 <sup>a</sup>		15759	264.14±0.59 <sup>a</sup>		15759	21.32±0.02 <sup>a</sup>	
Twin	3360	3.94±0.02 <sup>b</sup>		3360	26.79±0.10 <sup>b</sup>		3360	253.91±1.11 <sup>b</sup>		3360	21.29±0.04 <sup>b</sup>	
<b>Season</b>			NS			***			***			***
Winter	15703	4.19±0.01		15703	25.24±0.06 <sup>a</sup>		15703	233.83±0.66 <sup>b</sup>		15703	20.58±0.02 <sup>b</sup>	
Spring	3416	4.21±0.02		3416	29.79±0.10 <sup>b</sup>		3416	284.23±1.08 <sup>c</sup>		3416	22.03±0.04 <sup>c</sup>	
<b>Birth year</b>												***
2017	4446	4.14±0.01 <sup>b</sup>		4446	26.56±0.09 <sup>b</sup>		4446	249.03±1.04 <sup>b</sup>		4446	21.03±0.04 <sup>b</sup>	
2018	4178	4.06±0.02 <sup>a</sup>	***	4178	30.61±0.10 <sup>d</sup>	***	4178	294.95±1.08 <sup>d</sup>	***	4178	22.43±0.04 <sup>d</sup>	
2019	3929	4.15±0.02 <sup>b</sup>		3929	27.46±0.10 <sup>c</sup>		3929	259.09±1.09 <sup>c</sup>		3929	21.41±0.04 <sup>c</sup>	
2020	3197	4.31±0.02 <sup>c</sup>		3197	26.89±0.10 <sup>b</sup>		3197	250.92±1.12 <sup>b</sup>		3197	21.02±0.04 <sup>b</sup>	
2021	3369	4.34±0.02 <sup>d</sup>		3369	26.04±0.10 <sup>a</sup>		3369	241.14±1.14 <sup>a</sup>		3369	20.63±0.04 <sup>a</sup>	
<b>Herd Size</b>			***			***			***			***
0-150	2578	4.04±0.02 <sup>b</sup>		2578	25.93±0.11 <sup>a</sup>		2578	243.23±1.23 <sup>a</sup>		2578	20.92±0.04 <sup>a</sup>	
150-300	6446	4.06±0.01 <sup>a</sup>		6446	27.96±0.08 <sup>b</sup>		6446	265.48±0.90 <sup>b</sup>		6446	21.58±0.03 <sup>c</sup>	
>300	10095	4.50±0.01 <sup>c</sup>		10095	28.65±0.07 <sup>c</sup>		10095	268.37±0.79 <sup>c</sup>		10095	21.41±0.03 <sup>b</sup>	
<b>Intercept</b>	19119	4.27±0.03		19119	27.72±0.17		19119	260.63±1.82		19119	21.19±0.06	

Notes: The mean values which have different superscripts are significantly different. \*\*\*P < 0.001. \*\*P < 0.01. \*P < 0.05. SE = standard error; n = number of observations.



it can be presumed that larger enterprises provide better conditions for the pregnancy, care, and feeding of sheep.

### **Weaning weight**

When examining the results of weaning weight, it is observed that the impact of all fixed factors is significant. Detailed results based on the least square means are presented in Table 2. Upon scrutinizing the results of weaning weight obtained through least-square means, our study identified that male lambs had a weaning weight of  $28.52 \pm 0.07$  kg, while females had a weaning weight of  $26.51 \pm 0.07$  kg. The influence of gender on weaning weight was found to be significant for both male and female lambs ( $P < 0.001$ ). Studies conducted on Akkaraman and different genotypes show a similar trend, indicating that males tend to have higher weaning weights compared to females, with our findings showing weaning weights at 90 days surpassing results obtained by other researchers (Behrem, 2021; Ceyhan et al., 2019; Tüney Bebek & Keskin, 2021). The variability in weaning weights based on gender suggests that breeders may place greater emphasis on feeding male lambs, anticipating a significant portion of them going for slaughter.

When examining the results, it was determined that the weaning weights of singleton and twin-born lambs were  $28.23 \pm 0.05$  kg and  $26.79 \pm 0.10$  kg, respectively. The birth type significantly influences weaning weight ( $P < 0.001$ ). It is possible to attribute the better development of singleton animals in weaning weight to factors such as inadequate milking due to insufficient maternal milk for twins and the lack of necessary care by the breeder in raising twin-born lambs (Koyuncu & Duymaz, 2017). Numerous studies (Aksoy et al., 2023a; Çolakoğlu & Özbeyaz, 1999; Noyan & Ceyhan, 2021) have consistently reported that singleton lambs have higher weaning weights than twin-born lambs.

In our study, least square means were calculated for weaning weights based on the fixed factor of season, reporting weights of  $25.24 \pm 0.06$  kg in Winter and  $29.79 \pm 0.10$  kg in Spring, as presented in Table 2. The difference between groups based on the birth season was found to be significant ( $P < 0.001$ ). It is observed that lambs born in Spring have higher weaning weights compared to those born in Winter. A study on İvesi sheep reported changes in weaning weight based on birth months (Gül et al., 2020). The variability in weaning weight is evident in our study and other research, suggesting its dependence on birth month, region, care, feeding, and genotype. Another influential factor, the birth year, was found to have a significant impact on weaning weight ( $P < 0.001$ ). The results by birth year were calculated as  $26.56 \pm 0.09$  kg in 2017,  $30.61 \pm 0.10$  kg in 2018,  $27.46 \pm 0.10$  kg in 2019,  $26.89 \pm 0.10$  kg in 2020, and  $26.04 \pm 0.10$  kg in 2021, according to least square means. The highest weaning weight was observed in 2018, while the lowest weaning weight occurred in 2021. Similar effects of years on growth have been noted in other studies (Aksoy et al., 2023b; Tüfekci, 2023). The COVID-19

pandemic disrupted global food and feed supply chains, leading to shortages and bottlenecks that subsequently drove up prices. Disruptions also resulted in decreased demand for some animal products such as wool and meat, impacting livestock and grazing industries, and making it difficult for sheep farmers to feed their flocks. The high increase in feed prices during the COVID-19 pandemic and afterward could be considered a fundamental reason for breeders not providing additional supplementary feed to ewes, leading to this situation in 2020 and 2021.

In our study, weaning weights were determined as  $25.93 \pm 0.11$  kg in 0-150,  $27.96 \pm 0.08$  kg in 150-300, and  $28.65 \pm 0.07$  kg in 300 or more head farms, based on flock size. The farms with 300 or more heads had the highest weaning weights, while those with 0-150 heads had the smallest, as presented in Table 2. The impact of flock size on weaning weight was found to be significant ( $P < 0.001$ ). It is observed in the study that larger farms show more careful management, care, and feeding, positively reflecting on weaning weight compared to medium and small farms.

### **Average daily weight gain**

As seen in Table 2 based on least-square means, our study determined that males have a significantly higher average daily weight gain (ADWG) than females, and the difference between males and females is significant ( $P < 0.001$ ). When evaluated by birth type, we found a significant difference between singleton and twin groups ( $P < 0.001$ ), with singleton lambs exhibiting a higher ADWG than twin lambs in our study. Regarding the birth season, the highest ADWG was observed in lambs born in the Spring. The influence of the birth season on ADWG is statistically significant ( $P < 0.001$ ). Examining the ADWG increase over the years, the highest ADWG was observed in 2018, while the lowest ADWG was seen in 2021. Additionally, the impact of years on ADWG is significant ( $P < 0.001$ ). Analyzing ADWG based on flock size, the highest ADWG is observed in farms with 300 or more heads, similar to the pattern observed in weaning weight. The environmental factors play a crucial role in the ADWG increase, and their impact is significant ( $P < 0.001$ ).

### **Kleiber ratio**

In our study, when determining the Kleiber ratio, we found that the ratio is  $21.57 \pm 0.03$  in male lambs and  $21.04 \pm 0.03$  in female lambs, with a significant difference between the two groups ( $P < 0.001$ ). The influence of sex is significant, consistent with results obtained in other studies (Mahala et al., 2020; Sofla et al., 2011). According to birth type, the Kleiber ratio for singleton and twin lambs is  $21.32 \pm 0.02$  and  $21.29 \pm 0.04$ , respectively, as shown in Table 2. However, our results for singleton and twin lambs differ from those reported by Behrem (2021), with our study showing a lower Kleiber ratio. The slight but significant difference between singleton and twin

lambs is consistent with results from other studies ( $P < 0.001$ ) (Sofla et al., 2011).

Analyzing the results according to the birth season, the highest Kleiber ratio is observed in lambs born in the Spring, with a ratio of  $22.03 \pm 0.04$ , while in lambs born in the Winter, it is  $20.58 \pm 0.02$  (Table 2). The difference in the Kleiber ratio between groups born in Winter and Spring is significant ( $P < 0.001$ ), indicating a seasonal effect on the Kleiber ratio, similar to findings in other studies (Sofla et al., 2011). Examining the Kleiber ratio based on birth years, the values for 2017, 2018, 2019, 2020, and 2021 are  $21.03 \pm 0.04$ ,  $22.43 \pm 0.04$ ,  $21.41 \pm 0.04$ ,  $21.02 \pm 0.04$ , and  $20.63 \pm 0.04$ , respectively. The highest Kleiber ratio was observed in 2018, consistent with GCAA and SKA results. The impact of birth years on the Kleiber ratio is significant ( $P < 0.001$ ), similar to findings in another study (Behrem, 2021). Regarding flock size, the highest Kleiber ratio is observed in enterprises with 150-300 heads, and the difference between groups based on flock size is significant ( $P < 0.001$ ).

The Kleiber ratio holds significant importance in sheep breeding as it functions both as an indirect selection criterion for feed conversion and an insightful indicator of growth efficiency. Breeding for an improved Kleiber ratio or growth rate in sheep has the potential to enhance the genetic composition of the breed. Health care and feeding practices are also closely tied to the Kleiber ratio, adding another layer of importance to its consideration in sheep breeding. Studies suggest that the Kleiber ratio in sheep is influenced by both genetic and non-genetic factors, underscoring the need to incorporate it into selection and management strategies to achieve desired growth rates. However, some research indicates that the variation in growth rate and the Kleiber ratio in certain sheep breeds may not be primarily linked to genetic factors. In a broader context, the Kleiber ratio plays a pivotal role in evaluating overall growth, as emphasized in studies by Eskandarinasab et al. (2010) and Mahala et al. (2020).

## Conclusions

The observed influence of environmental factors on pre-weaning growth traits and the Kleiber ratio underscores their significant impact on the economic traits of the Akkaraman sheep breed. Enhancing these environmental factors holds the potential to positively influence productivity in terms of these traits. The study's outcomes emphasize the importance of not overlooking environmental factors when incorporating the Akkaraman breed into selection programs. The insights gained from this research offer valuable information for enhancing productivity and ensuring sustainability in the context of growth characteristics specific to the Akkaraman breed. Given its resilience to

arid climates and challenging pasture conditions, coupled with its status as the most widely bred indigenous breed in Türkiye, increased recognition and further exploration of its genetic attributes, particularly in terms of growth and adaptation characteristics, are poised to make substantial contributions to Türkiye's overall development.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Acknowledgements

The animals were under the National Community-based Small Ruminant Breeding Programme. Therefore, the authors kindly acknowledge the contribution of the General Directorate of Agricultural Research and Policies (Ministry of Agriculture and Forestry) of the Republic of Türkiye, who fund and run the National Community-based Small Ruminant Breeding Program.

## References

- Aksoy, Y., Şekeroğlu, A., Duman, M., & Çoban, Ö. B. (2023a). A study on the Determination of Some Reproductive Traits of Ewes and The Growth Performance of Lambs Akkaraman Raised under Farm Conditions in the Province of Niğde. *Turkish Journal of Agriculture - Food Science and Technology*, 11(6), 1168–1175. <https://doi.org/10.24925/turjaf.v11i6.1168-1175.5991>
- Aksoy, Y., Şekeroğlu, A., Duman, M., & Çoban, Ö. B. (2023b). A study on the Determination of Some Reproductive Traits of Ewes and The Growth Performance of Lambs Akkaraman Raised under Farm Conditions in the Province of Niğde. *Turkish Journal of Agriculture - Food Science and Technology*, 11(6), 1168–1175. <https://doi.org/10.24925/turjaf.v11i6.1168-1175.5991>
- Assan, N. (2020). *Africa Animal Genetic Resources View project Harnessing Genetic Diversity in Local and Improved Goat Types through Improved Nutrition for Efficiency in Meat Production in Zimbabwe. View project*. <https://doi.org/10.14196/aa.v9i5.645>
- Bağkesen, Ö., & Koçak, S. (2018). Ramlıç ve Dağlıç Koyunlarda Kırkım Sonu Canlı Ağırlık, Yapağı Verimi ve Özellikleri. *Kocatepe Veterinary Journal*, 11(2), 148–155. <https://doi.org/10.30607/kvj.402769>
- Behrem, S. (2021). Effects of Environmental Factors Growth Traits of Akkaraman. *Livestock Studies*, 61(1), 22–27. <https://doi.org/http://doi.org/10.46897/livestockstudies>
- Behrem, S., & Gül, S. (2022). Effects of age and body region on wool characteristics of Merino sheep crossbreds in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 46(2), 235–247. <https://doi.org/10.55730/1300-0128.4171>
- Biçer, O., Keskin, M., Gül, S., Gündüz, Z., Zeynep OFLAZ, N., & Behrem, S. (2019). Comparison of yield characteristics

