MEDITVETJ

MAKÜ AUGUST 2024 VOLUME 9 ISSUE 2

MEDITERRANEAN
VETERINARY JOURNAL

e - ISSN: 3023-8447

Mediterranean Veterinary Journal
Volume: 09 . *Number:* 02 . 2024
Published tri-annual
Former name
Veterinary Journal of Mehmet Akif Ersoy University

Publisher
On behalf of Budur Mehmet Akif Ersoy University
Faculty of Veterinary Medicine
Prof. Dr. Hüseyin DALGAR
Rector

Address
Burdur Mehmet Akif Ersoy University
Faculty of Veterinary Medicine
Degirmenler, 15030, BURDUR

'This Journal is indexed and abstracted by TUBİTAK-ULAKBİM TR-Dizin, Zoological Record, EBSCO, CAB Abstract, CiteFactor, Google Scholar, Science Library Index, International Institute of Organized Research, Researchbib, SciLit, SJIFactor, COSMOS IF and SOBIAD.'

E-mail: meditvetj@mehmetakif.edu.tr

Web Adress: https://dergipark.org.tr/en/pub/meditvetj

Online Submission

https://dergipark.org.tr/en/journal/4407/submission/step/manuscript/new

The iThenticate/Turnitin plagiarism analysis program is used to check submitted articles for originality before they are accepted for publication.

The reviewers of MEDIVETJ, 2024, 9 (2)

Ahmed Ibrar, Selcuk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Konya, Türkiye
Akal Eser, Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Samsun, Türkiye
Aydoğdu Uğur, Balıkesir University, Faculty of Veterinary Medicine, Department of Internal Medicine, Balıkesir, Türkiye
Doğan Fırat, Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Virology, Hatay, Türkiye
Ergün Yaşar, Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Hatay, Türkiye
Garip Mustafa, Selcuk University, Faculty of Veterinary Medicine, Department of Animal Science, Konya, Türkiye
İder Merve, Selcuk University, Faculty of Veterinary Medicine, Department of Internal Medicine, Konya, Türkiye
Karapınar Zeynep, Balıkesir University, Faculty of Veterinary Medicine, Department of Virology, Balıkesir, Türkiye
Kayar Tamer, Aksaray University, Faculty of Veterinary Medicine, Department of Animal Science, Konya, Türkiye
Keleş Hikmet, Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, Afyonkarahisar, Türkiye
Kuter Eren, Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Burdur, Türkiye
Özenç Erhan, Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Afyonkarahisar, Türkiye
Özkadif Sema, Çukurova University, Faculty of Veterinary Medicine, Department of Anatomy, Adana, Türkiye
Özyiğit Özgür, Bursa Uludag University, Faculty of Veterinary Medicine, Department of Pathology, Bursa, Türkiye
Riaz Rozhan, Kafkas University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kars, Türkiye
Sevim Ömer, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Aydın, Türkiye
Tekin Koray, Ankara University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Ankara, Türkiye
Top Şermin, Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Şanlıurfa, Türkiye
Toprak Burhan, Bozok University, Faculty of Veterinary Medicine, Department of Anatomy, Yozgat, Türkiye
Yavuz Orhan, Dokuz Eylül University, Faculty of Veterinary Medicine, Department of Pathology, İzmir, Türkiye
Yılmaz Emre, Ataturk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Erzurum, Türkiye
Yurdagül Özsoy Şule, Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Pathology, Aydın, Türkiye

*Volume 9, Issue 2 of 2024 has an alphabetical list of the editorial board and reviewers.

CONTENTS

Research Articles

Case reports

Medit Vet J, 9(2): 270-276, 2024

Research Article

Determination of feed value of chemically treated sun-dried grape pomace

Kadir Emre Buğdaycı[1], Derya Merve Karagöz[1], Fatma Karakaş Oğuz[1], Mustafa Numan Oğuz[1], Esra Çağan Ulusan[1], Aykut Nacak[1]

[1]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
ammonia
digestibility
grape pomace
sodium hydroxide
urea

Received : 27 December 2023
Revised : 1 July 2024
Accepted : 1 July 2024
Published : 31 August 2024
Article Code : 1410829

Correspondence:
KE. BUĞDAYCI
(kebugdayci@mehmetakif.edu.tr)

ORCID
KE. BUĞDAYCI : 0000-0002-1715-6904
DM. KARAGÖZ : 0000-0002-1179-1317
F. KARAKAŞ OĞUZ : 0000-0002-9077-8531
MN. OĞUZ : 0000-0001-8802-3423
E. ÇAĞAN ULUSAN : 0000-0002-2176-6772
A. NACAK : 0000-0002-4736-2899

ABSTRACT

This study aimed to elucidate the effects of various chemical treatments on the in vitro dry matter digestibility (IVDMD) and concomitant feed value of dried grape pomace (DGP). Sun-dried grape pomace, derived from grapes processed in Denizli, Türkiye province was used in the experiment. One control group and three treatment groups were established, each with eight replicates. The first, second and third experimental groups were treated with 1.5% ammonia (NH_3), 3% urea (CH_4N_2O) and 3% sodium hydroxide (3%) (NaOH), respectively. The dry matter content was set at 50% for all groups. The control group received no additional chemicals. All groups were kept in airtight, closed plastic bags at room temperature for one week. Adding NH_3 and CH_4N_2O significantly improved crude protein levels (24.45 and 19.84%, respectively) and in vitro protein degradability (59.84 and 65.69%, respectively) in the experimental groups ($p<0.001$). Treatment with CH_4N_2O did not exert the same deleterious effect on the ether extract concentration of desiccated grape pomace ($p<0.004$) as treatment with NaOH. Moreover, relative feed value declined significantly more after NaOH treatment compared to treatments containing nitrogen ($p<0.001$). Additionally, NaOH treatment yielded the lowest in vitro protein degradability of DGP at 40.32% ($p<0.001$). With an in vitro dry matter degradability of 47.18%, the findings indicate that among the chemical treatments investigated, application of NH_3 resulted in the lowest degradability value ($p<0.001$). Consequently, it can be inferred that the feed value and digestibility of desiccated grape pomace are highly influenced by the nitrogen concentration or basic nature of the supplemental chemical compounds.

INTRODUCTION

The utilization of nutritionally valuable by-products in animal nutrition is common in the agriculture sector. Grape pomace is, however, one of these underutilized agricultural by-products. Although grape production areas in Türkiye have decreased since 2013 (Sümbül and Yıldız, 2022), FAO statistics put it 7th in the world with 390 thousand hectares in 2021 (Faostat, 2023). Türkiye produced 4 million 165 thousand tons of grapes in 2022 (2 million 99 thousand for table, 1 million 681 thousand for drying, and 383 thousand for wine). This production constitutes 15.5% of the country's total output in the fruits, beverages, and spices category (TUIK, 2023). The waste product from pressing grapes for wine or juice is called grape pomace. Varış et al. (2000) state that approximately 15-25% of grapes used in wine production are left as grape pomace. Nerantzis and Tataridis (2006) describe grape pomace' composition as 24.9% stem, 22.5% seed, and 42.5% peels. The chemical composition of pomace varies depending on the grape variety, climate conditions, and winemaking techniques (Deng et al., 2011). Due to its high moisture content, grape pomace is often recommended to be processed by drying or ensiling for use in animal feed (Özdüven et al., 2005).

Bahrami et al. (2010) found that incorporating 10% grape pomace in the diet did not adversely affect lamb growth. The addition of 20% grape pomace to heifer rations had no negative effect on fattening performance or feed intake (Voicu et al., 2014). Zhen-Zhen et al. (2015) noted that incorporating 8-16% grape pomace in lamb diets improves feed conversion ratio, daily live weight gain, and several carcass characteristics. The present experiments evince that grape pomace can serve as a partial replacement for roughage, and that augmenting its feed value may engender improved performance. Chemical analyses have demonstrated that the crude protein (CP) content of grape pomace ranges from 6.6% (Besharati and Taghizadeh, 2010) to 17.27% (Aghsaghali et al., 2011). Similar variations in acid detergent fiber (ADF) and neutral detergent fiber (NDF) content have been reported. For instance, Besharati and Taghizadeh (2010) reported pomace's ADF and NDF levels as 18.4% and 18.7%, respectively, while Atalay (2020) reported them as 53.88% and 58.8%. According to Sarıçiçek and Kılıç (2002), grape pomace had a 48-hour dry matter (DM) degradability of 18.57%, an organic matter degradability of 16.19%, a low crude protein degradability of 19.80%, and a high pepsin solubility of 42.80%. *In vitro* studies conducted by Kılıç and Abdiwali (2016) revealed that DGP exhibited higher true digestibility, DM digestibility, and relative feed value (RFV), along with increased dry matter intake (DMI), compared to grape seeds.

Recent investigations have evinced that the application of different chemical compounds to low-quality or alternative

To cite this article:
Buğdaycı KE, Karagöz DM, Karakaş Oğuz F, Oğuz MN, Çağan Ulusan E, Nacak A. Determination of feed value of chemically treated sun-dried grape pomace. Medit Vet J. 2024; 9(2): 270-276.

forages can decrease ADF and NDF levels, thereby enhancing the digestibility of DM and organic matter (Rezaii et al., 2023; Uzatıcı et al., 2022; Maduro Dias et al., 2021). Rezaii et al. (2023) found that treatment of cumin straw with CH_4N_2O led to an increase in its NDF level. Uzatıcı et al. (2022) demonstrated that applying 1-3% sodium hydroxide (NaOH) to cane (*Phragmites australis*) reduced its NDF and ADF content while enhancing the digestibility of total DM (1-3%) and organic matter (2 and 3%). Rezaii et al. (2023) also observed that hydrogen peroxide treatment of cumin straw increased its total digestible nutrients (TDN) but decreased both the amount of ADF and DM digestibility. Maduro Dias et al. (2021) reported that treating ginger lily components with 8% NaOH not only decreased the plant's ADF and NDF levels but also increased its *in vitro* digestibility of dry matter (IVDMD) and organic matter. Similar results were observed in the *Arundo donax* plant treated with 8% NaOH (Teixeira et al., 2021).

The NaOH utilized in the present study is classified as an acidity regulator for cats, dogs, and ornamental fish according to Annex I of the EU Feed Additives Regulation published on January 21, 2013 (Code 1j524). However, NaOH can additionally be employed in product processing as part of its designated application under the 'special use' categorization (EC 2003). Conversely, CH_4N_2O is also permitted for use in ruminants (EC 2012). It was observed that there exists no regulation governing the use of liquid NH_3 in animal feed. The purpose of this investigation is to explore whether grape pomace, a by-product of the grape industry, can be utilized as a roughage supplement in animal husbandry. To this end, an assessment was conducted on the nutritional value, *in vitro* protein degradability, and IVDMD of DGP in response to interventions involving NH_3, CH_4N_2O, and NaOH, all of which constitute economically viable additives for producers.

MATERIALS and METHODS

DGP from the province of Denizli-Türkiye was used in the study. Three experimental groups and one control group were established, each consisting of eight replicates. Solutions of 1.5% ammonia (DGP-NH_3), 3% urea (DGP- CH_4N_2O), and 3% sodium hydroxide (DGP-NaOH) were prepared in distilled water and applied (by hand mixing) to the first, second, and third experimental groups, respectively. The DM content was standardized to 50% for all groups. The study methodology employed herein adheres to the approaches delineated by Martens et al. (2022) and Uzatıcı et al. (2022) for determining the requisite quantities of NaOH and CH_4N_2O, as well as for standardizing the treatments at 50% DM. The treatment level was established at 1.5%, thereby ensuring that the application of liquid NH_3 in this investigation did not perturb the 50% DM standardization. The control group received no chemical additives, and distilled water was used to adjust moisture levels. All the study groups were stored in airtight plastic bags at room temperature for one week (Martens et al., 2022; Teixeira et al., 2021).

Determination of nutritional content of groups

The levels of DM, crude ash (CA), CP, and ether extract (EE) in the samples were determined using the techniques outlined in AOAC (2000). The quantification of crude fiber (CF), NDF, ADF, and acid detergent lignin (ADL) was performed using an ANKOM A2000 Fiber Analyzer (ANKOM Technology, NY, USA) (Ahsan, 2023).

Determination of roughage qualities and relative feed value

To calculate the forage quality (including total carbohydrate, non-structural carbohydrate, cellulose, hemicellulose, net energy lactation (NEL), and total digestible nutrient (TDN) content and (RFV) of the control and experimental groups, the following formulas were used (Ahsan, 2023; Horrocks and Vallentine, 1999).

- Total carbohydrates (in dry matter) = DM% – (CP% + CA%+ EE%)
- Non-structural carbohydrates (%, DM basis) = 100 – (NDF% + CP %+ CA% + EE%)
- Cellulose (%, DM basis) = ADF% –ADL%
- Hemicellulose (%, DM basis) = NDF% – ADF%
- NEL (Mcal/kg) = [1.044 – (0.0119 × ADF%)] × 2.205
- TDN (%, DM basis) = (–1.291 × ADF%) + 101.35
- Digestible dry matter-DDM (%, DM basis) = 88.9 – (0.779 × ADF%)
- Dry matter intake-DMI (%, DM basis) = 120 ÷ NDF%
- Relative feed value-RFV (%, DM basis) = DDM% × DMI% × 0.775

RFV is a key metric used in the evaluation and marketing of roughage. According to Kılıç and Addi Abdiwali (2016), RFV values below 75 indicate poor quality, 75-86 signify 4th quality, 87-102 3rd, 103-124 2nd, 125-151 as good, and values greater than 151 are considered 1st quality.

Determination of in vitro rumen digestibility and crude protein degradability of groups

The IVDMD and organic matter digestibility (IVOMD) were determined for each group using the ANKOM Daisy II incubator. For this purpose, duplicate samples were weighed and placed into filter bags (25 mm pore size propylene polyester) compatible with the device. These filter bags were then placed in the rumen fluid incubator bottle. The ruminal contents utilized were obtained by combining those from three distinct bovine subjects (13-month-old Holstein steers) that had been slaughtered subsequent to receiving a diet consisting of barley and corn silage. The fluid was transported to the laboratory within 20 minutes, using a thermos maintained at a constant temperature of 39°C. The filter bags were subsequently subjected to a 48-hour incubation period in an ANKOM Daisy II incubator containing ruminal content, buffer A, and buffer B. The quantities of each component (KH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $CaCl_2 \cdot 2H_2O$, and CH_4N_2O) utilized in the preparation of the Buffer A solution were as specified in the prescribed methodology. The constituents of the buffer B solution were Na_2CO_3 and $Na_2S \cdot 9H_2O$. Anaerobic condi-

tions were maintained throughout the procedure by means of a continuous carbon dioxide gas flow. Finally, the values for IVDMD and IVOMD were calculated based on the process (Ahsan, 2023).

Statistical analysis

For statistical analysis, the software SPSS 18.0 (IBM Corp., Armonk, NY, US) was employed. The Kolmogorov-Smirnov test was utilized to assess whether the data followed a normal distribution. One-way analysis of variance (ANOVA) was performed to compare the differences in values across the groups. Duncan's multiple range test was used to determine the significance of differences in mean values between groups. Results are presented as marginal means and their associated standard errors. A p-value of less than 0.05 was considered statistically significant (Dawson and Trapp, 2001).

RESULTS

The nutrient content, and levels of fiber fractions (NDF, ADF, and ADL) of grape pomace treated with various chemicals are depicted in Table 1. Compared to the control and NaOH treatment groups, the application of NH_3 and CH_4N_2O substantially increased CP levels (24.45 and 19.84%, respectively) ($p<0.001$), with improvements ranged from 61-90%. Regarding EE content, the two N-containing groups (DGP- CH_4N_2O and DGP-NH_3) exhibited similarity. The difference in EE content between these groups and the control and DGP-NaOH groups did not reach statistical significance. Remarkably, all experimental groups exhibited significantly higher ADF, NDF, and ADL levels when compared with the control group ($p<0.035$). Moreover, the DGP- CH_4N_2O group demonstrated the highest CA content in comparison to the control and other experimental groups ($p<0.001$).

The effect of chemical treatments on total carbohydrates (CHO), non-fibrous carbohydrates (NFC), cellulose and hemicellulose levels in grape pomace is shown in Table 2. In the study, no alteration was seen in calculated structural carbohydrate levels (cellulose and hemicellulose). Table 3 presents the feed value and *in vitro* digestibility of chemically treated grape pomace. Notably, all chemically treated experimental groups exhibited considerably lower values for digestible DDM, DMI, RFV, NEL, and TDN compared to the control group ($p<0.001$). Interestingly, no significant differences were found between the experimental groups treated with N-containing chemicals (DGP- CH_4N_2O and DGP-NH_3). The IVPD values for the two N-containing experimental groups (DGP- CH_4N_2O and DGP-NH_3) were 65.69% and 59.84%, respectively. Although a significant difference existed between these values, they were naturally significantly higher ($p<0.001$) than that of the DGP-NaOH group (40.32%). Conversely, the IVDMD and IVOMD values of both N-containing experimental groups were significantly reduced compared to the control group ($p<0.001$).

Table 1. Nutrient content and NDF, ADF, and ADL levels of grape pomace treated with chemicals (%, DM basis).

		Experimental Groups			
Nutrients	Control	DGP-NH_3	DGP-CH_4N_2O	DGP-NaOH	p
DM	94.04 ± 0.04 c	94.94 ± 0.24 b	95.90 ± 0.03 a	94.90 ± 0.02b	0.001
CF	31.31 ± 0.23 b	33.55 ± 0.36 a	32.19 ± 0.40 b	30.19 ± 0.37 c	0.001
EE	3.78 ± 0.15 bc	4.35 ± 0.23 a	4.06 ± 0.11 ab	3.50 ± 0.13 c	0.004
CP	12.86 ± 0.15 c	24.45 ± 0.22 a	19.84 ± 0.47 b	12.32 ± 0.18 c	0.001
CA	6.14 ± 0.07 b	5.88 ± 0.05 b	9.97 ± 0.30 a	6.52 ± 0.38 b	0.001
ADF	19.21 ± 0.33 b	20.53 ± 0.39 a	21.11 ± 0.43 a	21,61 ± 0.41 a	0.001
ADL	13.07 ± 0.31 b	14.53 ± 0.40 a	14.72 ± 0.58 a	14.75 ± 0.48 a	0.035
NDF	23.08 ± 0.36 b	24.51 ± 0.40 a	24.88 ± 0.41 a	25.73 ± 0.74 a	0.005

DM: Dry matter, CF: Crude fiber, EE: Ether extract, CP: Crude protein, CA: Crude ash, ADF: Ash-free acid detergent fiber, ADL: Acid detergent lignin, NDF: Ash-free neutral detergent fiber after amylase treatment.

Table 2. The effect of chemical treatments on total CHO, NFC, cellulose, and hemicellulose levels in grape pomace (%, DM basis).

		Experimental Groups			
Nutrients	Control	DGP-NH_3	DGP-CH_4N_2O	DGP-NaOH	p
Total CHO	71.25 ± 0.27 b	60.25 ± 0.37 d	62.02 ± 0.56 c	72.55 ± 0.46 a	0.001
NFC	54.12 ± 0.44 a	40.79 ± 0.41 c	41.22 ± 0.67 c	51.91 ± 0.75 b	0.001
Hemicellulose	3.86 ± 0.07	3.98 ± 0.09	3.76 ± 0.25	4.11 ± 0.41	0.788
Cellulose	6.13 ± 0.07	6.00 ± 0.18	6.39 ± 0.34	6.86 ± 0.39	0.155

Total CHO: Total carbohydrates, NFC: Non-fibrous carbohydrates.