- of brown and black headed Awassi sheep Kahverengi ve siyah başlı İvesi koyunlarının verim özellikleri yönünden karşılaştırılması. In *Mustafa Kemal University Journal of Agricultural Sciences* (Vol. 24, Issue 1). <http://dergipark.org.tr/mkutbd>
- Ceyhan, A., Şekeroğlu, A., & Duman, M. (2019). Niğde İlinde Yetiştirilen Akkaraman Irkı Koyunların Bazı Döl Verim Özellikleri ve Kuzuların Büyüme Performansı. *Turkish Journal of Agriculture - Food Science and Technology*, 7(10), 1509–1514. <https://doi.org/10.24925/turjaf.v7i10.1509-1514.2249>
- Çolakoğlu, N., & Özbeyaz, C. (1999). Akkaraman ve Malya Koyunlarının Bazı Verim Özelliklerinin Karşılaştırılması. *Tr. J. of Veterinary and Animal Science*, 23, 351–360.
- Eskandarinasab, M., Ghafouri-Kesbi, F., & Abbasi, M. A. (2010). Different models for evaluation of growth traits and Kleiber ratio in an experimental flock of Iranian fat-tailed Afshari sheep. *Journal of Animal Breeding and Genetics*, 127(1), 26–33. <https://doi.org/10.1111/j.1439-0388.2008.00789.x>
- Gül, S., Keskin, M., & Güzey, Y. Z. (2019). Path analysis of the relationship between weaning weight and some morphological traits in Awassi lamb. *J. Agr. Nat.* 22(2).
- Gül, S., Keskin, M., Biçer, O., Gündüz, Z., & Behrem, S. (2020). Effects of different lambing season on some reproductive characteristics of ewes and growth performance of lambs in Awassi sheep. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, 60(1), 32–36. <https://doi.org/10.46897/lahaed.797929>
- Gül, S., Oflaz, N., Keskin, M., & Behrem, S. (2022). Aksaray ilinde koyun yetiştiriciliğinin yapısal özellikleri. *Erciyes Tarım ve Hayvan Bilimleri Dergisi*, 5(2), 1-11.
- Güngör, I., Alkoyak, K., Öz, S., & Koncagül, S. (2021). Growth, survival rate, and some reproductive characteristics of Hair goat under breeder conditions in Kahramanmaraş Province. *Turkish Journal of Veterinary and Animal Sciences*, 45(6), 1022–1029. <https://doi.org/10.3906/vet-2104-51>
- Heasman, L., Clarke, L., Stephenson, T. J., & Symonds, M. E. (1999). The influence of maternal nutrient restriction in early to mid-pregnancy on placental and fetal development in sheep. *Proceedings of the Nutrition Society*, 58(2), 283–288. <https://doi.org/10.1017/S0029665199000397>
- Joy, A., Dunshea, F. R., Leury, B. J., Clarke, I. J., Digiacomo, K., & Chauhan, S. S. (2020). Resilience of small ruminants to climate change and increased environmental temperature: A review. In *Animals* (Vol. 10, Issue 5). MDPI AG. <https://doi.org/10.3390/ani10050867>
- Koyuncu, M., & Duymaz, Y. (2017). Kuzularda Yaşama Gücünün İyileştirilmesi. *Hayvansal Üretim*, 58(1), 46–56.
- Kutlu, M. A., Çelik, Ş., & Kaygusuzoglu, E. (2022). Bingöl İlinde Halk Elinde Yetiştirilen Akkaraman Irkı Kuzuların Büyüme Performansının İncelenmesi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, 25(6), 1502–1509. <https://doi.org/10.18016/ksutarimdog.a.vi.1011360>
- Mahala, S., Saini, S., Kumar, A., Sharma, R. C., & Gowane, G. R. (2020). Genetic trends for the growth rates and Kleiber ratio in Avikalin sheep. *Small Ruminant Research*, 189. <https://doi.org/10.1016/j.smallrumres.2020.106143>
- Mondal, S., & Reddy, I. J. (2017). Impact of climate change on livestock production. *Biotechnology for Sustainable Agriculture: Emerging Approaches and Strategies*, 234–256. <https://doi.org/10.1016/B978-0-12-812160-3.00008-8>
- Noyan, M., & Ceyhan, A. (2021). Yarı Entansif Koşullarda Yetiştirilen Akkaraman Irkı Kuzuların Büyüme Performansı. In *J. Agric. Food, Environ. Anim. Sci* (Vol. 2, Issue 2). <http://www.jafeas.com>
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Şirin, E., Aksoy, Y., Uğurlu, M., Çiçek, Ü., Öneç, A., Ulutaş, Z., Şen, U., & Kuran, M. (2017). The relationship between muscle fiber characteristics and some meat quality parameters in Turkish native sheep breeds. *Small Ruminant Research*, 150, 46–51. <https://doi.org/10.1016/j.smallrumres.2017.03.012>
- Selçuk, S. E., Özmen, Ö., & Çolak, R. (2023). Molecular Mechanisms Affecting Development of Animal Fibers and Some Studies on Goats. *Livestock Studies*, 63(1), 30–38.
- Sofla, S. S., Savar-Sofla, S., Nejati-Javaremi, A., Abbasi, A., Vaez-Torshizi, R., & Chamani, M. (2011). Investigation on Direct and Maternal Effects on Growth Traits and the Kleiber Ratio in Moghani Sheep. *World Applied Sciences Journal*, 14(9), 1313–1319.
- Sönmez, R., Kaymakçı, M., Eliçin, A., Tuncel, E., Wassmuth, R., & Taşkın, T. (2009). Türkiye Koyun Islahı Çalışmaları. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, 23(2), 43–65. <https://doi.org/10.20479/uuzfd.09856>
- Supakorn, C., & Pralomkarn, W. (2012). Genetic parameter estimates for weaning weight and Kleiber ratio in goats. In *Songklanakarın J. Sci. Technol* (Vol. 34, Issue 2). <http://www.sjst.psu.ac.th>
- Tüfekci, H. (2023). Yetiştirici Koşullarında Akkaraman Irkı Koyunlarda Döl Verimi ile Kuzularda Büyüme ve Yaşama Gücü Özelliklerinin Belirlenmesi. *Akademik Ziraat Dergisi*, 12(1), 139–144. <https://doi.org/10.29278/azd.1188633>
- TUIK. (2023). *Livestock Production, Number of Sheep and Goat*. <https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1>
- Tüney Bebek, D., & Keskin, M. (2021). Güney Karaman koyunlarının bazı üreme, besi ve süt verim özellikleri. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*, 26(1), 1–7. <https://doi.org/10.37908/mkutbd.729230>
- Unlusoy, I., & Ertugrul, O. (2016). The effects of exon 2 of inhibin βB gene and exon 3 of FSHB gene on litter size in Akkaraman and Bafra sheep breeds. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 22(5).
- Ünal, N. (2002). Survival Rate, Growth Characteristics and Some Body Measurements of Akkaraman and Sakız (Chios) x Akkaraman F<sub>1</sub> Lambs. *Turkish Journal of Veterinary & Animal Sciences*, 26(1), 109–116. <https://journals.tubitak.gov.tr/veterinary/vol26/iss1/17/>
- Yalcin, B. C. (1986). *Sheep and goats in Turkey*. The Food and Agriculture Organization (FAO).
- Yardımcı, M., & Özbeyaz, C. (2001). A study on the comparison of the milk yield and udder characteristics in Akkaraman and Sakız x Akkaraman F<sub>1</sub> crossbreds. *Journal of Lalahan Livestock Research Institute*, 41(2), 63–77.

# Investigation of Some Environmental Factors on Reproductive Characteristics, Milk Traits and Kleiber Ratios of Kilis Goats Reared in the Fertile Crescent of Türkiye

Sabri GÜL<sup>1,\*</sup> , Mahmut KESKİN<sup>1</sup> , Şerafettin KAYA<sup>1</sup> , Mustafa DİKME<sup>2</sup> 

<sup>1</sup>Hatay Mustafa Kemal University, Agricultural Faculty, Department of Animal Science, Antakya/Hatay, Türkiye

<sup>2</sup>Hatay Mustafa Kemal University, Graduate School of Natural and Applied Sciences, Animal Science Department, Antakya/Hatay, TÜRKİYE

\*Corresponding Author

## Article History

Received: 30.05.2024

Accepted: 25.06.2024

First Online: 03.07.2024

## Corresponding Author

E-mail: sabrigul@gmail.com

Tel.: +0903262455845

## Keywords

Environmental factors

Developmental characteristics

Milk yield

Keiber ratio

## Abstract

This study was carried out to determine the effect of environmental factors on some yield traits in Kilis goats. For this purpose, 6000 heads of Kilis goats each year, and a total of 30000 goats and kids born from them for 5 years, were the subject of the research. Milk control in goats was calculated by the ICAR-AT method. At the end of the study, Average birth weight was 3.56 kg, weaning weight was 15.06 kg and daily live weight gain was 191.64 g in kids. KR value found to be 23.6±0.03 in kids. Lactation milk yield in mothers was determined as 367.64 kg. As a result, it was determined that environmental factors were effective on the reproductive parameters and milk yield characteristics of Kilis goats and the developmental characteristics of kids. The crucial determination of these factors in the breeding of this breed will increase the accuracy of selection. In addition, the success will also be increased by starting the transition from the traditional breeding approach to the genomic breeding method and by combining these two strategies.

## Introduction

Agriculture plays a very important role in preventing poverty, ending hunger, ensuring food security, and making the whole world a happy and liveable place. At the same time, it is all the positive events that we can count on behalf of agriculture, science, engineering, health, technology, social life, culture, environment, and humanity. Agricultural production has created a wonderful product variety because of centuries-old traditions, the accumulation of experience in this field and the synthesis of the richness of the geography with the climate in Türkiye. Hosting different cultures in agricultural production, Türkiye has always made a name for itself among the countries of the world with its existing plant and animal genetic resources. In this geography, goat breeding has always kept its importance and raised as a hobby as well as a source of livelihood. For breeding, several studies have been conducted and are still being conducted (Gül *et al.*, 2016; Keskin *et al.*, 2016, 2017; Özdemir & Keskin, 2018; Kurtay, 2019; Gül *et al.*, 2022; Behrem *et al.*, 2022; Gül *et al.*, 2023). These studies span certain time frames, and most of them are brief. However, animal

improvement is a long-term process, and it must be ongoing.

Reproductive characteristics and milk yields are always considered to be the most important factor in increasing productivity in some environmental conditions. The level of yield performance in farm animals depends on genetic and environmental factors. At the beginning of environmental factors are birth type, maternal age, herd management, year, season, and sex. Success in selection and breeding will rise with the identification of these parameters and the elimination of their negative impacts.

Growth characteristics such as birth weight, weaning weight and average daily weight gain are the most important indicator phenotypes for sustainable and economical production in goat breeding and for the farmer to make a more accurate selection decision. In addition, a major part of the income in goat breeding comes from selected animals. For this reason, the selection of animals with high daily weight gain and better feed conversion efficiency should be the main components of a successful goat enterprise. Measuring

the feed efficiency of animals in extensive farming is very difficult and impractical. For this purpose, different selection strategies have been developed according to the growth characteristics and feed conversion rates of animals (Ghafouri-Kesbi, 2011; Illa *et al.*, 2018; Gül *et al.*, 2023).

Kleiber ratio (KR) is a developed and widely used metric to calculate the feed conversion of animals and is calculated by dividing the average daily gain weight by the metabolic weight ( $ADG/WW^{0.75}$ ) and the growth rate divided by the body weight (Kleiber, 1947). The Kleiber ratio, which is an indicator of feed conversion efficiency, can be used in the selection program to increase the biological efficiency of the herd.

The Kilis goat breed has been a very good adaptation to harsh environmental conditions and is widely raised in almost every region of Türkiye. Kilis goat breed is known for high reproductive characteristics and milk yield and is mostly raised in Gaziantep, Kilis, Şanlıurfa and Hatay provinces located on the fertile crescent of Türkiye (Keskin *et al.*, 2004; Gül, 2008; Gül *et al.*, 2022; Keskin *et al.*, 2022).

A breeding program was started in 2011 by the General Directorate of Agricultural Research and Policies to improve milk and reproductive traits in Kilis goats under breeder conditions. In this context, some yield characteristics are investigated in goats.

In this study, within the scope of the breeding project carried out in Gaziantep between 2018-2022, the effects of different environmental factors on the reproductive and milk yield of Kilis goats and the developmental characteristics of kids were investigated, and the results were presented.

Among these environmental factors, seasonal differences between years affect the productivity of the whole flock, while birth type, maternal age, sex, and birth weight affect individual performance. Therefore, estimating the magnitude of all such parameters is critical to developing efficient and successful breeding plans.

In this study, the impact of various environmental conditions on the reproductive and milk yield of Kilis goats as well as the developmental traits of kids were examined, and the results were reported as part of the breeding project conducted in 2018-2022.

## Materials and Methods

### Animal Material and Management

Gaziantep is located at the junction of the Mediterranean Region and South-eastern Anatolia

Region and between 36° 28' and 38° 01' east longitudes and 36° 38' and 37° 32' north latitudes. The climate is transitional between Mediterranean and Continental climates, and the Mediterranean climate predominates. Summers are hot and dry. Winters are very cold and rainy. The average precipitation is 550 millimetres. The temperature ranges between -17°C and 48.8°C. The data used in this study were obtained from goats (50 flocks) included in the improvement of Kilis goats under breeder conditions project within the scope of the National Public Small Ruminant Improvement Project in Gaziantep province. The animal material of the project consists of 6000 heads of females and 300 heads of male goats each year. The data of 30.000 goats and their offspring were analysed in this study. In the research region, there are a total of 55 different herds. The herds have a minimum size of 100 animals and a maximum of 250.

Mating in goats was carried out between August and September as free mating. The bucks were separated from the flocks at least 45 days before the mating. The number of goats mated per buck in a mating season varied from 20 to 25. Goats were exposed to bucks from the beginning of August to the end of September.

Animals were kept under extensive conditions. The goats are grazing in forest and open areas from early morning until late evening according to the seasonal conditions. Water needs were met from natural water resources in the pasture. Although the pasture areas differ in terms of the amount and quality of grass, they consist of pine forests, oak forests, maquis and annual grasses. During the heavy winter days and when the pasture is weak, 500-750 g of barley, bran, cotton seed grain and wheat straw were given as a feed mix per animal. Kids were suckled in the morning and evening and kept in the barn for up to one month of age. All offspring were fed with tree branches (leafy olive and oak branches), barley, wheat straw and kid-growing from 2 weeks of age and weaned at 60 days.

### Data Collection

The kids were ear-tagged shortly after birth, and the mother's ear tag number, birth weight, birth date and gender were all noted. Weaning weights were recorded on the 60<sup>th</sup> day to reveal the growth performance of the kids. Milk yield controls were started in goats that gave birth from the 15th day and continued until they were dry. Milking was done by hand by the breeder, and daily milk yield was determined at 28 days intervals according to the ICAR-AT method. The Fleischman method was used to determine lactation milk yield using the daily milk yield data (Gül, 2008). In this study, the data regarding the fertility parameters were calculated according to Keskin *et al.* (2017) as follows;

Pregnancy rate: (number of does give birth/number of does for mating) \* 100

Infertility rate: (number of does not give birth/number of does for mating) \* 100

Birth rate: (number of does give birth/number of does for mating) \* 100

Single kidding rate: (number of does with single kid/number of does giving birth) \* 100

Twinning rate: (number of does with twin kids/number of does giving birth) \* 100

Triplet rate: (number of does with triplet kids/number of does giving birth) \* 100

Litter size: (number of kids born/number of does giving birth) \* 100

Kid yield: (number of kids born/number of does for mating) \* 100

### Statistical analyses

Statistical analysis on growth characteristics and lactation milk yield The data obtained from the project were evaluated with the SPSS statistical program in terms of basic statistical analysis (SPSS, 2012). For this purpose, Duncan's multiple comparison tests were used to compare birth and weaning weights. The mathematical model of Statistical analysis on growth characteristics and lactation milk yield is given in the following equation (Equation 1 and 2).