Table 3. Feed value and *in vitro* digestibility of grape pomace treated with chemicals (%, DM basis).

	Experimental Groups				
	Control	DGP-NH_3	DGP-CH_4N_2O	DGP-NaOH	p
Feed value					
DDM	73.93 ± 0.26 a	72.77 ± 0.32 b	72.44 ± 0.33 b	70.39 ± 0.35 c	0.001
DMI	5.21 ± 0.08 a	4.91 ± 0.08 b	4.84 ± 0.08 b	4.12 ± 0.06 c	0.001
RFV	299.25 ± 5.80 a	277.56 ± 5.98 b	272.25 ± 6.13 b	225.40 ± 4.59 c	0.001
NEL	1.79 ± 0.01 a	1.74 ± 0.01 b	1.74 ± 0.01 b	1.67 ± 0.01 c	0.001
TDN	76.54 ± 0.43 a	74.11 ± 0.87 b	74.08 ± 0.55 b	70.69 ± 0.59 c	0.001
In vitro digestibility					
IVPD	47.63 ± 1.60 c	59.84 ± 1.34 b	65.69 ± 0.98 a	40.32 ± 1.51 d	0.001
IVDMD	59.43 ± 0.60 a	47.18 ± 0.69 c	54.39 ± 0.76 b	56.94 ± 1.59 ab	0.001
IVOMD	58.45 ± 0.61a	46.22 ± 0.73 b	47.91 ± 1.02 b	55.39 ± 1.69 a	0.001

DDM: Digestible dry matter (%, DM basis), DMI: Dry matter intake (% body weight), RFV: Relative feed value, NEL: Net energy for lactation (Mcal/kg), TDN: Total digestible nutrients (%, DM basis), IVPD: In vitro protein degradability (%, DM basis), IVDMD: In vitro true dry matter digestibility (%, DM basis), IVOMD: In vitro true organic matter digestibility (%, DM basis).

DISCUSSION

The CP levels observed in the control and DGP-NaOH groups, 12.86% and 12.32% respectively, were comparable to the 12.5% level reported for dried grape pomace (DGP) by Kılıç and Abdiwali (2016). Several studies (Pop et al., 2015; Basalan et al., 2011; Baumgartel et al., 2007) indicate that there may be a difference in CP levels between 2.1 and 6.2% in favor of red grape pomace. Furthermore, the findings of Hanusovsky et al. (2020) evinced that the crude protein content of pomace derived from the same grape variety can exhibit a disparity of approximately 2.7% when cultivated in distinct geographical locales. The soil and climate where the grape grow will influence the nutritional level, although variations in processing methods could also play a role. The CP levels were found to be elevated in DGP treated with NH_3 or CH_4N_2O. It is plausible that the high nitrogen content of these two chemical compounds may be responsible for the observed increase in CP levels. The EE level obtained by NaOH treatment of DGP is congruent with the findings of Sarıçiçek and Kılıç (2002) (3.67%). Notably, the EE ratio of the DGP-NH_3 group (4.35%) is substantially higher than that of the control and NaOH groups. This phenomenon could be attributable to either the interaction between NH_3 and oil or the hydrolysis of lipids facilitated by NaOH. Basalan et al. (2011) reported that the EE content in grape pomace seed, membrane plus soft tissue, and stem is 6.2%, 4.6%, and 1.2%, respectively.

Numerous studies have reported that the CF content of pomace is influenced by factors such as the grape variety, the proportion of stems present in the pomace, and the region where the grapes are cultivated (Hanusovsky et al., 2020; Pop et al., 2015; Baumgartel et al., 2007). According to Baumgartel et al. (2007) and Pop et al. (2015), red grape pomace exhibited a CF content of 31.2%, stemless red grape pomace had 31.31%, and stalked white grape pomace contained 32.28%. These values are consistent with those observed for the untreated pomace (control) and DGP-CH_4N_2O groups in the present study. However, according to reports, the CF content of grape pomace from Slovakia's Zwegelt and Austria's Green Veltliner is 23.3% and 12%, respectively (Hanusovsky et al., 2020). Sarıçiçek and Kılıç (2002) reported 18.6% CA in grape pomace. The grape species could be the cause of the high CA level. The results of the study are consistent with the CA values of white-stemmed and red-stemmed grape pomace (6.46% and 6.51%, respectively) in the control, DGP-NH_3, and DGP-NaOH groups. The high CA of the DGP- CH_4N_2O group in the study may be due to the purity level of the CH_4N_2O used or the seed content of the pomace mixture. Özcan et al. (2017) reported that the mineral content of grape seed is higher compared to the rest of the grape. Similar to those measured from Green Veltliner grape pomace produced in Austria (Hanusovsky et al., 2020), the values of ADF, NDF, ADL in the experimental groups of this study are in agreement with those reported.

There is a lack of study about the effects of chemical treatment on the structural carbohydrates of DGP. However, certain studies have demonstrated that the ADF, NDF, and ADL levels of pomace are influenced by the country where the grape is produced (the soil or climate in which it grows) (Hanusovsky et al., 2020), as well as its variety (Kılıç and Abdiwali, 2016; Winkler et al., 2015; Baumgartel et al., 2007). Hanusovsky et al. (2020) reported that structural carbohydrates in a single grape variety grown in Slovakia can exhibit variations of 4.9 to 5.9%. The present study's ADF, NDF, and ADL values for all chemical groups were lower compared to the results of earlier studies (Kılıç and Abdiwali, 2016; Winkler et al., 2015; Baumgartel et al., 2007) on grape pomace. The literature indicates that the ADF content of white grape pomace was 43.7% (Winkler et al., 2015), while the NDF content was 50.7% (Baumgartel et al., 2007). Regarding DGP, the ADL was reported as 32.4% (Kılıc and Abdiwali, 2016).

Chemical treatments did not significantly alter the amounts of cellulose and hemicellulose in the groups. The cellulose content observed in DGP aligns with the findings of Kılıç and Abdiwali (2016). Similarly, the hemicellulose level mirrors the results reported for Austrian Pinot Blanc grape pomace by Honisovsky et al. (2020). The NFC values of the control and DGP-NaOH groups resemble those of Austrian Green Veltliner grape (54%). Conversely, the NFC values of the DGP-NH_3 and DGP- CH_4N_2O groups more closely match the values of Pinot Blanc grape pomace (41.5%) (Hanusovsky et al., 2020). The reduction in pomace's NFC value induced by both N-containing chemicals may correlate with the concurrent increase in its nitrogen content.

Kılıç and Abdiwali (2016) reported that DGP had DDM, DMI, and RFV of 62.61, 2.74, and 133.06%, respectively. These values are lower than those observed in our trial and control groups in the present study. Within the present study, the DGP-NaOH group exhibited the lowest relative feed value (RFV) at 225.40. However, it should be noted that Linn and Martin (1999) have posited that an RFV score exceeding 151 is indicative of roughage meeting the highest quality classification criteria. Investigations examining the *in vitro* dry matter digestibility (IVDMD) of grape pomace are relatively scarce. Nonetheless, the IVDMD levels observed in the control and DGP-NaOH groups were congruent with the report by Kılıç and Abdiwali (2016) (62.61%). This variation may be attributed to differences in grape species and the proportion of stems to seeds. Çakmakçı and Barut (1997) noted that NaOH treatment of low-nutritional-value forages solubilized some hemicellulose without affecting the cellulose concentration. Various studies have shown that NaOH treatment of alternative forages enhances their IVDMD (Uzatıcı et al., 2022; Maduro et al., 2021; Teixeira et al., 2021). The IVDMD results for DGP-NaOH are consistent with literatures. However, the IVDMD of pomace was significantly reduced following NH_3 treatment compared to the control and other trial groups. The reduction in digestibility might be attributed to the low hemicellulose levels (3-4%) in the DGP used in the present study. Currently, literature lacks data on protein degradability for DGP. In the present study, the IVPD in the DGP-NH_3 and DGP-CH_4N_2O groups were significantly higher compared to the DGP-NaOH and control groups. Nevertheless, it is evident that both nitrogen (N)-containing chemicals, NH_3 and CH_4N_2O significantly enhance the CP level in pomace.

CONCLUSION

The nutritional composition of DGP is significantly affected by chemical treatments. N-containing chemicals such as CH_4N_2O and NH_3 were found to increase DGP's IVPD and CP levels. Conversely, IVDMD decreased with NH_3 treatment, while NaOH treatment led to its increase. All groups exhibited RFV values meeting the criteria for top-level roughages in the study. However, NaOH treatment resulted in a lower RFV value for DGP compared to other chemical treatments. Further studies on the feed value and IVDMD of DGP may benefit from maintaining lower moisture content, thereby prolonging the incubation period, which holds potential for enhancing the literature through extended chemical application periods.

DECLARATIONS

Ethics Approval

The research is not an animal experiment, ethics committee authorisation is not required.

Conflict of Interest

The authors have no conflict of interest with any person, institution or organisation.

Consent for Publication

Publication is appropriate

Author contribution

Idea, concept and design: KEB

Data collection and analysis: KEB, DMK, EÇU, AN

Drafting of the manuscript: KEB

Critical review: KEB, MNO, FKO

Data Availability

The data is available from the corresponding author on reasonable request.

Acknowledgements

Not applicable.

REFERENCES

Aghsaghali, A. M., Maheri-Sis, N., Mansouri, H., Razeghi, M. E., Telli, A. S., & Golshani, A. A. (2011). Estimation of the nutritive value of grape pomace for ruminants using gas production techniques. African Journal of Biotechnology, 10(33), 6246-6250.

Ahsan, U. (2023). Effect of single or combined homo- and heterofermentative silage additives on the quality, nutritive value, and *in vitro* digestibility of ensiled wheat harvested at early dough stage of maturity. Mehmet Akif Ersoy University Journal of Health Sciences Institute, 11(2), 267-274. https://doi.org/10.24998/maeusabed.1345229

AOAC. (2000). Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists.

Atalay, A. I. (2020). Determination of nutritive value and anti-methanogenic potential of Turkish grape pomace using i*n vitro* gas production technique. Journal of Animal and Plant Sciences, 30(4), 944-949. https://doi.org/10.36899/JAPS.2020.4.0110

Bahrami, Y., Foroozzandeh, A. D., Zamani, F., Modarresi, M., Eghbal-Saeid, S., & Chekani-Azar, S. (2010). Effect of diet with varying levels of dried grape pomace on dry matter digestibility and growth performance of male lamb. Journal of Animal and Plant Sciences, 6(1), 605-610.

Basalan, M., Güngör, T., Owens, F. N., & Yalçınkaya, I. (2011). Nutrient content and *in vitro* digestibility of Turkish grape pomaces. Animal Feed Science and Technology, 169, 194–198. https://doi.org/10.1016/j.anifeedsci.2011.07.005

Baumgartel, T., Kluth, H., Epperlein, K., & Rodehutscord,

M. (2007). A note on digestibility and energy value for sheep of different grape pomace. Small Ruminant Research, 67, 302-306. https://doi.org/10.1016/j.smallrumres.2005.11.002

Besharati, M., & Taghizade, A. (2010). Effect of adding polyethylene glycol and polyvinylpyrrolidone on organic matter digestibility, metaboliz.able energy and net energy for lactation of grape pomace using *in vitro* gas production technique. Proceeding of the British Society of Animal Science, 1(1), 251. https://doi.org/10.1017/S2040470010003948

Çakmakçı, S., & Barut, N. (1997). The methods of increasing nutritional value and digestibility of low quality forages. Akdeniz University Journal of the Faculty of Agriculture, 10, 345-357.

Dawson, B., & Trapp, R.G. (2001). Basic and clinical biostatistics, 3rd ed. Lange Medical Books/McGraw-Hill Medical Publishing Division, New York, 994, 180.

Deng, Q., Penner, M. H., & Zhao, Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. Food Research International, 44(9), 2712-2720. https://doi.org/10.1016/j.foodres.2011.05.026

EC (2003). Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal L 268:29, 2003. http://data.europa.eu/eli/reg/2003/1831/oj

EC (2012). Commission Implementing Regulation (EU) No 839/2012 of September 2012 concerning the authorisation of urea as feed additive for ruminants. Official Journal of the European Union L252:11, 2012. https://eur-lex.europa.eu/LexUriServ/Lex-UriServ.do?uri=OJ:L:2012:252:0011:0013:EN:PDF

Faostat (2023). Food and Agriculture Organization of the United Nations, Rome, Italy. (https://www.fao.org/faostat/en/#compare) Erişim: 29.09.2023

Hanusovsky, O., Galik, B., Biro, D., Simko, M., Juracek, M., Rolinec, M., Zabransky, L., Philipp, C., Puntigam, R., Slama, J. A., & Gierus, M. (2020) The nutritional potential of grape by-products from the area of Slovakia and Austria. Emirates Journal of Food and Agriculture, 32(1), 1-10. https://doi.org/10.9755/ejfa.2020.v32.i1.2051

Horrocks, V., Valentine, J. F. (1999). Harvested forages. Academic Press.

Kılıç, U., & Abdiwali, A. A. (2016). Determination of *in vitro* true digestibilities and relative feed values of wine industry grape residues as alternative feed source. Journal of the Faculty of Veterinary Medicine, Kafkas University, 22(6), 895- 901. https://doi.org/10.9775/kvfd.2016.15617

Linn J. G., Martin N. P. (2024, April 8). Forage Quality Tests and Interpretations. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://conservancy.umn.edu/bitstream/handle/11299/207442/MN2500_AGFO_2637_revised1989.pdf?sequence=1&isAllowed=y

Maduro Dias, C. S. A. M., Madruga, J. S., Vouzela, C. F. M., Borba, A. E. S. (2021). Effect of sodium hydroxide treatment in ginger lily forage (Hedychium gardnerianum, Sheppard ex Ker-Gawl) as forage for animal feeding. African Journal of Agricultural Research, 17(2), 268-272. https://doi.org/10.5897/AJAR2020.15332.

Martens, S. D., Wildner, V., Schulze, J., Richardt, W., Greef, J. M., Zeyner, A., & Steinhöfel, O. (2022). Chemical treatment of straw for ruminant feeding with NaOH or urea – investigative steps via practical application under current European Union conditions. Agricultural and Food Science, 31, 260–281. https://doi.org/10.23986/afsci.115262

Nerantzis, E. T., & Tataridis, P. (2006). Integrated enology utilization of winery by products into high added value products. Journal of Science and Technology, 1(3), 79-89.

Özcan, M. M., Al Juhaimi, F., Gülcü, M., Uslu, N. & Geçgel, Ü. (2017). Determination of bioactive compounds and mineral contents of seedless parts and seeds of grapes. South African Journal of Enology and Viticulture, 38(2), 212-220. http://dx.doi.org/10.21548/38-2-1605

Özdüven, M. L., Coşkuntuna, L. & Koç, F. (2005). Determination of fermentation and feed value characteristics of grape pomace silage. Trakya University Journal of Natural Sciences, 6(1), 45-50.

Pop, I. M., Pascariu, S. M., Simeanu, D., Radu-Rusu, C., & Albu, A. (2015). Determination of the chemical composition of the grape pomace of different varieties of grapes. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie, 63, 76-80.

Rezaii, F., Bayatkouhsar, J., Ghanbari, F., & Arab, F. (2023). The effect of water, urea, sodium hydroxide, and hydrogen peroxide processing on the cumin residues animal digestibility. International Journal of Recycling Organic Waste in Agriculture, 12(4), 525- 537. https://doi.org/10.30486/IJROWA.2022.1953020.1418

Sarıçiçek, B. Z., & Kılıç, Ü. (2002). A study on determining *in situ* degradability of grape pomace. Atatürk University Journal of Agricultural Faculty, 33(3), 289-292.

Sümbül, A., & Yıldız, E. (2022). Present situation and production projection of table, dry and wine grapes grown in Turkey. Erciyes Agriculture and Animal Science, 17-22. https://doi.org/10.55257/ethabd.1095080

Teixeira, S. M. P., Maduro Dias, C. S. A. M., Vouzela, C. F. M., Madruga, J. S., & Borba, A. E. S. (2021). Nutritional valorisation of cane (Arundo donax) by treatment with sodium hydroxide. South African Journal of Animal Science, 51 (4), 437-443. http://dx.doi.org/10.4314/sajas.v51i4.3

TÜİK, (2023) İstatistik veri portalı. https://data.tuik.gov.tr/Search/Search?text=%C3%BCz%C3%BCm Erişim: 29.09.2023

Uzatıcı, A., Canbolat, O., & Kamalak, A. (2022). Effect of

sodium hydroxide treatment on chemical composition and feed value of common reed (phragmites australis) straw. Fermentation. 8, 749, 1-7. https://doi.org/10.3390/fermentation8120749

Varış, S., Altıntaş, S., & Butt, S.J. (2000). Cibre ve cibre torba kültürü. Hasad Dergisi, 186, 40-43.

Voicu, D., Habeanu, M., Uta, R. A., Voicu, I., & Gras, M. A. (2014). Effect of the dietary dry grape pomace on the performance and health state of fattening steers. Scientific Papers. Series D. Animal Science, 57, 118-124.

Winkler, A., Weber, F., Robert, R., & Dusel, G. (2015). Determination of polyphenol and crude nutrient content and nutrient digestibility of dried and ensiled white and red grape pomace cultivars. Archives of Animal Nutrition, 69:3, 187-200. https://doi.org/10.1080/1745039X.2015.1039751

Zhen-Zhen, L., Chen, Z., Fa-Di, L., Chong, L., De-Fu, T., Xiu-Xiu, W., Ting, L., Zhi-Yuan, M., & Dong-Fang, M. A. (2015). Effect of feeding grape pomace on nutrients digestibility, body weight gain, and carcass quality in lambs. Acta Petrologica Sinica, 24 (4), 114-120.

Medit Vet J, 9(2): 277-282, 2024

Research Article

Investigation of the shape of goat (capra hircus) astragalus via geometric morphometry method

Tuğba Oktay[1], Yasin Demiraslan[2], Özcan Özgel[2]

[1]Department of Anatomy, Institute of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2]Department of Anatomy, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
astragalus
capra hircus
gender
shape

Received : 15 January 2024
Revised : 20 June 2024
Accepted : 28 July 2024
Published : 31 August 2024
Article Code : 1420043

Correspondence:
T. OKTAY
(tugbaoktay32@gmail.com)

ORCID
T. OKTAY : 0000-0002-0846-4403
Y. DEMİRASLAN : 0000-0003-3612-6142
Ö. ÖZGEL : 0000-0003-0394-5678

The present study was presented at the XIIth National/IIIth International Veterinary Anatomy Congress on September 27-29, 2023.