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + p_k + \delta_l + e_{ijklm} \quad (\text{Eq. 1})$$

In this model,

$Y_{ijklm}$ , an individual observation,

$\mu$ , the overall mean

$\alpha_i$ , the fixed effect of sex (male, female),

$\beta_j$ , the fixed effect of birth type (single, twin, triplet),

$p_k$ , the fixed effect of the birth year (2018, 2019, 2020, 2021, 2022)

$\delta_l$ , the fixed effect of maternal age (1, 2, 3, 4, 5 $\geq$ ),

$e_{ijklm}$ , the random error.

(Eq. 2)

$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij}$$

In this model,

$Y_{ij}$ , an individual observation,

$\mu$ , the overall mean

$\alpha_i$ , the fixed effect of maternal age (2, 3, 4, 5 $\geq$ ),

$\beta_j$ , the fixed effect of the birth year (2018, 2019, 2020, 2021, 2022)

## Results

All births were completed in February and March in approximately 45 days. The fertility characteristics of Kilis goats obtained between 2018-2022 are given in Table 1. The project requires that each herd must have 6000 head of goats available for mating.

The number of goats giving birth varied according to the years and birth was observed in all the goats in the last year of the study. Accordingly, the rate of multiple births in herds has also increased. Selection is made in herds every year, considering the birth weight, weaning weight and milk yield of the mothers. Multiple birth rates in herds may have increased due to selection.

The growth characteristics of kids calculated by the years are given in Table 2. As can be seen from this table, the birth weights of kids differed over the years, and these differences were found to be statistically significant ( $P < 0.01$ ).

An increase is observed in the birth weight of kids when they reach the last year of the study. It can be said that this increase in birth weight is because of the selection made every year in the herd and the improvement in care and feeding. In this table (Table 2), a different situation was displayed in terms of weaning weights in kids from birth weights. While this feature was fluctuating ( $P < 0.01$ ) until the beginning of 2022 in the project, there was a decrease in the last year. Especially in our country and the region, the severe seasonal conditions (winter) made it difficult for the goats to go out to the pasture and prevented the growth of annual grasses grown in the pasture in spring. Adverse weather conditions and deficiencies in supplementary feeding also affected milk yield, so the development of kids slowed down. This negativity experienced in the last year of the study also negatively affected the average daily earnings due to weaning, and the values obtained by years were found to be statistically significant ( $P < 0.01$ ).

Table 3 shows the growth characteristics of kids according to maternal age, sex, and birth type.

Even though the kid's birth weights are very similar, the age of the mother has an impact on the birth weights. ( $P < 0.01$ ). In this group, the lowest weight was determined in aged 4 ( $3.53 \pm 0.01$  kg) years and the highest in aged 5 and over ( $3.59 \pm 0.01$  kg) ( $P < 0.01$ ). The effect of maternal age was also seen on weaning weights and average daily gains, and both traits were found to be statistically significant ( $P < 0.01$ ). The highest weaning weight and ADG were observed in 2-year-old mothers ( $15.28 \pm 0.02$  kg and  $195.57 \pm 0.40$  g).

It was determined that sex affected the birth weights of kids ( $P < 0.01$ ). Although this difference between males and females was expected, it was very low ( $3.60 \pm 0.01$  kg vs  $3.52 \pm 0.01$  kg). The weaning



**Table 1.** Fertility characteristics by the years

Characteristics/Year	2018	2019	2020	2021	2022
Number of does for mating	6000	6000	6000	6000	6000
Number of does giving birth	5968	5980	5963	5951	6000
Number of born kids	7333	7314	7626	7458	7644
Number of weaned kids	7102	6696	7241	6914	7096
Number of single-born kids	4630	4644	4262	4438	4334
Number of twin-born kids	1338	1336	1635	1504	1639
Number of triplet-born kids	---	---	66	9	27
The fertility rate suitable for mating goats (%)	122.2	121.9	127.1	124.3	127.4
The fertility rate for giving birth (%)	122.9	122.3	127.9	125.3	127.4
Single kidding rate (%)	77.58	77.66	71.48	74.58	72.23
Twin kidding rate (%)	22.42	22.34	27.42	25.27	27.32
Triplet kidding rate (%)	---	---	1.10	0.15	0.45
Survival rate at weaning (60. days)	0.97	0.92	0.95	0.93	0.93
Kidding rate per does (%)	1.22	1.22	1.27	1.24	1.27
Kidding rate per birth (%)	1.23	1.22	1.28	1.25	1.27

**Table 2.** Developmental characteristics of the kids by the years (mean  $\pm$  se)

Year	n	BW	n	WW	ADG	KR
2018	7098	3.50 $\pm$ 0.01 <sup>b</sup>	7098	14.95 $\pm$ 0.02 <sup>c</sup>	190.82 $\pm$ 0.43 <sup>c</sup>	25.0 $\pm$ 0.03 <sup>d</sup>
2019	7331	3.47 $\pm$ 0.01 <sup>a</sup>	6769	14.79 $\pm$ 0.02 <sup>a</sup>	188.70 $\pm$ 0.43 <sup>b</sup>	23.0 $\pm$ 0.08 <sup>a</sup>
2020	7625	3.60 $\pm$ 0.01 <sup>c</sup>	7247	15.46 $\pm$ 0.02 <sup>e</sup>	197.52 $\pm$ 0.33 <sup>e</sup>	24.0 $\pm$ 0.07 <sup>c</sup>
2021	7458	3.59 $\pm$ 0.01 <sup>c</sup>	6920	15.23 $\pm$ 0.01 <sup>d</sup>	193.94 $\pm$ 0.28 <sup>d</sup>	23.3 $\pm$ 0.08 <sup>b</sup>
2022	7643	3.64 $\pm$ 0.01 <sup>d</sup>	7100	14.86 $\pm$ 0.01 <sup>b</sup>	186.99 $\pm$ 0.23 <sup>a</sup>	22.9 $\pm$ 0.07 <sup>a</sup>
P		0.000		0.000	0.000	0.000
Overall	37155	3.56 $\pm$ 0.01	35134	15.06 $\pm$ 0.01	191.64 $\pm$ 0.14	23.6 $\pm$ 0.03

BW, birth weight; WW, weaning weight; ADG, average daily gain; KR, Kleiber ratio

weight of kids was affected by sex ( $P < 0.01$ ). This value was calculated to be 15.20  $\pm$  0.01 kg for females and 14.92  $\pm$  0.01 kg for males. It was determined that gender was also effective in weaning weight and ADG. There is no significant numerical difference was observed between the sex groups in terms of both characteristics, but differences were found to be statistically significant ( $P < 0.01$ ).

The birth type has affected the birth weight, weaning weight and ADG. Average birth weight was found to be 3.59  $\pm$  0.01 kg in single born, 3.52  $\pm$  0.01 kg in twins and 2.76  $\pm$  0.01 kg in triplets. There was no statistical difference between single and twin-born kids, the difference between these two groups and triplets was found to be significant ( $P < 0.01$ ). It was determined that was found to have an impact on WW and ADG as well. There was no statistical difference between single and twins in terms of both characteristics ( $P > 0.05$ ), but a statistical difference was found between these two groups and those born with triplets ( $P < 0.01$ ).

The Kleiber ratio was developed as a reflection of feed conversion efficiency. While 2-year-old goats had the greatest KR value (24.1  $\pm$  0.07), it was found that the

lowest KR value varied depending on the mother's age. a statistical difference was observed between 3 and over-aged and 2 aged in terms of KR ( $P < 0.01$ ). KR values were calculated to be close in female and male kids (23.7  $\pm$  0.04 vs 23.5  $\pm$  0.04). However, this difference was statistically significant ( $P < 0.01$ ). An interesting situation was revealed when KR values were evaluated according to sex. Normally, it was expected that single-born (23.2  $\pm$  0.04) are to be higher than twins (24.3  $\pm$  0.04) and triplets, while triplets received a higher value (25.3  $\pm$  0.02).

Lactation milk yields adjusted for 210 days according to years and ages are given in Table 4.

It was observed that there is a systematic change in the milk yield of Kilis goats depending on age, and the effect of year and age has been determined. Accordingly, the effect of year and age on milk yields was determined ( $P < 0.01$ ). Lactation milk yields decreased in the last year of the study. Due to the long-lasting snowfall in the region, the goats could not be taken to the pasture, and due to the harsh weather conditions, seasonal grasses could not be grazed in sufficient quantities. An insufficient amount of supplementary feeding also caused a decrease in milk

**Table 3.** Developmental characteristics of kids in Kilis goats by the age of the mother, sex, and birth type (mean  $\pm$  se)

Age	n	BW (kg)	n	WW (kg)	ADG (g)	KR
2	6002	3.54 $\pm$ 0.01 <sup>a</sup>	5746	15.28 $\pm$ 0.02 <sup>d</sup>	195.57 $\pm$ 0.40 <sup>d</sup>	24.1 $\pm$ 0.07 <sup>b</sup>
3	8173	3.58 $\pm$ 0.01 <sup>b</sup>	7729	15.03 $\pm$ 0.01 <sup>b</sup>	190.97 $\pm$ 0.29 <sup>b</sup>	23.6 $\pm$ 0.06 <sup>a</sup>
4	9174	3.53 $\pm$ 0.01 <sup>a</sup>	8662	14.78 $\pm$ 0.01 <sup>a</sup>	187.55 $\pm$ 0.30 <sup>a</sup>	23.4 $\pm$ 0.06 <sup>a</sup>
5 and over	13806	3.59 $\pm$ 0.01 <sup>b</sup>	12997	15.17 $\pm$ 0.01 <sup>c</sup>	193.02 $\pm$ 0.23 <sup>c</sup>	23.6 $\pm$ 0.05 <sup>a</sup>
P		0.000		0.000	0.000	0.000
<b>Sex</b>						
Male	18729	3.60 $\pm$ 0.01	17756	15.20 $\pm$ 0.01	193.23 $\pm$ 0.21	23.7 $\pm$ 0.04
Female	18426	3.52 $\pm$ 0.01	17378	14.92 $\pm$ 0.01	190.01 $\pm$ 0.20	23.5 $\pm$ 0.04
P		0.000		0.000	0.000	0.000
<b>Birth type</b>						
Single	22230	3.59 $\pm$ 0.01 <sup>b</sup>	20667	15.11 $\pm$ 0.01 <sup>b</sup>	191.90 $\pm$ 0.20 <sup>b</sup>	23.2 $\pm$ 0.04 <sup>a</sup>
Twin	14892	3.52 $\pm$ 0.01 <sup>b</sup>	14434	15.00 $\pm$ 0.01 <sup>b</sup>	191.29 $\pm$ 0.20 <sup>b</sup>	24.3 $\pm$ 0.04 <sup>ab</sup>
Triplet	33	2.76 $\pm$ 0.01 <sup>a</sup>	33	13.45 $\pm$ 0.01 <sup>a</sup>	178.16 $\pm$ 3.45 <sup>a</sup>	25.3 $\pm$ 0.02 <sup>b</sup>
P		0.000		0.000	0.003	0.000
<b>Overall</b>	<b>37155</b>	<b>3.56 <math>\pm</math> 0.01</b>	<b>35134</b>	<b>15.06 <math>\pm</math> 0.01</b>	<b>191.64 <math>\pm</math> 0.14</b>	<b>23.6 <math>\pm</math> 0.03</b>

BW, birth weight; WW, weaning weight; ADG, average daily gain; KR, Kleiber ratio

**Table 4.** Milk yield of Kilis goats (l)

Year	LMY	Age	LMY
2018	324.53 $\pm$ 0.93 <sup>a</sup>	2	348.19 $\pm$ 1.27 <sup>a</sup>
2019	359.06 $\pm$ 0.97 <sup>b</sup>	3	370.62 $\pm$ 1.11 <sup>c</sup>
2020	373.54 $\pm$ 1.05 <sup>c</sup>	4	381.19 $\pm$ 0.94 <sup>d</sup>
2021	408.19 $\pm$ 1.02 <sup>d</sup>	5 and over	362.98 $\pm$ 0.85 <sup>b</sup>
2022	359.63 $\pm$ 1.30 <sup>b</sup>		
P	0.000		0.000
<b>Overall</b>		<b>367.64<math>\pm</math>0.51</b>	

LMY, lactation milk yield

yield. It was determined that there is an increase in milk yield with the effect of selection made in the herd and the improvement of environmental factors.

## Discussion

Table 1 shows reproductive characteristics of Kilis goats. Factors influencing reproductive performance in any animal can be classified as intrinsic or extrinsic. Extrinsic factors are associated with the environment, management practices, and farm conditions, whereas intrinsic factors are related to the genotype of the animal. Tatar *et al.* (2019) calculated the pregnancy rate as 95.30%, the birth rate as 99.30%, the single birth rate as 53.35%, the twin birth rate as 41.73%, the triplet birth rate as 4.08%, and the fertility rate as 150.70% in Kilis goats. Keskin *et al.* (2018) reported that the fertility range in Kilis goats was between 123-136%. Therefore, our results are in line with the reports of researchers.

The growth performance of kids determined by the years are given in Table 2. The variation in birth weight, weaning weight and average daily gain in different years can be considered a reflection of rainfall, environmental conditions, pasture quality, grass, and feed production. In animal production, effective reproduction is crucial for overall production. Goat

reproduction is influenced by genetic factors and environmental with a high sensitivity to the latter (Song & Sol, 2006). Gül *et al.* (2016) reported that birth and weaning weights in Kilis goats are affected by the age of the mother, gender and year, and the average birth and weaning weights are 3.0 kg and 16.0 kg. Similarly, Özdemir & Keskin (2018) stated that the developmental characteristics of Kilis goat kids are affected by the environment. The differences between the numerical values obtained can be explained as there is a wide variation in Kilis goats and it is caused by environmental effects. Our results for growth performance correlated with a review of the literature.

Table 3 shows the developmental characteristics of kids of Kilis goats by the maternal age, sex, and birth type. Does age have a significant effect on the birth weight of kids. The significant influence of maternal age on the birth weight of kids was reported in previous studies (Elmas *et al.*, 2020; Ceyhan *et al.*, 2021; Gül *et al.*, 2022). Also, the dam's maternity ability affects the weaning weight of kids (Gül *et al.*, 2017).