ABSTRACT

The aim of this study is to determine the effect of sex on shape of goat astragalus via geometric morphometry method. A total of 37 astragalus bone samples collected from 16 female and 21 male goats were used as material. Bone samples were cleaned from skin and soft tissue and then macerated by boiling. Astragalus bone samples were photographed from a distance of 20 cm dorsally by focusing on the center of the bone. The photographs were transferred to the computer in JPEG format. Principal Component Analysis, Regression Analysis, Canonical Variate Analysis, and Discriminant Function Analysis were conducted using the Cartesian coordinate values, which were obtained by homologous landmark marking. The first two principal components accounted for 75.967% of the total shape variation. Shape variation was determined in different regions of the astragalus. According to the scatter plot of male and female individuals, male individuals were completely placed within the confidence interval ellipse of female individuals. It was found that allometric effect on the shape of astragalus bone was not statistically significant. As a result of Canonical Variate Analysis, mahalanobis and procrustes distances were detected as 2.9216 ($p<0.0001$) and 0.0645 ($p=0.0035$), respectively. This test indicated two female individuals in the group of males. The proximal of male goat astragalus was wider than that of female goats. The results of the Discriminate Function Analysis revealed that 8 of the female individuals and 7 of the male individuals were incorrectly grouped according to the cross validation scores. Geometric morphometry and the related analyses allowed to examine the differences between the astragalus bone samples of male and female goats. The fact that the astragalus bone of male goats was wider than that of females supported the studies using linear measurements in the literature. Consequently, the result indicating that sex factor had a limited grouping effect on astragalus shape in goats was obtained.

INTRODUCTION

Capra hircus is a species known as domestic goat belonging to the genus Capra of the family Bovidae (Payne and Wilson, 1999). Goats, known as the first domesticated animal species (Zeder and Hesse, 2000), have been in relation with humans since about 10.000 years ago according to the archeological findings. Human beings have reared goats as of ancient times to obtain animal products. The reason for being a common species when compared to other animal species is their easy adaptation to environmental conditions and their resistance to diseases and parasites (Naderi et al., 2008).

Geometric morphometry is the analysis of shape variables including all geometric information in the data (Slice, 2007). This method is based on the quantification of the shape of the material by using two- or three-dimensional coordinates of anatomical landmarks and semi-landmarks (Gonzalez et al., 2009). In geometric morphometry, measurements are taken with three-dimensional digitalization tools or special software (Aytek, 2017). This method directly focuses on shape differences in individuals and/or groups (Özkan, 2022).

Astragalus is the second largest tarsal bone after the calcaneus. It consists of three parts: corpus tali, collum tali, and caput tali (Motagi et al., 2015). The trochlea of the astragalus articulates with the tibia and its plantar and lateral parts articulate with the calcaneus (König and Liebich, 2013).

Especially in forensic deaths and archaeological remains, bones are often found as fragmented and mixed (Figus et al., 2017). For these reasons, biological profiles are created from different bones that can be an alternative to bones such as pelvic and cranial bones, which are important in sex determination (Brzobohat, 2015). It is known that individuals of different sexs have different gait kinetics, postures, and joint angles (Graci et al., 2012). It is thought that these differences may be reflected in the general shape of the astragalus (Sorrentino et al., 2020). There have been studies in the literature in which linear measurements of bones such as astragalus and calcaneus were performed (Davies et al., 2014; Nathena et al., 2017). However, it is known that studies conducted with linear measurements have some limitations (Lee et al., 2015). Geometric morphometry eliminates the limitations of linear measurements (Kranioti et al., 2009) as it provides too much data on shape (Aytek, 2017).

The geometric morphometry method was preferred as it eliminates the limitations of linear measurements and it was aimed to investigate its role in sex determination.

To cite this article:
Oktay T, Demiraslan Y, Özgel Ö. Investigation of the shape of goat (capra hircus) astragalus via geometric morphometry method. Medit Vet J. 2024; 9(2): 277-282.

MATERIALS and METHODS

A total of 37 right astragalus bones from 16 adult (2-3 years old) female goats and 21 adult (2-3 years old) male goats were used in the study. Bone materials were cleaned from skin and soft tissue and then boiled. After boiling, the tissues remaining on the bone were well cleaned.

Geometric Morphometric Analysis

Astragalus bones were photographed from a distance of 20 cm from the dorsal side and focusing on the center of the bone (Canon 600D 18x55 lens, Japan). The photographs were transferred to computer in JPEG format. These images were converted to tps format using TpsUtil (Version 1.79) software (Rohlf, 2019). Cartesian coordinates of homologous LMs (sign element) were determined by marking on the images with TpsDig2 (Version 2.31) software (Rohlf, 2018). Principal Component Analysis (PCA) was performed on the coordinates. MorphoJ (Klingenberg, 2011) software was used to determine the LM levels and directions where shape differences were observed between groups. Regression analysis was performed to determine the allometric effect (Jeanjean et al., 2022). Canonical Variate Analysis (CVA) and Discriminate Function Analysis (DFA) were then performed to analyze the accuracy of classification.

LMs marked on dorsal surface of the astragalus (Figure 1);

1. Most proximal end of lateral proximal condyl
2. Lateral side of the center
3. Most lateral end of distal lateral condyl
4. Most medial end of distal lateral condyl
5. Most concave point between distal lateral and medial condyle
6. Most lateral end of distal medial condyl
7. Most medial end of distal medial condyl
8. Distal start point of the medial eminence
9. Peak point of the medial eminence
10. Proximal start point of the medial eminence
11. Most proximal end of medial proximal condyl
12. The most proximal concave point between the lateral and medial condyle

RESULTS

A total of 20 principal components were calculated in the study. PC1 accounted for 53.891% of the total shape variation. The first 2 principal components accounted for 75.967% of the total shape variation.

Figure 2 shows the shape variation graph obtained from principal component analysis. Accordingly, LM2, LM8, LM9, LM10, and LM11 were determined as the landmarks with the most significant amount of first order variation. Secondarily responsible landmarks were determined as LM1 and LM7. The other landmarks contributed the least to the amount of variation. Therefore, variation was determined in the central lateral edge, medial eminence and medial proximal condyle of the astragalus (Figure 2).

According to the scatter plot of male and female individuals in goats, male individuals were completely placed within the confidence interval ellipse of female individuals (Figure 3).

Size had an allometric effect of 3.0326% on the shape. The allometric effect was not statistically significant in the 10.000 rounds permutation test (P=0.3200).

As a result of CVA, the mahalanobis and procrustes distances were 2.9216 (P<0.0001) and 0.0645 (P=0.0035), respectively. Figure 4 shows the frequency graph obtained as a result of this test. The graph showed two female individuals in the group of males. More than 90% of the individuals were grouped correctly. There were differences between female and male goat astragalus bones at LM8, LM9, LM10, and LM11. These landmarks represented the mediopoximal portion of the astragalus. The proximal part of the astragalus was wider in males than in females (Figure 5).

According to the cross validation scores in DFA, 8 female individuals and 7 male individuals were incorrectly grouped (Figure 6). Females and males were grouped correctly by 50% and 65%, respectively. According to these results, sex factor had a limited grouping effect on astragalus shape.

DISCUSSION

Geometric morphometry and the related analyses allowed to examine the differences between the astragalus bones of male and female goats. The results revealed the shape of the astragalus in goats, while the allometric effect of size on the shape of the astragalus was not statistically significant. According to the results, the shape of the astragalus was found in the medioproximal part of the astragalus between male and female individuals, and the proximal part of the astragalus was wider in male goats than in females. When male and female comparative studies with linear measurements in the literature were examined, it was observed that many bones including the astragalus were morphologically wider in male individuals among human beings (Bilge, 2008; Bass, 1995). The current study conducted by using geometric morphometry support the studies using linear measurements in the literature in this regard.

Sorrentino and colleagues (2020), conducted a study using the geometric morphometric technique on a total of 98 human astragalus, (44 females and 54 males) from different geographical locations to investigate the role of shape, form and size in determining sex. They also demonstrated that the fragmented astragalus can be reconstructed. In the study, significant differences were found in sex dimorphism between astragalus from Sassari and Bologna regions and astragalus from New York and Bologna regions, while there was no significant difference in sexual dimorphism in the comparison between Sassari and New York regions. These results suggested that a population-specific approach should be used to assess sexual

dimorphism in the human astragalus (Sorrentino et al. 2020). In addition, the data of the current study supported studies using traditional linear measurements to determine sex (Bidmos and Dayal, 2004; Lee et al., 2012a). In the present study, even though there were differences in some LM points in terms of sexual dimorphism, it was understood that the sex factor had a limited grouping effect on astragalus shape.

The morphological and morphometric features of bones may differ in certain populations. This situation is important for surgical operations. There are studies in the literature in which the talus bone is grouped into different types according to joint characteristics in populations located in different geographies (Lee et al., 2012b; Azra et al., 2020). According to these studies, there are differences in the right and left hind leg joint types of the same individual. These differences in the joints also affect the movement of the joint (Boyan et al., 2016). There are studies in the literature showing that different locomotor behaviors have an effect on the shape of the bone (Vermeulen et al., 2022). For this reason, talus bones from the same side (right) were used as material in our study. According to the findings of our study, it was revealed that the talus bones of the right side of goats did not provide clear results in determining gender.

Haruda (2017) conducted a comparative study with both traditional morphometry and geometric morphometry to reveal the taxonomic differences of sheep and goat talus bone. According to the findings of this study, the talus bone of sheep varied in size. It was also determined that the talus of sheep was larger in size than that of goats. Talus variation between sheeps and goats has been described. In our study, whether the talus bone would be effective in determining gender in goats was investigated using geometric morphometry.

When the literature is examined, there are comparative studies on cranial bones in goats with geometric morphometry method (Yaprak et al., 2022; Parés-Casanova, 2015; Parés-Casanova and Domènech-Domènech, 2021), comparative studies on mandible (Demiraslan et al., 2020; Evcim 2020) and studies comparing the difference of astragalus between sheep and goat (Haruda, 2017). However, there is no study in the literature that examined the sexual dimorphism of the astragalus with geometric morphometry on goats. Therefore, the results obtained in the current study could not be compared with a study conducted on goats using geometric morphometry technique.

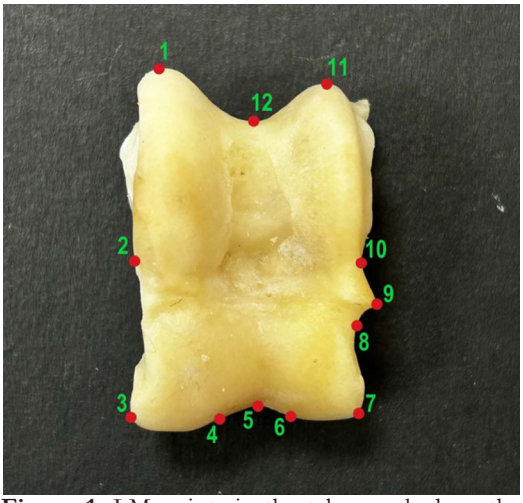

Figure 1. LM points in the talus marked on the photograph

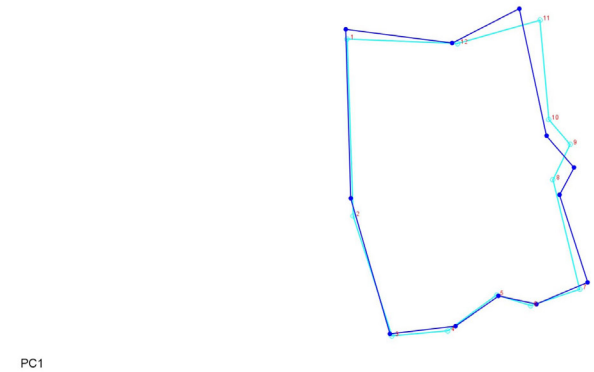

Figure 2. Line graph of the shape change of the talus in PC1 (Dark blue: mean shape, Light blue: variation)

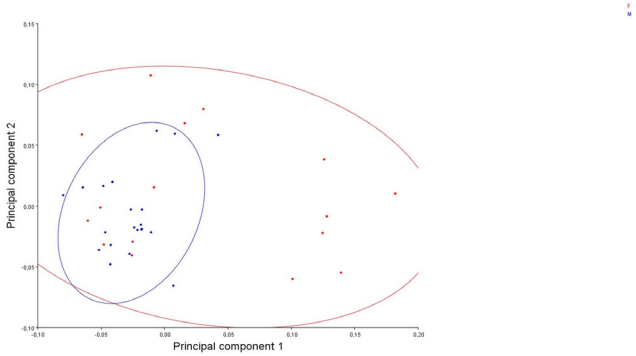

Figure 3. Scatter plot of PC1 of female and male individuals (Red: female, Blue: male)

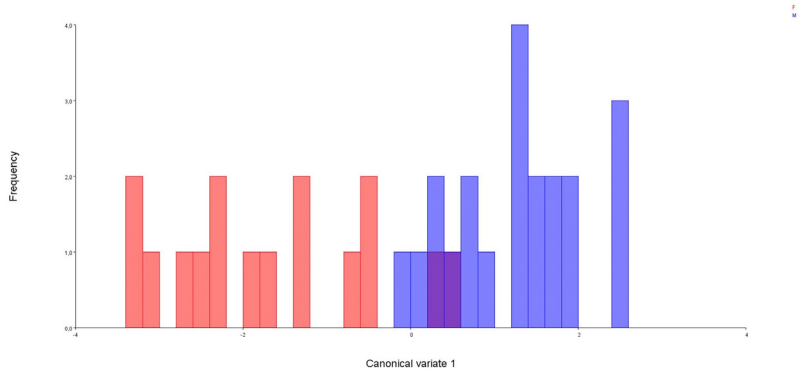

Figure 4. CVA graph of female and male individuals (Red: female, Blue: male)

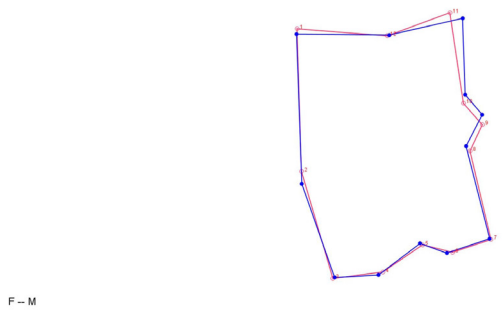

Figure 5. Line graph of female and male talus (Red: female, Blue: male)

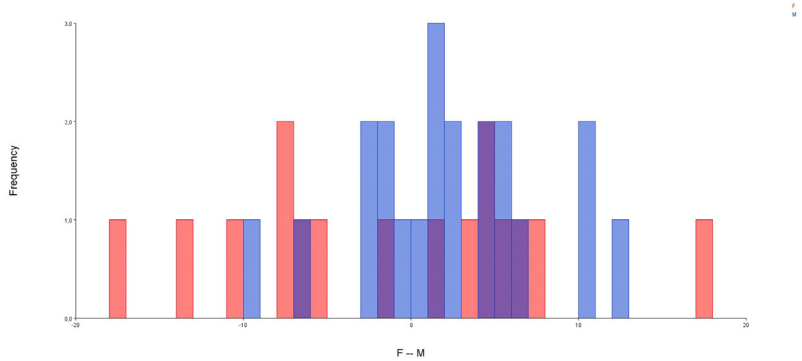

Figure 6. Graph of cross validation score in CFA for female and male individuals (Red: female, Blue: male)

CONCLUSION

In the present study, the sexual dimorphism of the astragalus bones of domestic goats was examined by geometric morphometry. It was found that the astragalus had a limited effect on sex determination. Thus, the study has supported the studies in the literature that have been conducted with linear measurements. In addition, it was determined that the size of the Astragalus had no significant effect on the shape of the astragalus. It is thought that the present study will contribute to the literature for future studies on small ruminants.

The limitations of the study are the lack of material and the unilateral examination of the bone.

DECLARATIONS

Ethics Approval

According to Article 8 of the Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees dated 15 February 2014 and numbered 28914, Ethics Committee Approval is not required (E-93773921-020-354464).

Conflict of Interest

There is no conflict of interest in current practice.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: YD

Data collection and analysis: TO

Drafting of the manuscript: YD

Critical review: ÖÖ

Data Availability

Not applicable.

Acknowledgements

The present study was presented at the XIIth National/IIIth International Veterinary Anatomy Congress on September 27-29, 2023.

REFERENCES

Aytek, A. İ. (2017). Geometrik morfometri. Mimarlar Arkeologlar Sanat Tarihçileri Restoratörler Ortak Platformu E-Dergisi, 11(17), 1-7.

Azra, M. Abhaya Prabhu. N, Balachandra. (2020). An anatomical study on types of calcaneal facets on talus and co relation between squatting facets and angles of neck. Indian Journal of Clinical Anatomy and Physiology 5(4), 434-438. DOI: 10.18231/2394-2126.2018.0101

Bass, W. M. (1995). Human Osteology: A laboratory and field manual (4th ed.). Missouri Archeological Society: Columbia.

Bidmos, M. A. and Dayal, M. R. (2004). Further evidence to show population specificity of discriminant function equations for sex determination using the talus of South African blacks. Journal of Forensic Sciences, 49(6), 1165–1170. PMID: 15568686

Bilge, Y. (2008). Adli Tıp. Üçbilek Matbaası: Ankara

Boyan, N. Ozsahin, E. Kizilkanat, E. Soames, R. Oguz, O. (2016). Morphometric measurement and types of articular facets on the talus and calcaneus in an Anatolian population. International Journal of Morphology. 34(4), 1378-1385

Brzobohata, H. (2015). Sex classification using the three-dimensional tibia form or shape including population specificity approach. Forensic Sciences, 60(1), 29–40. https://doi.org/10.1111/1556-4029.12641 PMID: 25387800

Davies, C. M., Hackman, L., Black, S. M. (2014). The foot in forensic human identification - A review. Foot, 24(1), 31–36. https://doi.org/10.1016/j.foot.2013.12.001 PMID: 24382464

Demiraslan, Y., Özgel, Ö., Gürbüz, İ., Zümre, Ö. (2021). The mandibles of the Honamli and Hair goat (Capra hircus); a geometric morphometric study. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 68, 321-328. DOI: 10.33988/auvfd.759964

Evcim, B. (2020). Koyun, keçi ve tavşanda mandibula'nın geometrik özellikleri. Yüksek Lisans Tezi, Aydın Adnan Menderes Üniversitesi Sağlık Bilimleri Enstitüsü Anatomi (Veteriner) Programı, Aydın.

Figus, C., Traversari, M., Scalise, L. M., Oxilia, G., Vazzana, A., Buti, L., et al. (2017). The study of commingled non-adult human remains: Insights from the 16th–18th centuries community of Roccapelago (Italy). Journal of Archaeological Science: Reports, 14, 382-391. https://doi.org/10.1016/j.jasrep.2017.06.023

Gonzalez, P. N., Bernal. V., Perez, S. I. (2009). Geometric morphometric approach to sex estimation of human pelvis. Forensic Science International, 189 (1-3), 68-74. doi: 10.1016/j.forsciint.2009.04.012.

Graci, V., Van Dillen, L. R., Salsich, G. B. (2012). Gender differences in trunk, pelvis and lower limb kinematics during a single leg squat. Gait Posture, 36(3), 461–466. https://doi.org/10.1016/j.gaitpost.2012.04.006 PMID: 22591790

Haruda, A. F. (2017). Separating sheep (ovis aries l.) and goats (capra hircus l.) using geometric morphometric methods: an investigation of astragalus morphology from late and final bronze age central asian contexts. International Journal of Osteoarchaeology, 27(4), 551–562. https://doi.org/10.1002/oa.2576

Jeanjean, M., Haruda, A., Salvagno, L., Schafberg, R., Valenzuela-Lamas, S., Nieto-Espinet, A., Forest, V., Blaise, E., Vuillen, M., Mureau, C., Evin, A. (2022). Sorting the flock: Quantitative identification of sheep and goat from isolated third lower molars and mandibles through geometric morphometrics. Journal of Archaeological Science, 141. https://doi.org/10.1016/j.jas.2022.105580

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric Morphometrics. Mol Ecol Resour, 11,

353-357.