It is well known that males are heavier birth weights than females. Researchers working on Kilis goats (Keskin *et al.*, 2017; Gül *et al.*, 2022; Keskin *et al.*, 2022) and other breeds (Kuthu *et al.* 2013; Dinçel *et*



*al.*, 2019; Topbaş & Dağ, 2019; Erdem *et al.*, 2022) have published a research article on this subject. Also, sex affects growth characteristics due to the positive relationship between male gonadal cells and growth hormones related to growth performance (Mourad and Anous, 1998; Koşum *et al.*, 2004; Gökdal *et al.*, 2013).

In multiple pregnancies, due to the presence of multiple fetuses, the secretion of placental lactogen, progesterone and prolactin hormones, which are mammary gland stimulants, increases and more milk is produced (Lobo *et al.* 2017). However, females carrying multiple fetuses during pregnancy tend to produce more milk due to the lactation reflex and physiological mechanism that prepares the udder to produce more milk (Idowu and Adewumi 2017).

The analysis of the variance table revealed that maternal age, sex, and birth type had a significant effect on birth weight, weaning weight, and ADG. Keskin *et al.* (2018) reported that birth weight in Kilis goats was affected by maternal age, sex, and birth type. The birth weight in kids ranged between 3.4 and 3.7 kg, and weaning weight was between 11.9 and 12.8 kg in Kilis province. Gül *et al.* (2022) stated that environmental factors were effective on the developmental characteristics of Kilis goat kids in Gaziantep and calculated the average birth weight as 3.5 kg, weaning weight as 15.2 kg and average daily body weight gain of 193.4 g in kids. Our findings of the study were in partial agreement with the findings of many researchers (Zelege, 2007; Adenaike and Bemji 2011; Güngör *et al.*, 2021; Keskin *et al.*, 2022; Tüfekci, 2023). The slight variations between the findings from this study and those from other studies are believed to be caused by the large variety of breeds, individual effects, and environmental factors.

The values we calculated for KR in Kilis goats were higher than the reports of researchers in different breeds (Supakorn & Pralomkarn, 2012; Kahadda *et al.*, 2018; Hammoud *et al.*, 2019). However, it is in agreement with the values obtained from Kilis goats by Gül *et al.* (2023). According to the results of this study, it can be said that environmental factors played a role on the Kleiber ratios in the kids of Kilis goats.

Descriptive values for lactation milk yield in Kilis goats are given in Table 4. Milk yield in animals increases with age due to increases in metabolic activity, hormone levels used in milk synthesis, secretory cells and food intake (Idowu and Adewumi 2017). Milk yield in goats increases physiologically up to 4-5 lactations and tends to decrease in subsequent lactation periods (Kaymakçı, 2006). Özdemir & Keskin (2018) reported lactation milk yield in Kilis goats as 212.8 kg in Gaziantep and 306.8 kg in Kilis provinces; Gül *et al.* (2016), on the other hand, found this value to be 293.7 l in the control group and 408.3 l in the supplemental feeding group in Hatay province;

Daşkıran *et al.* (2022) reported the milk yield of Kilis goats as 201.05 kg in breeder conditions. Our findings the present study is higher than Özdemir & Keskin (2018) and Daşkıran *et al.* (2022), in line with the report of Gül *et al.* (2016) in the supplementary feeding group.

Kilis goats are an important genetic resource that has been taken to different regions in Türkiye. Studies have been carried out on this breed in the regions where it is grown. Koşum *et al.* (2004) in İzmir, Alizadehasl and Ünal (2011) in Ankara, Gül *et al.* (2016) in Hatay, Tatar *et al.* (2019), in Diyarbakir, Keskin *et al.* (2018) Kilis province and Gül *et al.* (2023) in Gaziantep province conducted studies on progeny, milk yield and kid yield in Kilis goats. When all studies are compared, it is seen that milk yield performances in goats are very close to each other, but it can be said that the differences are caused by care, feeding and environmental factors. In addition, as seen in this study, there are serious variations between regions in Kilis goats. From this point of view, it becomes necessary to go a long way in terms of improvement.

## Conclusion

Livestock activities should be evaluated with their economic, social, cultural and environmental aspects. If one of these components is absent, discussing sustainability will be challenging. The first step towards a well-structured and sustainable animal breeding and conservation program is to uncover detailed information about intra- and inter-species genetic diversity. This shows how important it is to reveal the genetic structure of the breeds.

Data from this study were provided under extensive farm conditions. The inclusion of modern breeding methods in this system, which is completely carried out in traditional and extensive conditions, will provide an increase in the quantity and quality of production and continuity in animal breeding.

Some reproductive traits and milk yield of Kilis goats that is one of the local genetic resources were investigated in this study. It is seen that there is a significant variation in Kilis goats. The reproductive parameters and milk yield are generally affected by factors such as year, age, management, nutrition and regional conditions in does. Also, it has been determined that non-genetic factors are effective on the growth characteristics of kids. It is crucial for selection to identify these environmental elements that have an impact on animal growth and development and to take them into account when choosing animals. In addition, taking the first steps from the classical breeding method to the genomic breeding method and using these two methods together will increase the success. If we make a general evaluation of Kilis goats, it can be easily said

that this breed has an important place for the country's economy.

### Funding Information

This project was supported by Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies under National Public Small Ruminant Improvement Project in Gaziantep province (Project number: 27KLS2012-01).

### Author Contributions

Sabri GÜL: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing -original draft; Mahmut KESKİN: Data Curation, Methodology, Visualization, Writing -review and editing; Şerafettin KAYA: Data Curation, Methodology, Visualization, Writing -review and editing; Mustafa DİKME: Data Curation, Methodology, Visualization, Writing -review and editing

### Conflict of Interest

“The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.”

### Acknowledgements

We would like to thank TAGEM, Gaziantep Sheep and Goat Breeders' Association, and the technical staff of project for their valuable contributions to the project.

### References

- Adenaike AS, Bemji MN (2011) Effects of environmental factors on birth weights and weaning weights of West African dwarf goats under intensive and extensive management systems, *Advances in Agri. Biotech.*, 12011: 9-14.
- Alizadehasl M, Ünal N (2011) Some morphological traits of Kilis, Norduz and Honamlı indigenous goats breeds. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, 51: 81-92.
- Behrem S, Keskin M, Gül S, Ünay E, Satılmış M, Ünal M, Saçlı Y, Erişek, A (2022) Investigation on the Hair characteristics of Damascus and Kilis goats. *Journal of Natural Fibers*, 19: 9562-9568.
- Ceyhan A, Çınar M, Serbester U (2022) Kid growth performance and reproductive characteristics of Hair goats raised under breeder conditions. *Turkish Journal of Veterinary and Animal Sciences*, 46: 592-598.
- Dinçel D, Ardıçlı S, Samlı H, Ogan MM, Balcı F (2019) Comparison of Kilis goats raised in Gaziantep and Kilis provinces due to some morphological and physiological characteristics. *Turkish Journal of*

*Agriculture - Food Science and Technology*, 7: 1541-1547.

- Erdem E, Bulut ETÖ, Erat S (2022) Comparison of growth and development characteristics of hair and Damascus kids reared under extensive conditions. *Kocatepe Veterinary Journal*, 15: 84-93.
- Gül S (2008) Comparison of different goat genotypes of performances under east mediterranean region conditions. Hatay Mustafa Kemal University Natural Sciences, Department of Animal Science, Doctoral thesis. 125p, Hatay, Türkiye.
- Gül S, Keskin M, Göçmez Z., Gündüz Z (2016) Effects of supplemental feeding on performance of Kilis goats kept on pasture condition. *Italian Journal of Animal Science*, 15: 110-115.
- Gül S, Keskin M, Kaya, Ş (2022) Effects of environmental factors on growth performance of Kilis goat in Gaziantep Province. *Livestock Studies*, 62: 16-20.
- Gül S, Arzık Y, Kızılaslan M, Behrem S, Keskin M (2023) Heritability and environmental influence on pre-weaning traits in Kilis goats. *Tropical Animal Health and Production*, 55: 85. <https://doi.org/10.1007/s11250-023-03509-3>.
- Güngör İ, Alkoyak K, Öz S, Koncagül S (2021) Growth, survival rate, and some reproductive characteristics of Hair goat under breeder conditions in Kahramanmaraş Province. *Turkish Journal of Veterinary and Animal Sciences*, 45: 1022-1029.
- Daşkıran İ, Yılmaz A, Günbey VS (2022) A study on lactation milk yield and milk quality characteristics of Kilis goats in farm conditions. *Journal of Tekirdag Agricultural Faculty*, 19: 678-687.
- Ghafouri-Kesbi F, Abbasi MA, Afraz F, Babaei M, Baneh H, Arpanahi RA (2011) Genetic analysis of growth rate and Kleiber ratio in Zandi sheep. *Tropical Animal Health Production*, 43: 1153-1159.
- Gökdal Ö, Atay O, Özüğür A K, Eren V (2013) Growth and survival characteristics of Hair goat and its crosses with Saanen and Alpine goats under rural farm conditions. *Journal of Animal Production*, 54 (1): 30-37.
- Hammoud MH, Salem MMI (2019) Estimates of variance components and heritabilities of pre-weaning growth traits of Anglo-Nubian and Baladi kids. *Egyptian Journal of Animal Production*, 56: 121-127.
- Idowu ST, Adewumi OO (2017) Genetic and non-genetic factors affecting yield and milk composition in goats. *Advances in Dairy Research*, 5: 1000175.
- Illa SK, Gollamoori G, Nath S (2018) Direct and maternal variance components and genetic parameters for average daily body weight gain and Kleiber ratios in Nellore sheep. *Tropical Animal Health and Production*, 51: 155–163.
- Kaymakçı M (2006) Goat Breeding. Ege University, Agricultural Faculty. İzmir Province Sheep-Goat

- Breeders' Association Publications No: 2, Meta Edition, Bornova-İzmir.
- Keskin M, Şahin, A, Biçer O, Gül S (2004) Comparison of the Behaviour of awassi lambs in cafeteria feeding system with single diet feeding system. *Applied Animal Behaviour Science*, 85: 57-64.
- Keskin M, Gül S, Can E, Gündüz Z (2016) Milk yield and reproductive traits of Shami and Kilis x Hair goat crossbred genotypes under semi-intensive conditions. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, 56: 20-24.
- Keskin M, Gül S, Biçer O, Daşkıran İ (2017) Some reproductive, lactation, and kid growth characteristics of Kilis goats under semiintensive conditions. *Turkish Journal of Veterinary and Animal Sciences*, 41: 248-254.
- Keskin M, Gül S, Daşkıran İ, Gündüz İ, Biçer O, Behrem S (2018) Kilis goat breeding programme and some yield parameters in farm condition. 1<sup>st</sup> International GAP Agriculture and Livestock Congress. P.9. 25-27 April, Şanlıurfa-Türkiye.
- Keskin M, Gül S, Kaya Ş (2022) Five-year term evaluation of the project named "Kilis Goat National Breeding Project in Kilis Province. *Livestock Studies*, 62: 7-15.
- Khadda BS, Singh B, Singh DV, Singh, SK, Singh CB, Singh JL (2018) Genetic and non-genetic parameters of Kleiber ratio in Pantja goat under field conditions of Tarai region of Uttarakhand. *Indian Journal of Animal Sciences*, 88: 1370–1373.
- Kleiber, M., 1947. Body size and metabolic rate. *Physiological reviews*, 27, 511–541.
- Koşum N, Taşkın T, Akbaş Y, Kaymakçı M (2004) Heritability estimates of birth and weaning weights in Saanen, Bornova and Saanen x Kilis goats. *Pakistan Journal of Biological Sciences*, 7 (11): 1963-1966.
- Kurtay T (2019). Determination of growth and life characteristics of Damascus x Kilis F<sub>1</sub> kids in breeders conditions. Dicle University, Natural Sciences, Master thesis, Department of Animal Science. 53 p. Diyarbakır-Türkiye.
- Kuthu ZH, Javed K, Babar ME, Sattar A, Abdullah M (2013) Environmental effects of growth traits of Teddy goats. *The Journal of Animal & Plant Sciences*, 23: 692-698.
- Lôbo AMBO, Lôbo RNB, Facó O, Souza V, Alves AAC, Costa AC, Albuquerque MAM (2017) Characterization of milk production and composition of four exotic goat breeds in Brazil. *Small Ruminant Reserach*, 153: 9-16.
- Mourad M, Anous MR (1998) Estimates of genetic and phenotypic parameters of some growth traits in Common African and Alpine crossbred goats. *Small Ruminant Research*, 27: 197-202.
- Özdemir FH, Keskin, M (2018) Comparison of Kilis goats raised in Gaziantep and Kilis provinces due to some morphological and physiological characteristics. *Journal of Agricultural Faculty of Mustafa Kemal University* 23: 115-123.
- Özkan E, Saatçı M, Ağaoğlu M, Korkmaz Ö, Akbaş AA, Özçelik MM, Gezer G, Yılmaz G (2020) Reproductive performance and kid growth until weaning in Hair goat reared on-farm conditions in Turkey. *Turkish Journal of Veterinary and Animal Sciences*, 44: 370-376.
- Song H, Sol HS (2006) Reproductive performance of Korean native goats under natural and intensive conditions. *Small Ruminant Research*, 65: 284-287.
- SPSS (2012) IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp.
- Supakorn C, Pralomkarn W (2012) Genetic parameter estimates for weaning weight and Kleiber ratio in goats. *Songklanakarın Journal of Science Technolgy*, 34: 165-172.
- Tatar AM, Tuncer SS, Şireli HD (2019) Comparison of yield characteristics of Damascus and Kilis goats in dry climatic conditions. *Austral Journal of Veterinary Sciences*, 51: 61-66.
- Topbaş H, Dağ B (2019) Some reproductive characteristics of Honamlı goats. *Selcuk Journal of Agriculture and Food Sciences*, 33: 223-226.
- Tüfekçi H (2023) Determination of some yield characteristics of Hair goats under extensive conditions. *MAS Journal of Applied Sciences*, 8: 329–335.
- Zelege ZM (2007) Environmental influences on preweaning growth performances and mortality rates of extensively managed Somali goats in Eastern Ethiopia. *Livestock Reserach Rural Development*, 19(12).

# Evaluation of the Relationship of Some Environmental Factors and Number of Inseminations per Pregnancy with Milk Yield in Holstein Cows

Onur ERZURUM<sup>1,\*</sup>, Tamer KAYAR<sup>2</sup>

<sup>1</sup> Selcuk University Karapınar Vocational School Veterinary Department, Konya, Türkiye.