König, H. E., and Liebich, H. G. (2013). Veterinary anatomy of domestic mammals: textbook and colour atlas. (4th ed). Stuttgart, Schattauer Verlag, 197-218.

Kranioti, E. F., Bastir, M., Sanchez-Meseguer, A., Rosas, A. (2009). A geometric-morphometric study of the cretan humerus for sex identification. Forensic Science International, 189, 2–9. https://doi.org/10.1016/j.forsciint.2009. 04.013 PMID: 19446415

Lee, J. Y. Jung, M. H. Lee, J. S. Choi, B. Y. Cho, B. P. (2012b). Types of calcaneal articular facets of the talus in Korean. Korean journal of physical anthropology. 25(4), 185-192.

Lee, U. Y., Han, S. H., Park, D. K., Kim, Y. S., Kim, D. I., Chung, I. H., et al. (2012a). Sex determination from the talus of Koreans by discriminant function analysis. Forensic Science International, 57, 166–171. https://doi.org/10.1111/j. 1556-4029.2011.01914.x PMID: 21981282

Lee, U. Y., Kim, I. B., Kwak, D. S. (2015). Sex determination using discriminant analysis of upper and lower extremity bones: New approach using the volume and surface area of digital model. Forensic Science International, 253-135. e1–135.e4. https://doi.org/10.1016/j.forsciint. 05.017 PMID: 26117502

Motagi, M. V., Kottapurath, S. R., Dharwadkar, K. (2015). Morphometric analyses of human dry tali of South Indian origin. International Journal of Medical Science and Public Health, 4(2), 237-240. doi: 10.5455/ijmsph.2015.3009201443

Naderi, S., Rezaei, H. R., Pompanon, F., Blum, M. G. B., Negrini, R., Naghash, H. R., Balkiz, O., Mashkour, M., Gaggiotti, O. E., Ajmone-Marsan, P., Kence, A., Vigne, J. D. Taberlet, P. (2008). The goat domestication process inferred from large-scale mitochondrial DNA analysis of wild and domestic individuals. Proceedings of the National Academy of Sciences, USA 105, 17659–17664. doi: 10.1073/pnas.0804782105

Nathena, D., Michopoulou, E., Kranioti, E. F. (2017). Sexual dimorphism of the calcaneus in contemporary Cretans. Forensic Science International, 277, 260. e1–260.e8. https://doi.org/10.1016/j. forsciint. 04.005 PMID: 28625510

Özkan, E. (2022). Kedilerde Pelvis ve Femur Radyolojik Görüntülerinin Osteometrik ve Geometrik Morfometrik Yöntemle Değerlendirilmesi. Doktora Tezi. İstanbul Üniversitesi-Cerrahpaşa Lisansüstü Eğitim Enstitüsü, Veterinerlik Anatomisi Anabilim Dalı Anatomi. İstanbul.

Parés-Casanova, P. M. (2015). Geometric morphometrics to the study of skull sexual dimorphism in a local domestic goat breed. Journal of Fisheries & Livestock Production, 3(3), 141. doi:10.4172/2332-2608.1000141

Parés-Casanova, P. M., and Domènech-Domènech, X. (2021). A comparative analysis of sphenoid bone between domestic sheep (ovis aries) and goat (capra hircus) using geometric morphometrics. Anatomia, Histologia, Embryologia, 50, 556–561. doi: 10.1111/ahe.12661

Payne, W. J. A. and Wilson, R. T. (1999). An introduction to animal husbandry in the tropics (5th ed.). Blackwell Science, s: 824. Oxford.

Rohlf, F. J. (2018) TpsDig Version 2.31, Ecology & Evolution. SUNY at Stone Brook, USA.

Rohlf, F. J. (2019). TpsUtil program Version 1.79, Ecology & Evolution. SUNY at Stone Brook. USA.

Slice, D. E. (2007). Geometric morphometrics, Annual Review Anthropol, 36(1), 261-281. DOI: 10.1146/annurev.anthro.34.081804.120613

Sorrentino, R., Belcastro, M. G., Figus, C., Stephens, N. B., Turley, K. (2020). Exploring sexual dimorphism of the modern human talus through geometric morphometric methods. PLoS ONE, 15(2), e0229255. https://doi. org/10.1371/journal.pone.0229255

Vermeulen, V. Kozma, E. Delsupehe, A. Pieter, C. Emmelie, S. Alexander, V. T. Lieven, D. W. Vereecke, E. (2022). Scapular morphology of great apes and humans: A three-dimensional computed tomography-based comparative study. Jornal of Anatomy. 242(2), 164-173. DOI: 10.1111/joa.13784

Yaprak, A., Demiraslan, Y., Özcan, Ö. (2022). Investigation of the skull basally in honamli, Hair, Kilis and Saanen Goats using geometric morphometric methods. Harran Üniversitesi Veteriner Fakültesi Dergisi, 11(2), 179-184. DOI:10.31196/huvfd.1161196

Zeder, M. A. and Hesse, B. (2000). The initial domestication of goats (Capra hircus) in the Zagros mountains 10, 000 years ago. Science, 287(5461), 2254-2257. doi: 10.1126/science.287.5461.2254.

Medit Vet J, 9(2): 283-287, 2024

Research Article

Performance, some blood paremeters, fatty acid profile and TBARS value of broiler chickens fed chia seed

Onur Çelikoğlu[1], Fatma Karakaş Oğuz[2]

[1]Animal Hospital, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
broiler chick
chia seed
fatty acid
meat shelf life
performance

Received : 26 February 2024
Revised : 8 July 2024
Accepted : 14 August 2024
Published : 31 August 2024
Article Code : 1443133

Correspondence:
F. KARAKAŞ OĞUZ
(fkarakasoguz@mehmetakif.edu.tr)

ORCID
O. ÇELİKOĞLU : 0000-0003-2743-7424
F. KARAKAŞ OĞUZ : 0000-0002-9077-8531

This article is a part of the first author's master's thesis with the same title and was supported by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinatorship with project number 0487-YL-17.

ABSTRACT

This study aims to determine the effect of chia seeds added to broiler rations at the level of 5% on live weight, live weight gain, feed consumption, feed conversion ratio, breast meat fatty acid profile and meat shelf life. A total of 112 daily-aged Sasso chicks were used in the experiment. Chicks were divided into 2 groups of 56 animals. Each group is divided into 8 subgroups of 7 broilers. The experimental group ration was prepared to contain 5% chia seeds starting from the 15th day. During the experiment, feed and water were given ad libitum. Before the experiment, a 14-day initial ration was applied to all groups. The trial lasted 49 days. In the study, there was no statistical difference between the groups in terms of body weight, live weight gain, feed consumption and feed conversion rates ($P>0.05$). At the end of the experiment, the breast meat Linolenic acid ratio of the experimental group (2.02%) was significantly higher than the control group (1.07%) ($P<0.05$).The arachidonic acid ratio was found to be significantly lower in the experimental group than in the control group ($P<0.05$). While the TBARS value of breast meats kept at +4°C for seven days increased in the control group, it decreased significantly in the experimental group ($P<0.05$). As a result of this study, it can be said that chia seeds have a positive effect on the shelf life of broiler meat.

INTRODUCTION

Chia is an annual plant that grows in tropical or temperate regions. Chia seeds are about 2 mm long, oval in shape, grey, black, brown or white and have dots on them. Chia seeds contain 42.1g of carbohydrates, 30.7g of fat and 16.5g of protein per 100 grams and contain approximately 486 kcal of metabolic energy. Chia is a good source of polyunsaturated (PUFA) omega-3 fatty acids. In a study in which chia seed oil, linseed oil and fish oil were added to rations, it was observed that omega-3 fatty acids increased by 100%-200% in the eggs of chickens consuming chia seed oil (Ayerza, 2009). The Sasso chicken breed, which is made to be a durable and slow-growing poultry, is an alternative to fast-growing hybrid chickens fed a corn-based ration. Sasso is not a breed name, but the name of a French company that has been breeding chickens for decades. It is also known as French village chicken. Chia seed has been largely used as a food, oil source, and raw material for medicinal compounds. Its benefits result primarily from the high concentrations of essential fatty acids, dietary fiber, antioxidants, flavonoids, anthocyanins, vitamins, carotenoids, and minerals (Terevinto et al 2023). It is thought that chia seeds, which have many superior properties, may be effective on meat quality and shelf life, especially with their antioxidant effect. For this reason, in this study, the effect of chia seed on performance, fatty acid profile in breast meat and shelf life of breast meat was investigated.

MATERIALS and METHODS

The study was conducted with the decision and permission of Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee dated 06.12.2017 and numbered 347. A total of 128 1-day-old Sasso breed chicks were used at the beginning of the study. Broiler chicks were vaccinated on the first day. In the study, broiler chicks were given two types of feed: starter and growth feed. Lighting was provided for 24 hours during the study. Daylight and fluorescent lamps were used for lighting the research unit. Three electric heaters (2200 W) were used for heating. The average temperature of the experimental room (for 49 days) was 26.5° C and the average humidity was 68.20%. The starter feed was used between days 1-14 and the growth feed was used between days 15-49. Feed and water were provided ad libitum. Feeders and drinkers were checked daily. Starter feed was used until the fourteenth day and then the experiment started. All chicks were weighed individually and feed intake (FI) was recorded at d 14, 21, 28, 35, 42 and 49 as group. Body weight gain (BWG) and feed conversion ratio (FCR) were subsequently calculat-

To cite this article:
Çelikoğlu O, Karakaş Oğuz F. Performance, some blood paremeters, fatty acid profile and TBARS value of broiler chickens fed chia seed. Medit Vet J. 2024; 9(2): 283-287.

ed based on the performance values. At the beginning of the study, all chicks were weighed and distributed so that there were chicks of similar weight in each group. During the study, live weight weighings were done individually every week with a UWE HGM-20K balance with ±1 g accuracy.