<sup>2</sup> Aksaray University Faculty of Veterinary Medicine Department of Animal Science, Aksaray, Türkiye.

## Article History

Received: 06.06.2024

Accepted: 29.06.2024

First Online: 08.07.2024

## Corresponding Author

E-mail: onurerzurum@selcuk.edu.tr

Tel: +90 555 6117792

## Keywords

Fertility

Holstein

Insemination per pregnancy

Milk yield

## Abstract

In order to investigate the relationship between some environmental factors and the number of artificial inseminations per pregnancy with milk yield, data of 90 healthy Holstein breed dairy cows in a private dairy farm in Karapınar district of Konya province were taken. For the utilization of the data, age, number of artificial inseminations, lactation yield, calving season and sequence of lactation records were taken from the herd management program in the enterprise and analysed. As a result of the analysis, the number of artificial inseminations per pregnancy in 2016 was 1.79 and lactation milk yield was 10079,41 kg; the number of artificial inseminations per pregnancy in 2017 was 2.16 and lactation milk yield was 9767.94 kg. There was a statistically significant relationship between the number of artificial inseminations per pregnancy and milk yield during lactation period in both years. As a result, the increase in the number of artificial inseminations per pregnancy negatively affected the average milk yield of lactation period. Genetic and environmental factors are thought to play a major role in this effect.

## Introduction

There are many factors that affect the profitability of dairy cows, such as fertility and milk yield, calving interval, dry period, number of artificial inseminations per pregnancy, age at first service. The most important of these factors is the ability to produce a good milk yield and one calf per year from healthy animals, which depends on reproductive performance. Reproductive performance is measured on the basis of numerical values such as service period, number of artificial inseminations per pregnancy, calving interval (Gökcan

*et al.*, 2021). These measurements are made possible by ensuring herd continuity and cow conception (Bakır and Kibar, 2019). After the calving period, the period until the formation of a new pregnancy is called the service period (Gül and Karaca, 2022). Tüzemen (2020) reported that this period should be 85 days.

The lactation period in which the cow begins to produce milk at the end of calving is accepted as a standard of 305 days (Alpan and Aksoy, 2009). Cows with a lactation period of less than 305 days are evaluated on the basis of the number of milking days if they are spontaneously dry, and if the milking period is stopped

due to disability, reform, forced slaughter and disease, correction factors are applied according to 305 days. If the lactation period is longer than 305 days, it is assumed that milking takes place twice a day and correction factors are applied according to 305 days (McDaniel *et al.*, 1965). The lactation period ends with the dry period, which should be 60 days. If the dry period is longer than 60 days or shorter than 40 days, the yield is reduced in the next lactation period (Homan and Wattiaux, 2008).

In dairy farms, if the conditions for resuming pregnancy are not met, the service period is extended and the number of artificial inseminations per pregnancy increases. As a result, the cost of AI per pregnancy also increases (Alkan *et al.*, 2021). In addition to artificial insemination at the right time to initiate pregnancy, there are other factors that affect reproductive performance. These factors are generally expressed as temperature stress, ration, inaccurate estrus detection, foot disease, udder health and metabolic diseases (Walsh *et al.*, 2011).

The number of inseminations per pregnancy refers to the number of artificial inseminations performed to get the cow pregnant again after calving. Ideally, this number should be less than 1.5 and this situation is considered successful. If the number of inseminations per pregnancy is 1.8 and above, it is considered unsuccessful (Kaya, 2013). Inchausti *et al.* (2010) stated that the number of artificial inseminations per pregnancy should be 1.61 to ensure the profitability of the farm. In the literature review, there are different reports on the number of inseminations per pregnancy (Bilgic and Yener, 1999; Kaygısız, 1997; Yıldırım, 1999; Duru and Tuncel, 2002; Orman, 2003; Yaylak, 2003; Sağlam and Ugur, 2007; Bayrıl and Yılmaz, 2010; Sahin and Ulutas, 2011; Tangorra *et al.*, 2022).

Kaya *et al.* (2003) found the average lactation yield of Holstein, which are known to have the highest milk yield, to be 6232 kg of Italian origin; Yaylak and Kumlu (2005) 6341 kg; Özkök and Uğur (2007) 6729.2 kg; Orman and Oğan (2008) 4535 kg; Şahin and Ulutaş (2010) 6976 kg; Tutkun (2015) 6198 kg; Sarar and Tapkı (2017) 6588 kg; Genç and Sosyal (2018) 6189 kg.

Looking at the data obtained by Karakçı (1990) in his study where the number of inseminations per pregnancy and the 305-day adjusted lactation milk yield average were analysed together in Holstein cows of different origins, a positive correlation can be mentioned between the number of inseminations per pregnancy and the 305-day adjusted lactation milk yield.

In this study, Holstein cattle were preferred as animal material due to the popularity of the breed and high lactation efficiency. There are studies in the literature (Erdem *et al.*, 2007; Sahin and Ulutas, 2010) that have investigated the effect of number of inseminations per pregnancy on reproductive traits.

However, the lack of sufficient number of studies investigating the effect of number of inseminations per pregnancy on milk yield shows that this study is original and it is believed that it will provide new information for future studies. This study was conducted to investigate the effect of some environmental factors, especially the number of inseminations per pregnancy, on the milk yield of Holstein cows in private dairy farms in Karapınar district of Konya province between 2016 and 2017.

## Materials and Method

The study material was obtained from the herd management programme of a private dairy farm in Karapınar district, Konya province, by collecting data from 90 healthy Holstein breed milking cows in 2016 and 2017. Data belonging to the same animals were selected to be used in the analyses. The records during this period included age, number of inseminations per pregnancy, number of days milked, milk yield during the lactation period, calving season and sequence of lactation.

The lactation periods of cows with lactation periods shorter than 305 days were calculated based on the number of days milked, those whose lactation period was terminated due to disease were calculated by applying correction factors according to 305 days, those with lactation periods longer than 305 days were calculated according to 2 milkings per day and by applying correction factors according to 305 days.

For calving season, December, January, February were grouped as winter season, March, April, May as spring season, June, July, August as summer season and September, October, November as autumn season. The number of inseminations per pregnancy was grouped as 1-2; 3-4; 5-6; 7 and above 4.

The SPSS 26 package was used for statistical analysis of the data obtained. The data were normally distributed (Tabachnick and Fidell, 2013). Correlation was used to determine if there was a relationship between the variables; regression analysis was used to determine the degree of relationship between the variables ( $P < 0.05$  significant;  $P < 0.01$  highly significant). The following formula has been used in regression analyses for both years.

$$y = a + bx_1 + bx_2 + \dots + bx_z$$

In which;  $y$  = dependent variable,  $a$  = constant,  $b$  = regression coefficient,  $x$  = independent variable

The ethics committee report required for the study was obtained with the decision of the Ethics Committee of the Experimental Animal Production and Research Centre (SUVDAMEK) of the Faculty of Veterinary Medicine, Selcuk University, dated 02.11.2023 and numbered 2023/115.

## Results and Discussion

It was determined that all values for both years exhibited a normal distribution, and the mean values and standard errors for the variables are provided in Table 1.

The results of the correlation analysis (Table 2) indicated that for the year 2016, there was a significant

correlation between lactation yield and both age and sequence of lactation ( $P < 0.05$ ). Additionally, there was a highly significant correlation between the number of inseminations per pregnancy and lactation yield adjusted to 305 days ( $P < 0.01$ ). Similarly, the calculations for the year 2017 also showed a highly significant correlation between lactation yield and these variables ( $P < 0.01$ ).

**Table 1.** Means and standard errors of the variables.

Variable	Mean $\pm$ Std Error	
	2016	2017
Lactation yield (kg)	10079.41 $\pm$ 3462.573	9767.94 $\pm$ 2467.123
Age	3.77 $\pm$ 1.374	4.94 $\pm$ 1.369
Inseminations per pregnancy	1.79 $\pm$ 0.977	2.16 $\pm$ 1.027
Number of days milked	364.41 $\pm$ 109.736	353.61 $\pm$ 69.185
305 days corrected for milk yield	8225.29 $\pm$ 1605.399	8315.06 $\pm$ 1600.389
Sequence of lactation	2.49 $\pm$ 1.603	3.52 $\pm$ 1.173

The correlations between age and the number of inseminations per pregnancy, as well as age and lactation yield adjusted to 305 days, were significant for the year 2016. Additionally, the correlation between age and sequence of lactation was found to be highly significant.

The correlation between the number of inseminations per pregnancy and the sequence of lactation was observed to be significant for both 2016 and 2017.

When examining the correlation between lactation yield adjusted to 305 days and the sequence of

lactation, a significant correlation was found for 2016, whereas no correlation was found for 2017.

According to the t-test results for the significance of the regression coefficients (Table 3), the lactation yield adjusted to 305 days can be considered a significant determinant of lactation milk yield for the years 2016 and 2017.

In studies investigating the number of inseminations per pregnancy, the following values were reported: Kaygısız (1997) found 2.19; Bilgiç and Yener (1999) found 1.4; Yıldırım (1999) found 1.95; Duru and Tuncel (2002) found 1.33; Orman (2003)

**Table 2.** Correlation analysis of variables.

2016	Lactation yield (kg)	Age	Inseminations per pregnancy	305 days corrected for milk yield	Calving season
Age	0.019*				
Inseminations per pregnancy	0.000**	0.031*			
305 days corrected for milk yield	0.000**	0.040*	0.328		
Calving season	0.167	0.333	0.276	0.320	
Sequence of lactation	0.005*	0.000**	0.031*	0.003*	0.464
2017					
Age	0.491				
Inseminations per pregnancy	0.000**	0.085			
305 days corrected for milk yield	0.000**	0.493	0.421		
Calving season	0.288	0.073	0.297	0.078	
Sequence of lactation	0.354	0.000**	0.045*	0.378	0.130

\* $p < 0.05$

\*\* $p < 0.01$

found 1.69; Yaylak (2003) found 1.87; Sağlam and Uğur (2007) found 1.60; Bayril and Yılmaz (2010) found 1.47; Şahin and Ulutaş (2011) found 1.59; Tangorra *et al.*

(2022) found 2.2-2.9. According to Kaya (2013), the value of 1.79 obtained for the year 2016 fell in between and did not show similarity to other studies. Similarly,

**Table 3.** Regression analyses for predicting variables with lactation milk yield.

Variable	Std error	$\beta$	t	P
<b>2016</b>				
Constant*	504.054		-16.242	0.001
Age	122.746	0.028	0.0569	0.571
Inseminations per pregnancy	144.801	-0.023	-0.567	0.572
305 days corrected for milk yield	0.047	0.535	24.322	0.001
Calving season	66.781	-0.015	-0.713	0.478
Sequence of lactation	162.880	-0.057	-1.144	0.256
<b>2017</b>				
Constant*	399.611		17.270	0.001
Age	81.972	-0.045	-0.993	0.323
Inseminations per pregnancy	58.329	-0.021	-0.853	0.396
305 days corrected for milk yield	0.031	0.679	33.851	0.001
Calving season	46.014	0.002	0.088	0.930
Sequence of lactation	96.392	0.019	0.418	0.677

\* milk yield in lactation

(For 2016 R:982 R<sup>2</sup>=0.965 F<sub>(8,90)</sub>=381.801 P:0.001)

(For 2017 R:982 R<sup>2</sup>=0.965 F<sub>(8,90)</sub>=381.801 P:0.001)

according to the same statement, the value of 2.16 obtained for the year 2017 showed similarity to the values found by Kaygısız (1997) and Tangorra *et al.* (2022) but was considered unsuccessful. According to Inchausti *et al.* (2010), the farm failed to reach the ideal number for economic profitability.

The results of 305-day milk yield in the studies carried out by some researchers; Kaya *et al.* (2003) found 6232 kg for Italian origin Holsteins; Yaylak and Kumlu (2005) found 6341 kg; Özkök and Uğur (2007) found 6729.2 kg; Orman and Oğan (2008) found 4535 kg; Şahin and Ulutaş (2010) found 6976 kg; Tutkun (2015) found 6198 kg; Sarar and Tapkı (2017) found 6588 kg; and Genç and Sosyal (2018) found 6189 kg. The average lactation milk yield for the year 2016 in this study was 10079.41 kg, and for the year 2017, it was 9767.94 kg. These values were higher than those reported in these studies and did not show similarity. In a study by Karakçı (1990) examining both the number of inseminations per pregnancy and lactation milk yield adjusted to 305 days in cows, the values were reported as follows: for Israeli origin Holsteins, 1.83 and 5119.44 kg; for German origin Holsteins, 1.74 and 4394.17 kg; for American origin Holsteins, 1.48 and 4382.76 kg. Israeli origin Holsteins had higher average milk yields during both the pregnancy per insemination and lactation periods compared to

German and American origin ones. Israeli origin Holsteins had the highest pregnancy per insemination and lactation yields. The average number of inseminations per pregnancy and lactation milk yield adjusted to 305 days in this study were 1.79 and 8225.29 kg for 2016, and 2.16 and 8315.06 kg for 2017, respectively. These values are consistent with the findings of Karakçı (1990). According to the study's findings, there is a negative correlation between the number of inseminations per pregnancy and milk yield. That is, while the number of inseminations per pregnancy increased in 2017, the lactation milk yield decreased. As a result of the regression analysis, an increase of 0.37 in the number of inseminations per pregnancy resulted in a loss of 311.47 kg in lactation milk yield, with a loss of 3.4 kg per animal. The analysis results indicate that the number of inseminations per pregnancy alone does not affect milk yield, and many genetic and environmental factors also affect milk yield.

In studies on the effect of calving season on milk yield, some have stated that it affects milk yield, while others have stated that it does not (Ozcan, 1994; Thaler *et al.*, 1996; Pelister, 1998; Reyes, 1998). It has been suggested that the effect of the season on milk yield may arise from factors such as temperature, body



weight, breed, and diet (Linwill and Pardue, 1992). In this study, it was found that the effect of the season on lactation milk yield was not statistically significant ( $P>0.05$ ).

There are researchers who report that sequence of lactation affects lactation milk yield (Kurt, 2001; Erdem *et al.*, 2007), as well as researchers who report that there is no effect (Bakir and Cetin, 2003; Tekerli and Gundogan, 2005). While the effect of sequence of lactation was found to be significant for lactation yield in 2016, it was found to be insignificant for 2017. The results obtained in the study support both of these statements expressed by researchers.

## Conclusion

In conclusion, according to the study, there is a high-level correlation between the number of inseminations per pregnancy and lactation milk yield, and the regression analysis indicates that an increase in the number of inseminations per pregnancy leads to a decrease in lactation milk yield. Furthermore, an increase in the number of inseminations per pregnancy increases labor requirements, time loss, and causes significant economic losses to the farm. Therefore, inseminations should be performed at the right time and with proper technique. Indeed, managerial errors in farms can also lead to an increase in the number of inseminations per pregnancy and a decrease in lactation milk yields. Additionally, investigating the effects of these and similar factors in subsequent studies will be beneficial for breeders.

## Conflict of Interest

The authors declare there is no conflict of interest.

## References

- Alkan, H., Satılmış, F., Yeşilkaya, Ö. F., Çiftçi, M. F., Alkan, K. K., & Erdem, H. (2021). The effect of carprofen treatment on conception rate after insemination in cows with increased days in milk. *Kocatepe Veterinary Journal*, 14(1), 83-90. <https://doi.org/10.30607/kvj.849190>
- Alpan, O., & Aksoy, A. R. (2009). *Siğir Yetiştiriciliği ve Besiciliği*. 5th Edt. Erzurum: Zafer Ofset Matbaacılık.
- Bakır, G., & Çetin, M. (2003). Reyhanlı Tarım İşletmesi'nde yetiştirilen Siyah Alaca sığırlarda döl ve süt verim özellikleri. *Turkish Journal of Veterinary & Animal Sciences*, 27(1), 173-180.
- Bakır, G., & Kibar, M. (2019). Muş ilinde özel süt sığırcılığı işletmelerinde üreme faaliyetlerinin belirlenmesi. *Journal of Agriculture and Nature*, 22(5), 772-780. <https://doi.org/10.18016/ksutarimdogra.vi.536831>
- Bayrıl, T., & Yılmaz, O. (2010). Kazova Vasfi Diren Tarım İşletmesinde yetiştirilen Siyah Alaca sığırların döl verimi özellikleri. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 21(3), 163-167.
- Bilgiç, N., & Yener, M. (1999). Ankara Üniversitesi Ziraat Fakültesi Zootečni Bölümü sığırcılık işletmesinde yetiştirilen Siyah Alaca ineklerde bazı süt ve döl verimi özellikleri. *Journal of Agricultural Sciences*, 5(2), 81-84.
- Duru, S., & Tuncel, E. (2002). Koçuş Tarım İşletmesinde yetiştirilen Siyah-Alaca sığırların süt ve döl verimleri üzerine bir araştırma. 2. döl verim özellikleri. *Turkish Journal of Veterinary & Animal Sciences*, 26(1), 103-107.
- Erdem, H., Atasever, S., & Ertuğrul, K. (2007). Gökhöyük Tarım İşletmesinde yetiştirilen Siyah Alaca sığırların süt ve döl verim özellikleri 2. döl verim özellikleri. *Anadolu Journal of Agricultural Sciences*, 22(1), 47-54.
- Genç, S., & Soysal, M. İ. (2018). Türkiye Siyah Alaca siğir popülasyonlarında süt ve döl verimi. *Journal of Tekirdag Agricultural Faculty*, 15(01), 76-85.
- Gökcan, E., Coşkun, G., & Aytekin, İ. (2021). Süt sığırlarında döl verimi ve senkronizasyon metotları. *In: International Siirt Scientific Research Congress*. Kasım, 5-7, Siirt, Türkiye.
- Gül, B., & Karaca, F. (2022) Farklı büyüklükteki süt ineği çiftliklerinde döl verimi parametrelerinin belirlenmesi. *Antakya Veteriner Bilimleri Dergisi*, 1(1), 1-8.
- Homan, E. J., & Wattiaux, M. A. (2008). *Teknik Süt Sığırcılığı Rehberi: Laktasyon ve Sağım*; ed. Ahmet G. Önel; trans. Bayazıt Musal. Aydın: Adnan Menderes Üniversitesi.
- Inchaisri, C., Jorritsma, R., Vos, P. L., Van der Weijden, G., & Hogeveen, H. (2010). Economic consequences of reproductive performance in dairy cattle. *Theriogenology*, 74(5), 835-846. <https://doi.org/10.1016/j.theriogenology.2010.04.008>
- Karakçı, N. (1990). *Halk elindeki değişik orijinli Siyah Alaca sığırların döl ve süt verim performansları üzerinde araştırmalar*. (Doctoral thesis), İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, İstanbul.
- Kaya, İ., Uzman, C., Kaya, A., & Akbaş, Y. (2003). Comparative analysis of milk yield and reproductive traits of Holstein Friesian cows born in Turkey or imported from Italy and kept on farms under the Turkish-ANAFI project. *Italian Journal of Animal Science*, 2(2), 141-150. <https://doi.org/10.4081/ijas.2003.141>
- Kaya, M. (2013). *Denizli ili özel işletme koşullarında yetiştirilen Holştayn ırkı sığırların süt verimi ve döl verimi özellikleri üzerine bazı çevresel faktörlerin etkisi*. (Master thesis), Adnan Menderes Üniversitesi, Sağlık Bilimleri Enstitüsü, Aydın.
- Kaygısız, A. (1997). Siyah Alaca sığırların Kahramanmaraş Tarım İşletmesi Şartlarındaki verim özellikleri. *Journal of Agricultural Sciences*, 3(2), 9-22.
- Kurt, S. (2001). *Tahirova Tarım İşletmesi'nde yetiştirilen Siyah Alaca sığırların bazı süt verim özellikleri*. (Master thesis), Çanakkale Onsekizmart Üniversitesi Fen Bilimleri Enstitüsü, Çanakkale.
- Linwill, D. E., & Pardue, F. E. (1992). Heat stress and milk production in South Carolina coastal plains. *Journal of Dairy Science*, 75(9), 2598-2604. [https://doi.org/10.3168/jds.S0022-0302\(92\)78022-9](https://doi.org/10.3168/jds.S0022-0302(92)78022-9)
- McDaniel, B., Miller, R., & Corley, E. (1965). DHIA factors for projecting incomplete records to 305 days. *Dairy Herd Improvement Ass Lett, ARS*, 41(6), 21.
- Orman, A. (2003). *Tahirova Tarım İşletmesindeki Holştayn ineklerin başlıca verim özellikleri ve bu özelliklere etki*



- eden bazı çevre faktörleri. (Doctoral thesis), UÜ Sağlık Bilimleri Enstitüsü, Bursa 2003.
- Orman, A., & Oğan, M. M. (2008). Environmental factors affecting milk and milk fat yields of Holstein cows. *Indian Veterinary Journal*, 85(6), 623-625.
- Özkök, H., & Uğur, F. (2007). Türkiye’de yetiştirilen Esmer ve Siyah Alaca sığırlarda süt verimi, ilk buzağılama yaşı ve servis periyodu. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 38(2), 143-149.
- Reyes, L. A. (1998). Performance of dairy cattle according to calving season in the Mexicali Valley, Lower California. *Cuban Journal of Agricultural Science*, 32(1), 19-24.
- Sağlam, M., & Uğur, F. (2007). Siyah Alaca sığırlarda gebelik başına tohumlama sayısı üzerine etkili faktörlerin analizi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 38(1), 37-39.
- Sarar, A. D., & Tapkı, İ. (2017). Türkiye’de yetiştirilen Holştayn ineklerde döl verim özelliklerine ait fenotipik ve genotipik parametre tahminleri. *Turkish Journal of Agriculture Food Science and Technology*, 5(12), 1476-1481. <https://doi.org/10.24925/turjaf.v5i12.1476-1481.1435>
- Şahin, A., & Ulutaş, Z. (2010). Polatlı Tarım İşletmesinde yetiştirilen Siyah Alaca ineklerde süt ve döl verim özellikleri. *Anadolu Journal of Agricultural Sciences*, 25(3), 202-212. <https://doi.org/10.7161/anajas.2010.25.3.202-212>
- Şahin, A., & Ulutaş, Z. (2011). Tahirova Tarım İşletmesinde yetiştirilen Siyah Alaca ineklerin süt ve döl verim özelliklerini etkileyen bazı çevresel faktörler. *Anadolu Journal of Agricultural Sciences*, 26(2), 156-168.
- Tabachnick, B. G. & Fidell, L. S. (2013). *Using Multivariate Statistics*. 6th ed. Boston, Pearson.
- Tangorra, F., Calcante, A., Vigone, G., Assirelli, A., & Bisaglia, C. (2022). Assessment of technical-productive aspects in Italian dairy farms equipped with automatic milking systems: A multivariate statistical analysis approach. *Journal of Dairy Science*, 105(9), 7539-7549. <https://doi.org/10.3168/jds.2021-20859>
- Tekerli, M., & Gündoğan, M. (2005) Effect of certain factors on productive and reproductive efficiency traits and phenotypic relationships among these traits and repeatabilities in West Anatolian Holsteins. *Turkish Journal of Veterinary & Animal Sciences*, 29(1), 17-25.
- Thaler, N. A., & Muhlbauer, M. D., Zardo, W. F., & Ramos, J. C. (1996). Factors affecting milk yield and lactation duration in a Flemish and Holstein herd in the upland of Santa Catarina State Brasil. *Ciência Rural* 26, 123-128.
- Tutkun, M. (2015). *Ceylanpınar tarım işletmesinde yetiştirilen siyah alaca ineklerin süt verimine ilişkin yönelim unsurlarının tahmini*. (Doctoral thesis) Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara.
- Tüzemen, N. (2020). Devrekâni Damızlık Sığır Yetiştiriciliği Birliğine üye işletmelerde bazı döl verimi özelliklerinin incelenmesi. *Journal of Animal Sciences and Products*, 3(2), 120-133.
- Yaylak, E. (2003). Siyah Alaca ineklerde sürüden çıkarılma nedenleri, sürü ömrü ve damızlıkta yararlanma süresi. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi*, 16(2), 179-185.
- Yaylak, E., & Kumlu, S. (2005). Siyah Alaca sığırların 305 günlük süt verimine vücut kondisyon puanı ve bazı çevre faktörlerinin etkisi. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 42(3), 55-66.
- Yıldırım, H. (1999). *Halk elindeki Holştayn ineklerin başlıca verim özellikleri ve bu özelliklere etki eden çevresel faktörler*. (Doctoral thesis) Uludağ Üniversitesi Sağlık Bilimleri Enstitüsü, Bursa.
- Walsh, S. W, Williams, E. J., & Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science*, 123(3-4), 127-13.

# The Investigation of the Fermentative, Chemical and Microbial Effects of Grape and Tangerine Pomace Added to High Moisture Alfalfa Silage

Erinç Gümüş<sup>1,\*</sup> , Yücel ÜNAL<sup>2</sup> , Musa YAVUZ<sup>3</sup> , Selim SIRAKAYA<sup>4</sup> , Behlül SEVİM<sup>4</sup>   
, Tugay AYAŞAN<sup>5</sup> 

<sup>1</sup> Aksaray University, Eski Vocational School, Department of Veterinary, Aksaray, Türkiye

<sup>2</sup> Aksaray University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Aksaray, Türkiye

<sup>3</sup> Isparta University of Applied Sciences, Faculty of Agriculture, Department of Feeds and Animal Nutrition, Isparta, Türkiye

<sup>4</sup> Aksaray University, Technical Sciences Vocational School, Department of Food Processing, Aksaray, Türkiye

<sup>5</sup> Osmaniye Korkut Ata University, Kadirli Faculty of Applied Sciences, Department of Organic Farming Management, Osmaniye, Türkiye

\*Corresponding Author

## Article History

Received: 12.11.2023

Accepted: 29.12.2023

## Corresponding Author\*

E-mail: erincgumus@gmail.com

## Keywords

Alfalfa  
Grape pulp  
Microbiota  
Silage  
Tangerine pulp

## Abstract

This study investigated the impact of grape pulp (GRP) and tangerine pulp (TNP) supplementation (10% each) on alfalfa silage quality. The control silage received no additives. After a two-month fermentation period, silage samples were analyzed for chemical composition, fermentation quality, microflora, and organic acids. According to our results, both silage additives effectively lowered pH levels and isobutyric acid, while increased Fleig score and lactic acid levels compared to control group. However, GRP supplementation significantly increased the levels of crude protein (CP), yeast-mold colony count, and butyric acid levels. Conversely, TNP supplementation resulted in higher levels of water-soluble carbohydrates (WSC), valeric acid and lower acetic acid levels in the alfalfa silage samples. In conclusion, both GRP and TNP supplements have distinct effects on the chemical composition, silage quality, microflora, and organic acid profiles of alfalfa silage. These findings provide valuable insights into optimizing alfalfa silage production and its utilization in animal nutrition. Further research could explore optimal inclusion rates and potential synergistic effects with other additives to enhance silage quality.

## Introduction

Alfalfa (*Medicago sativa* L.) is one of the most important feed crops globally because of its abundant nutritional availability and high protein content (Guo et al., 2019; Boga and Ayasan, 2022; Şengül et al., 2022). This plant is remarkable for its ability to be harvested three to four times per year (Guo et al., 2019). Withered forms of alfalfa are used more often than silage in dairy nutrition; however, silage may be preferable under wet conditions, such as harvesting in autumn (Gül et al., 2015). Nevertheless, preserving alfalfa in silage form poses problems owing to the limited content of water-soluble carbohydrates (WSC) (Canbolat et al., 2010). The use of additives such as fruit pulp in the ensiling process has been explored as a potential solution to improve silage fermentation

characteristics and overall quality (Besharati et al., 2020).

Grapes are among the most extensively cultivated fruits in the world, finding their way into both fresh consumption and the thriving wine and cider industries (Li et al., 2017). As a result of food processing, approximately 15-20% of grapes are left as pulp, contributing to the by-product stream of this versatile fruit (Canbolat et al., 2010). Grape pulp is a valuable source of WSC and various polyphenols including anthocyanins, flavanols, hydroxybenzoic and hydroxycinnamic acids, and stilbenes (Li et al., 2017). Polyphenols are recognized for their beneficial effects on silage fermentation processes, wherein they play a pivotal role in preventing degradation and actively participate in the reduction of silage pH (Ke et al.,

2015).

Tangerine is a prominent citrus fruit cultivated in Türkiye, accounting for nearly 5% of the world's total tangerine production (Ertek *et al.*, 2020). Tangerine pulp, on the other hand, is a by-product of tangerine fruit processing and has been shown to contain high levels of antioxidants, carotenoids, and dietary fiber (Rodrigo *et al.*, 2015). Citrus waste materials are susceptible to rapid spoilage and can potentially cause environmental pollution, causing challenges for their proper storage and handling, mainly because of their low dry matter content (Büyükkılıç Beyzi *et al.*, 2018). Therefore, these by-products can be utilized in animal nutrition either in their fresh form or as ensilaged products. Alternatively, they could be used as supplements to enhance the quality of other grass silage materials (Ülger *et al.* 2020).

Although there are studies on the use of grape and citrus by-products in different grass silages,

no study has compared the effects of grape pulp and tangerine pulp supplementation in alfalfa silage on the chemical composition, silage quality, microflora of silage, and organic acid profiles.

### Materials and Methods

The alfalfa was harvested using hand clippers, ensuring a precise cut of approximately 5 cm above ground level, in the fields of Eskil district in Aksaray Province, Türkiye, during late November 2022. The harvested material was chopped into pieces measuring 2-4 cm and divided into three portions. One portion of the chopped material was ensiled without supplementation (Control), whereas the other two portions were ensiled with the addition of 10% grape pulp (GRP) and 10% tangerine pulp (TNP). All samples were placed in polyethylene vacuum bags and the experiment was conducted with four replicates for each treatment. The chemical composition of fresh alfalfa is shown in Table 1.

**Table 1** Chemical analysis of fresh alfalfa before ensiling.

Parameters (%)*	Fresh Alfalfa	GRP	TNP
Dry Matter	18.69	24.73	20,49
Crude Protein	25.98	9.43	6.22
Ether Extract	3.17	5.94	2.93
Crude Ash	13.55	2.51	3.33
WSC	3.11	4.57	5.12
ADF	25.11	41.28	13.87
NDF	31.30	51.28	16.06

Results are given on a dry matter basis, \*GRP: Grape Pulp, TNP: Tangerine Pulp, WSC: Water Solvable Carbohydrates, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber,

The silages were stored for two months. Subsequently, upon opening the bags, 40 g of silage samples were taken from each bag and diluted with 360 mL of distilled water. The samples were then filtered through Whatman No.1 filter papers. The pH of the filtrate was assessed using a pH glass electrode (HI 1230 B, Hanna Instruments). The filtrate was diluted at a ratio of 1/100 for both the WSC and organic acid analyses. To facilitate the analysis of organic acids, we added 1.5 milliliters of 1M orthophosphoric acid to the filtrate. The mixture was then centrifuged at 10,000 rpm for 10 min. The filtrate was stored at -20°C until analysis. A gas chromatograph equipped with a flame ionization detector (GC-FID) was used for butyric, acetic, and propionic acid analyses. An autosampler (Thermo AI-1310, Thermo Scientific, USA) was used for this process. Lactic acid levels were determined according to the protocol described by Barnett (1951). Water-soluble carbohydrate levels in silages were

determined following the method described by Dubois *et al.* (1951). The WSC values were multiplied by 10 and divided by DM. The obtained values were multiplied by 10 and divided by the DM of the silage samples to determine the lactic acid content as a percentage of DM. A seven-day aerobic stability test was conducted on the samples following the method developed by Ashbell *et al.* (1991). The Fleig score of the silage was calculated using the following equation:  $220 + (2 \times \text{dry matter percentage} - 15) - (40 \times \text{pH})$ .

The chemical compositions of the fresh material and silage were determined according to AOAC (2000). After the alfalfa silage samples were dried at 105°C for 24 h to determine their DM content, the remaining samples were ground and further dried in an oven at 60°C until a constant weight was achieved (approximately 48 h). The ground samples were carefully placed in Ziplock bags for subsequent chemical analyses. For ash analysis, the samples were ashed in a 550°C ash furnace

(NÜVE) to determine the crude ash (CA) content. The nitrogen (N) content of the samples was assessed using the Kjeldahl method, and the resulting N values were multiplied by a conversion factor of 6.25 ( $N \times 6.25$ ) to obtain the crude protein (CP) content. The determination of crude oil values in the samples involved extraction of ether extract (EE) using petroleum ether in a Soxhlet apparatus. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the method described by Van Soest *et al.* (1991) using an Ankom 2000 fiber analyzer (Ankom Technology, Fairport, NY, USA).

After unsealing the silage samples, 10-gram sample was mixed with 90 mL of peptone water for microbiological cultivation using the spread plate technique. The resulting mixture was poured into Petri dishes according to the manufacturer's instructions. Subsequently, the yeast mold (Sırakaya & Büyükkılıç Beyzi, 2022), *Enterobacteria* (ISO 21528-2, 2018), *Clostridia* (ISO 7937, 2004), and lactic acid bacteria (ISO 15214, 1998) counts were determined. The yeast-mold was counted using Potato Dextrose Agar (Merck, Darmstadt, Germany) medium, which was then incubated for 5 days at a controlled temperature of  $25 \pm 1^\circ\text{C}$ . *Enterobacteria* were counted using Violet Red Bile Agar W/Glucose (Condalab, Madrid, Spain) medium and were subjected to incubation at a precisely controlled temperature of  $37 \pm 1^\circ\text{C}$  for a duration of 24 h. Similarly, MRS Agar (Merck, Darmstadt, Germany) was used to determine the population of lactic acid bacteria, which were then

incubated at a controlled temperature of  $37 \pm 1^\circ\text{C}$  for an extended period of 72 h. *Clostridia* were enumerated using Reinforced Clostridial Agar (Condalab, Madrid, Spain) medium, which was then incubated for 48 h at a controlled temperature of  $35 \pm 2^\circ\text{C}$ . For *Clostridia*, the pour-plate method was used, and incubation was performed in an oxygen-free environment.

All data were subjected to analysis of variance (ANOVA) using the General Linear Model of SPSS (version 25.0; IBM Corp., Armonk, NY, USA). Significant differences between individual means were identified using Tukey's multiple-range test. Differences were considered statistically significant at  $P < 0.05$ .

## Results

The effects of GRP and TNP supplementation on the nutritional composition of the alfalfa silage are presented in Table 2. These results suggest that the silage additives evaluated in this study did not produce statistically significant differences with respect to EE, CA, CF, ADF, NDF, and ADL content in alfalfa silage compared with Control ( $P > 0.05$ ). The CP values in the silage samples supplemented with GRP exhibited a notable increase compared to both Control and TNP-supplemented samples ( $P < 0.05$ ). However, WSC levels were statistically higher in the TNP-treated silage than in the GRP-treated silage and Control ( $P < 0.05$ ).

**Table 2** Effects of GRP and TNP supplementation on the chemical composition of alfalfa silage

Parameters (%)*	Silage Additives			P
	Control	GRP	TNP	
CP	21.93 $\pm$ 0.96 <sup>ab</sup>	23.82 $\pm$ 1.43 <sup>a</sup>	21.72 $\pm$ 0.90 <sup>b</sup>	0.022
EE	3.80 $\pm$ 0.48	4.57 $\pm$ 0.89	4.88 $\pm$ 1.81	0.368
CA	13.06 $\pm$ 0.61	12.68 $\pm$ 1.79	11.25 $\pm$ 1.45	0.133
WSC	1.68 $\pm$ 0.08 <sup>b</sup>	2.06 $\pm$ 0.15 <sup>b</sup>	2.59 $\pm$ 0.46 <sup>a</sup>	0.001
ADF	19.42 $\pm$ 1.08	18.82 $\pm$ 1.16	19.32 $\pm$ 0.87	0.632
NDF	27.45 $\pm$ 1.96	29.07 $\pm$ 1.62	28.22 $\pm$ 0.96	0.300
ADL	6.98 $\pm$ 1.60	7.46 $\pm$ 1.86	5.05 $\pm$ 0.85	0.058
CF	12.44 $\pm$ 2.65	11.35 $\pm$ 2.65	14.27 $\pm$ 0.47	0.144

Results are given on a dry matter basis, \*GRP: Grape Pulp, TNP: Tangerine Pulp, CP: Crude Protein, EE: Ether Extract, CA: Crude Ash, WSC: Water Solvable Carbohydrates, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, ADL: Acid Detergent Lignin, CF: Crude Fiber.

a–b Means in the same row followed by different superscript letters are significant difference ( $P < 0.05$ ).

The effects of GRP and TNP supplementation on alfalfa silage quality parameters are presented in Table 3. The present study revealed that DM and CO<sub>2</sub> values were unaffected by silage additives ( $P > 0.05$ ).

Conversely, the use of silage additives resulted in a significant reduction in pH levels compared with Control ( $P < 0.001$ ). Additionally, the silage additives led to a noteworthy enhancement in the Fleig score values when compared to the alfalfa silage samples in

Control (P<0.001).

**Table 3** Effect of GRP and TNP supplementation on DM, pH, Fleig Score and CO<sub>2</sub> values of alfalfa silage.

Parameters*	Silage Additives			P
	Control	GRP	TNP	
DM %	19.75±0.51	19.30±0.29	19.13±0.34	0.067
pH	6.06±0.16 <sup>a</sup>	5.23±0.11 <sup>b</sup>	5.36±0.17 <sup>b</sup>	0.000
Fleig Score	2.15±5.65 <sup>b</sup>	34.6±3.92 <sup>a</sup>	29.0±6.58 <sup>a</sup>	0.000
CO <sub>2</sub> , g/Kg	31.5±15.9	25.8±10.6	27.2±2.17	0.760

\*GRP: Grape Pulp, TNP: Tangerine Pulp, DM: Dry Matter.

a–b Means in the same row followed by different superscript letters are significant difference (P<0.05).

The fermentation profiles of the alfalfa silages enriched with GRP and TNP are presented in Table 4. According to these findings, silage additives did not exert a statistically significant influence on propionic acid levels in the alfalfa silage samples (P>0.05). In contrast, butyric, valeric, and isobutyric acid levels exhibited a notable response to GRP and TNP treatments in the alfalfa silage (P<0.05). Similarly,

acetic acid levels in the GRP- and TNP-treated groups were significantly lower, whereas lactic acid levels were higher in the same groups than in Control (P<0.001). TNP supplementation led to the most pronounced increase in lactic acid levels compared with Control and GRP-supplemented silage samples. Furthermore, the inclusion of TNP resulted in the lowest butyric acid levels among all treatments in this study.

**Table 4** Effect of GRP and TNP supplementation on fermentation profiles of alfalfa silages.

Parameters (%)*	Silage Additives			P
	Control	GRP	TNP	
Butyric Acid	0.17±0.04 <sup>a</sup>	0.21±0.05 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.003
Propionic Acid	0.07±0.02	0.06±0.01	0.05±0.01	0.141
Acetic Acid	1.08±0.23 <sup>a</sup>	0.30±0.05 <sup>c</sup>	0.73±0.17 <sup>b</sup>	<0.001
Lactic Acid	6.01±0.20 <sup>b</sup>	6.80±0.45 <sup>a</sup>	7.07±0.21 <sup>a</sup>	<0.001
Valeric Acid	0.040±0.000 <sup>b</sup>	0.041±0.001 <sup>a</sup>	0.040±0.000 <sup>b</sup>	0.007
Isobutyric Acid	0.057±0.003 <sup>a</sup>	0.052±0.002 <sup>b</sup>	0.049±0.001 <sup>b</sup>	0.002

Results are given on a dry matter basis, \*GRP: Grape Pulp, TNP: Tangerine Pulp

a–b Means in the same row followed by different superscript letters are significant difference (P<0.05).

The effects of GRP and TNP supplementation on the microbiological values of the alfalfa silage are presented in Table 5. The results indicated that *Clostridium* and lactic acid bacterial populations were not significantly affected by supplementation (P>0.05). In contrast, GRP (3.13) and TNP (0.00) supplementation resulted in fewer *Enterobacteria* colonies in the medium than Control (7.65) (P<0.001). Furthermore, yeast-mold colonies were significantly reduced in the alfalfa silage samples supplemented with TNP compared to both Control and GRP-treated silage samples (P<0.05).

## Discussion

In the present study, the silages supplemented with GRP exhibited the highest levels

of CP. Similarly, Bulut *et al.* (2023) also observed an increase in CP levels in sorghum-sudan grass silages supplemented with grape pulp, albeit not statistically significant, but evident in numerical terms, at various inclusion rates ranging from 10% to 40%. Similarly, in a study conducted by Li *et al.* (2017), the CP content of sweet sorghum silage treated with a combination of 10-15% grape pulp and lactic acid bacteria inoculant was higher than that of groups that were not supplemented with grape pulp. These results could be attributed to the inhibitory effect of polyphenols present in grapes on the proteolytic enzyme activity of bacteria in silages. This inhibition may lead to an improvement in the utilization of silage nitrogen content, resulting in higher levels of crude protein in the treated samples (Ke *et al.*, 2015; Li *et al.*, 2017). Moreover, WSC levels were significantly



higher in TNP-treated silage and GRP-treated alfalfa silage, not statistically but numerically. Similar to our findings, other researchers have also observed that by-products of grape pulp (Canbolat *et al.*, 2010) and citrus fruits (Tao *et al.*, 2021) were added to their

products to increase the WSC values. It has been previously reported that citric acid suppresses the growth of undesirable microorganisms, which may affect the utilization of WSC by these bacteria (Tao *et al.*, 2021).

**Table 5** Effect of GRP and TNP supplementation on microbiological values of alfalfa silage.

Parameters (log <sub>10</sub> cfu/g of FM)*	Silage Additives			P
	Control	GRP	TNP	
<i>Clostridia</i>	6.30±0.86	6.52±1.16	6.54±0.65	0.841
<i>Enterobacteria</i>	7.65±0.28 <sup>a</sup>	3.13±0.46 <sup>b</sup>	0.00 <sup>c</sup>	<0.001
Lactic acid bacteria	6.25±0.69	6.95±0.16	6.72±0.70	0.673
Yeast-Mold	+++	+++++	+	

\*GRP: Grape Pulp, TNP: Tangerine Pulp, FM: Fresh Matter, +: indicator of growing density

a–c Means in the same row followed by different superscript letters are significant difference (P<0.05)

In the present study, it was noted that the incorporation of silage additives led to a reduction in the pH levels of alfalfa silage when compared to Control. An investigation conducted on alfalfa silages demonstrated that pH levels decreased at both 0 and 5 d after air exposure as grape pulp levels increased (Canbolat *et al.*, 2010). Similarly, Li *et al.* (2017) found that the final pH of sweet sorghum silage decreased with both 10% GRP and lactic acid bacteria supplementation compared with Control. Moreover, investigations carried out on Marandu grass silage (Bernardes *et al.*, 2005), untreated lucerne (Besharati *et al.*, 2022), and elephant grass silage (Gomes *et al.*, 2017) demonstrated that the introduction of citrus pulp or its by-products resulted in decreased pH levels compared with Control. Both grapes and citrus fruits contain soluble sugars that act as substrates for lactic acid bacteria. SE On the other hand, in some situations, the buffering capacity of alfalfa might hinder the decrease in pH, even when a sugar source is added. Bulut *et al.* (2023) reported that both white and black grape pulp supplementation increased pH levels in sorghum sudan grass silage compared with silage samples without supplementation.

The Fleig score is an indicator of fermentation quality and preservation of alfalfa silage, and a higher Fleig score is associated with good preservation and enhanced silage fermentation (Gao *et al.*, 2021). In the current study, the untreated alfalfa silage samples had the lowest Fleig score values (2.15±5.65) compared to the silage samples treated with GRP (34.6±3.92) and TNP (29.0±6.58). This result was consistent with that of Gao *et al.* (2021), who documented that the Fleig scores of alfalfa silages were elevated in samples treated with various carbohydrate sources, including pectin, starch,

molasses, and fructose. Similarly, another investigation of raw lucerne silages observed an increase in Fleig score values with varying substitutions of lemon pulp (Besharati *et al.*, 2022). The Fleig score exhibits a negative correlation with pH and a positive association with the DM content of the silage (Gao *et al.*, 2021). Lower pH levels in the treatment groups could be the reason for the higher Fleig scores.

Butyric acid is an important indicator of silage quality and the fermentation process (Tao *et al.*, 2021). In the current study, the silage samples in Control exhibited a butyric acid level of approximately 0.17% in DM, whereas samples with GRP addition had a level of approximately 0.21% in DM, and those supplemented with TNP had a level of 0.08% in DM. Moreover, isobutyric acid levels decreased in the treated silages. Butyric acid levels of approximately %0.5 and %1 are acceptable for legume silages with lower than %30 DM (Kung *et al.*, 2018). In our study, the application of TNP significantly decreased butyric acid levels in ensiled alfalfa compared with Control and GRP. Similar to our results, another study conducted on Napier grass silage showed a decrease with the addition of citric acid residue at 45 days of ensiling (Tao *et al.*, 2021). Rapid production of lactic acid could result in the inhibition of clostridial fermentation, specifically the formation of butyric acid in silage, which is attributed to the heightened osmotic pressure and reduced pH levels (Kung *et al.*, 2018). Conversely, the levels of butyric acid were elevated in silage samples supplemented with GRP, in contrast to the TNP group. Grape pulp naturally contains yeasts (Zott *et al.*, 2010). Higher pH levels and an extended fermentation process, resulting from the absence of antifungal organic acids, may prove

inadequate in suppressing yeast proliferation within silage, consequently leading to an increase in silage temperature (Kung *et al.*, 2018). Elevated temperatures may trigger a shift in the end-product from acetate to butyrate during the fermentation process of food and feed (Wang *et al.*, 2020). This observation could potentially explain the higher presence of butyric acid, coupled with an elevated yeast population and reduced acetic acid levels in the alfalfa silages supplemented with GRP, compared to Control and TNP groups.

In the present study, both treatments led to elevated levels of lactic acid while concurrently suppressing acetic acid levels in alfalfa silage, compared to Control. The levels of WSC in fruit pulp facilitate the proliferation of lactic acid bacteria, leading to an increase in lactic acid production in the silage (Canbolat *et al.*, 2010). Numerous studies have demonstrated elevated lactic acid levels in various silages treated with citrus (Besharati *et al.*, 2022; Gomes *et al.*, 2017) and grape pulp (Canbolat *et al.*, 2010; Ke *et al.*, 2015). Acetic acid is an important volatile acid in silage owing to its inhibitory effect on yeast proliferation and its capacity to enhance aerobic stability when the silage is exposed to air (Kung *et al.*, 2018). In the present study, acetic acid levels were diminished in the treatment groups compared to Control. Furthermore, a study focusing on various levels of citric acid residue-treated Napier grass silage showed a reduction in the acetic acid content (Tao *et al.*, 2021). Similarly, Bulut *et al.* (2023) reported lower acetic acid levels in Sudan grass silage supplemented with 40% GRP. The higher acetic acid content in Control could be attributed to undesired bacterial activity, potentially stemming from elevated pH levels in these silages (Tao *et al.*, 2021).

In the current study, yeast levels were significantly higher in GRP-treated silage than with Control and TNP-treated silage. This observation is supported by the findings of Ke *et al.* (2015), who reported a greater number of yeast colonies in alfalfa silage treated with GRP. Furthermore, the lowest yeast levels in alfalfa silage were observed in TNP-treated silage. Similar to our results, Ke *et al.* (2017) demonstrated that treating alfalfa silage with 0.1% citric acid resulted in a decrease in yeast population. As previously stated, grapes can naturally contain yeasts. Additionally, higher pH and lower acetic acid levels in silage can contribute to the proliferation of yeast (Kung *et al.*, 2018). *Enterobacteria*, along with clostridial, yeast, and mold, constitute another group of undesirable bacteria that can lead to aerobic deterioration and subsequent nutritional losses (Tao *et al.*, 2021). In the

present study, both GRP- and TNP-treated alfalfa silage exhibited lower counts of *Enterobacteria* than Control. It has been reported that *Enterobacteria* are sensitive to pH levels below 5.0, which could elucidate the higher counts observed in Control (Bernardes *et al.*, 2005).

## Conclusion

Overall, post-ensiling alfalfa silages treated with both GRP and TNP showed improved fermentation quality. This improvement can be attributed to their capacity to lower pH levels by increasing lactic acid content, which may be linked to the WSC content in the fruit pulps. While GRP supplementation exhibited a more pronounced effect in preserving CP content, TNP-treated alfalfa silages demonstrated higher levels of WSC, lower concentrations of butyric acid, and a more effective inhibition of yeast-mold and *Enterobacteria* growth. This study demonstrated the efficacy of fruit pulp supplementation, particularly tangerine pulp supplementation, in improving the nutritional content and fermentation quality of alfalfa silage. These findings have implications for optimizing silage production practices, particularly in regions with abundant access to fruit-processing byproducts. Future research should further explore the nuanced interactions between fruit pulp composition and silage fermentation dynamics for more refined and tailored approaches to silage production.

## Acknowledgements

This study was supported by the Research Fund of Aksaray University (Project Number: 2022-02). The authors would also like to thank Fatih İlik for alfalfa supplementation and the students of the Eski Vocational School for their help in the silage preparation process.

## Author contributions

First Author: Conceptualization, Formal Analysis, Statistical Analysis, Writing -review and editing; Second Author: Funding Acquisition, Project Administration, Resources; Third Author: Data Curation, Formal Analysis, Investigation, Methodology, Visualization; Fourth Author: Formal Analysis, Supervision, Writing - review and editing; Fifth Author: Formal Analysis, Writing -review and editing. Sixth Author: Formal Analysis, Writing -review and editing.

## Conflict of Interest

The authors declare no conflicts of interest.



## References

- AOAC. (2000). Official Methods of Analysis. In AOAC INTERNATIONAL, Maryland, USA (2003) (Vol. 17). The Association of Official Analytical Chemists.
- Ashbell, G., Weinberg, Z. G., Azrieli, A., Hen, Y., & Horev, B. (1991). A simple system to study the aerobic determination of silages. *Canadian Agricultural Engineering*, 34, 171–175.
- Barnett, A. J. G. (1951). The colorimetric determination of lactic acid in silage. *Biochemical Journal*, 49(4), 527–529.
- Bernardes, T. F., Reis, R. A., & Moreira, A. L. (2005). Fermentative and microbiological profile of marandu-grass ensiled with citrus pulp pellets. *Scientia Agricola*, 62(3), 214–220. <https://doi.org/10.1590/S0103-90162005000300003>
- Besharati, M., Karimi, M., Taghizadeh, A., Nemati, Z., & Kaygisiz, A. (2020). Improve Quality of Alfalfa Silage Ensiled with Orange Pulp and Bacterial Additive. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*. <https://doi.org/10.18016/ksutarimdog.vi.673623>
- Besharati, M., Palangi, V., Salem, A. Z. M., De Palo, P., Lorenzo, J. M., & Maggiolino, A. (2022). Substitution of raw lucerne with raw citrus lemon by-product in silage: In vitro apparent digestibility and gas production. *Frontiers in Veterinary Science*, 9, 1006581. <https://doi.org/10.3389/fvets.2022.1006581>
- Boga, M., Ayasan, T. (2022). Determination of nutritional value of alfalfa varieties and lines by using the in vitro method and gas production technique. *Journal of the Hellenic Veterinary Medical Society*, 73(1), 3613-3620. <https://doi.org/10.12681/jhvms.24674>
- Bulut, R., Karsli, M. A., & Şenyüz, H. H. (2023). Determination of the effects of grape pomace addition to sorghum sudan grass on silage quality. *Livestock Studies*, 63(1), 47–54. <https://doi.org/10.46897/livestockstudies.1325043>
- Büyükkılıç Beyzi, S., Ülger, İ., Kaliber, M., & Konca, Y. (2018). determination of chemical, nutritional and fermentation properties of citrus pulp silages. *Turkish Journal of Agriculture - Food Science and Technology*, 6(12), 1833–1837. <https://doi.org/10.24925/turjaf.v6i12.1833-1837.2229>
- Canbolat, Ö., Kalkan, H., Karaman, Ş., & Filya, İ. (2010). Üzüm posasının yonca silajlarında karbonhidrat kaynağı olarak kullanılma olanakları. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 16(2), 269–276. <https://doi.org/10.9775/kvfd.2009.679>
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1951). A colorimetric method for the determination of sugars. *Nature*, 4265(168), 167–167.
- Ertek, N., Dağdemir, V., & Keskin, A. (2020). Türkiye’de mandalina piyasasının ekonomik analizi ve pazarlama marjları. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 52(2), 119–125. <https://doi.org/10.17097/ataunizfd.562118>
- Gao, R., Wang, B., Jia, T., Luo, Y., & Yu, Z. (2021). effects of different carbohydrate sources on alfalfa silage quality at different ensiling days. *Agriculture*, 11(1), 58. <https://doi.org/10.3390/agriculture11010058>
- Gomes, R. D. S., Almeida, J. C. D. C., Carneiro, J. D. C., Azevedo, F. H. V., Lista, F. N., Elyas, A. C. W., & Oliveira, T. S. D. (2017). Impacts of citrus pulp addition and wilting on elephant grass silage quality. *Bioscience Journal*, 675–684. <https://doi.org/10.14393/BJ-v33n3-33790>
- Gül, Z. D., Tan, M., Kaynar, D. F., & Kharazmi, K. (2015). Effects of some additives, harvest stage and wilting on quality characteristics of alfalfa silage. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 46(2), 113-118.
- Guo, G., Shen, C., Liu, Q., Zhang, S. L., Wang, C., Chen, L., Xu, Q. F., Wang, Y. X., & Huo, W. J. (2019). Fermentation quality and in vitro digestibility of first and second cut alfalfa (*Medicago sativa* L.) silages harvested at three stages of maturity. *Animal Feed Science and Technology*, 257. <https://doi.org/10.1016/j.anifeedsci.2019.114274>
- International Standard 7937 (2004). Microbiology of food and animal feeding stuffs horizontal method for the enumeration of Clostridium perfringens colony count technique.
- International Standard 21528-2 (2018). Microbiology of food cahin horizontal method for the detection and enumeration of Enterobacteriaceae colony count technique.

- International Standard 15214 (1998). Microbiology of food and animal feeding stuffs horizontal method for the enumeration of mesophilic lactic acid bacteria colony-count technique at 30 °C.
- Ke, W. C., Yang, F. Y., Undersander, D. J., & Guo, X. S. (2015). Fermentation characteristics, aerobic stability, proteolysis and lipid composition of alfalfa silage ensiled with apple or grape pomace. *Animal Feed Science and Technology*, 202, 12–19. <https://doi.org/10.1016/j.anifeedsci.2015.01.009>
- Ke, W. C., Ding, W. R., Xu, D. M., Ding, L. M., Zhang, P., Li, F. D., & Guo, X. S. (2017). Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. *Journal of Dairy Science*, 100(11), 8958–8966. <https://doi.org/10.3168/jds.2017-12875>
- Kung, L., Shaver, R. D., Grant, R. J., & Schmidt, R. J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, 101(5), 4020–4033. <https://doi.org/10.3168/jds.2017-13909>
- Li, P., Shen, Y., You, M., Zhang, Y., Yan, J., Li, D., & Bai, S. (2017). Effect of grape pomace on fermentation quality and aerobic stability of sweet sorghum silage. *Animal Science Journal*, 88(10), 1523–1530. <https://doi.org/10.1111/asj.12791>
- Rodrigo, M. J., Cilla, A., Barberá, R., & Zacarías, L. (2015). Carotenoid bioaccessibility in pulp and fresh juice from carotenoid-rich sweet oranges and mandarins. *Food & Function*, 6(6), 1950–1959. <https://doi.org/10.1039/C5FO00258C>
- Sırakaya, S., & Büyükkılıç Beyzi, S. (2022). Treatment of alfalfa silage with chitosan at different levels to determine chemical, nutritional, fermentation, and microbial parameters. *Journal of Animal and Feed Sciences*, 31(1), 73–80. <https://doi.org/10.22358/jafs/147014/2022>
- Şengül, Ö., Şengül, A. Y., Kökten, K., Çağan, E., & Beyzi, S. B. (2022). Possibilities of using dried mulberry pulp as an additive in alfalfa silage. *Medycyna Weterynaryjna*, 78(06), 6669–2022. <https://doi.org/10.21521/mw.6669>
- Tao, X., Chen, S., Zhao, J., Wang, S., Li, J., Sun, F., & Shao, T. (2021). Fermentation and aerobic stability of Napier grass silage treated with different levels of citric acid residue. *Grassland Science*, 67(2), 139–147. <https://doi.org/10.1111/grs.12298>
- Ülger, İ., Beyzi, S. B., Kaliber, M., & Konca, Y. (2020). Chemical, nutritive, fermentation profile and gas production of citrus pulp silages, alone or combined with maize silage. *South African Journal of Animal Science*, 50(1), 161–169. <https://doi.org/10.4314/sajas.v50i1.17>
- Van Soest P.V., Robertson J.B., & Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Wang, Q., Li, H., Feng, K., & Liu, J. (2020). oriented fermentation of food waste towards high-value products: A Review. *Energies*, 13(21), 5638. <https://doi.org/10.3390/en13215638>
- Zott, K., Claisse, O., Lucas, P., Coulon, J., Lonvaud-Funel, A., & Masneuf-Pomarede, I. (2010). Characterization of the yeast ecosystem in grape must and wine using real-time PCR. *Food Microbiology*, 27(5), 559–567. <https://doi.org/10.1016/j.fm.2010.01.0>