The chicks were weighed individually every week from the beginning to the end of the study using a UWE HGM-20K scale with ±1 g accuracy. Live weight gain determination was calculated by subtracting the weighing results of the previous week from the last weighing**.** Feed consumption during the study was calculated by subtracting the amount of feed re-

Table 1. Raw material and nutrient content of compound feeds (concentrate feed) used in the study and Chemical Analysis Results of Chia Seed and Ration (%)

Raw Materials	Starter	Magnification (without Chia)	Magnification (with Chia)
Corn	49.15	59.40	57.12
Vegetable Oil	6.50	4.95	3.50
Sunflower Meal, Unhulled (36% HP)	5.00	5.00	5.00
Soybean Meal (48%HP)	35.40	27.10	25.90
Dicalcium Phosphate	1.70	1.20	1.20
Dl-Methionine	0.20	0.20	0.20
Limestone	1.25	1.35	1.28
L-Lysine Hydrochloride	0.10	0.10	0.10
Sodium Bicarbonate	0.10	0.10	0.10
Salt	0.40	0.40	0.40
Vitamin-Mineral Mix	0.20	0.20	0.20
Chia seeds	0	0	5.00

Calculated Chemical Composition	Starter	Magnification (without Chia)	Magnification (with Chia)
Dry Matter	90.50	90.20	90.10
Crude Protein	23.00	20.00	20.00
Metabolic Energy	3201	3198	3201
Calcium	1.00	0.90	0.90
Available Phosphorus	0.45	0.35	0.36
Sodium	0.24	0.24	0.24
Chlorine	0.28	0.28	0.28
Methionine-Cystine	0.98	0.89	0.91
Lizin	1.32	1.10	1.10
Threonine	0.87	0.74	0.75
Tryptophan	0.31	0.26	0.27
Linoleic Acid	4.38	3.73	3.24

Results of the analyses	Starter	Magnification (without Chia)	Magnification (with Chia)	Chia Seeds
Crude Protein	23.2	19.42	19.87	21
Crude Fat	8.64	9.01	7.90	28.14
Crude Cellulose	4.05	4.01	4.84	34.4
Crude Ash	5.92	4.32	5.34	4.98
Dry Matter	89.68	89.32	89.64	94.45

Vitamin-Mineral composition (per kg feed): Vitamin A, 3,333 IU; Vitamin D_3, 0,833 IU; Vitamin E, 11.667 mg; Vitamin K_3, 1.333 mg; Vitamin B_1, 0.667 mg; Vitamin B_2, 2 mg; Vitamin B_3, 10 mg; Vitamin B_5, 2. 667 mg; Vitamin B_6, 1.333 mg; Vitamin B_{12}; 0.05 mg; Biotin, 0.15 mg; Folic Acid, 0.25 mg; Ascorbic Acid, 16.687 mg

maining in the following week from the amount of feed given to the animals each week. Feed weighing was done with a scale with ±1 g accuracy. Feed conversion ratio (Feed conversion ratio (g FI/g BWG) was obtained by dividing total feed consumption by live weight gain.

The experimental period using chia lasted 35 days in total. After the 14th day, 112 Sasso broiler chicks were used in the experiment. The chicks were divided into 2 groups of 56 animals. Each group was divided into 8 subgroups of 7 chicks. The experimental group ration was prepared to contain 5% chia seeds from the 14th day. Experiment diet analysis results are given in Table 1.

The feeds used in the study were analyzed in the Laboratory of the Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur. Dry matter (DM), crude ash (Ash), crude fiber (CF), ether extract (EE) and crude protein (CP) were analyzed. Dry matter, crude ash, ether extract, crude protein, and organic matter (OM) analyses of the feeds used in the study were carried out according to the methods reported in (AOAC, 2000) and crude fiber (CF) analysis was carried out according to (Crampton and Maynard, 1938). A total of thirty-two breast meat samples were taken from two animals with similar body weights randomly selected from each subgroup at slaughter. The experiment was terminated by decapitation on the 49th day. Blood was taken during decapitation. The brisket of the control and experimental groups was divided into two groups of eight pieces each, and the first group of the control and experimental groups were kept one hour after slaughter and the second group was kept in the refrigerator at +4 degrees Celsius for one week and then analyzed for Thiobarbiturate reactive substances (TBARS) in a spectrophotometer device (PERKIN ELMER Lambda 35) at Burdur Mehmet Akif Ersoy University Scientific and Technology Application and Research Center. The method described by (Zeb and Ullsh, 2019) was used for TBARS analysis. Fatty acids in brisket were analyzed by gas chromatography/mass spectroscopy (AGILENT 5975 C AGILENT 7890A GC).

PASW Statistics18 Independent group t-test was applied for statistical calculations of the groups, significance of the differences between the mean values of the groups and significance control of the difference between the groups. Significance was declared at $P < 0.05$

RESULTS

The effects of chia seed added to the ration at 5% level on body weight gain, feed intake and feed utilization averages are shown in Table 2. There was no difference between live weights at 14, 21, 28, 35, 42 and 49 days of the experiment.

Table 2. Average body weight gain (BWG), feed intake (FI)and feed conversion ratio of the groups during the experiment (14th day-49th day)

Parameters	Control Group ($X \pm Sx$)	Experimental Group ($X \pm Sx$)	P
Feed Consumption. g	3476.67 ± 37.58	3402.20 ± 70.51	0.519
Live weight gain. g	1615.79 ± 36.67	1648.21 ± 32.17	0.517
Feed conversion ratio (g FI/g BWG)	2.15 ± 0.06	2.06 ± 0.08	0.079

The effect of chia seed added to the ration at 5% level on the fatty acid composition of the breast meat of the animals is shown in Table 3 and the fatty acid composition of the feeds is shown in Table 4. The difference between linolenic acid and arachidonic acid values between the groups was statistically significant.

Table 3. Fatty Acid Profile Values of Meats of Experimental Groups. %

Parameters	Control Group ($\bar{x} \pm S_{\bar{x}}$)	Experimental Group ($\bar{x} \pm S_{\bar{x}}$)	p
Myristic Acid	0.90 ± 0.03	0.97 ± 0.14	0.633
Palmitic Acid	35.28 ± 1.25	37.80 ± 1.22	0.160
Palmitoleic Acid	0.47 ± 0.01	0.43 ± 0.01	0.102
Stearic Acid	5.16 ± 0.14	4.93 ± 0.14	0.290
Oleic Acid	25.81 ± 0.74	24.69 ± 0.71	0.290
Linoleic Acid	27.30 ± 0.75	26.42 ± 0.60	0.372
Linolenic Acid	1.07^{b}± 0.05	2.02^{a}± 0.27	0.002**
Arachidonic Acid	3.12^{a}± 0.23	2.11^{b}± 0.19	0.003**

**p<0.01 n=16 The difference between values with different letters in the same row is significant.

The effect of 5% chia seed supplementation to the ration on TBARS analysis results of control and experimental groups on day 1 after slaughter is shown in Table 5. The effect of chia

Table 4. Fatty Acid Composition of Feeds %

Component	Experimental feed	Control feed	Chia seeds
Myristic acid	-	-	0.253
Palmitic	14.001	11.983	8.7
Palmitoleic	0.951	0.966	0.144
Stearik	3.565	3.595	1.542
Oleic	18.252	19.452	5.033
Linoleic	53.601	60.334	20.996
Linolenic	9.335	3.245	61.484

seed supplementation on TBARS analysis results of control and experimental groups on day 7 after slaughter and TBARS analysis results between control and experimental groups on days 1 and 7 after slaughter are shown in Tables 5.

Table 5. TBARS Analysis Results of Control and Experimental Groups

MDA, nmol/g	Control ($\bar{x} \pm S_{\bar{x}}$)	Experimental ($\bar{x} \pm S_{\bar{x}}$)	p-value
d1 Post -Slaughter	10.75 ± 1.36	13.04 ± 1.78	0.326
d7 Post -Slaughter	13.01 ± 3.01	8.02 ± 0.90	0.135
p-value	0.507	0.025	

DISCUSSION

It was found that chia seed did not affect body weight, body weight gain, slaughter weight, feed intake, feed conversion ratio, carcass weight and hot carcass yield in the experimental groups.

Amela et al., 2016 reported that there was no statistical difference in terms of live weight and live weight gain in a study in which 10% chia meal and 10% chia meal+probiotic were added to broiler rations. Fernández et al., 2018 divided 96 Cobb 500 chickens into 4 groups and 16 subgroups. The first group received a control ration. The second group received 10% chia meal, the third group received 10% chia meal + hydroxytyrosol (7 mg/kg) and the fourth group received hydroxytyrosol (7 mg/kg). The incorporation of chia flour and/or hydroxytyrosol does not affected the evaluated parameters. The demucilagination or the inclusion of enzymes that degrade soluble fiber could contribute to demonstrating a positive effect on the productive performance. Peiretti and Meineri (2008) in their study on the effects of chia seed supplements on growth performance carcass characteristics, and fat and meat fatty acid profile of rabbits fed with chia seed supplements, added 0%. 10% and 15% chia seeds (*Salvia hispanica L.)* to rabbit rations and reported that there was no significant difference in terms of live weight and live weight gain. Urrutia et al. (2015) added 10.5% flaxseed and 10% chia seeds to the rations of Navaira breed lambs and reported that the additives did not affect live weight. It is seen that the findings obtained in the experiment are in agreement with the results of the above-mentioned studies (Amela et al., 2016, Urrutia et al., 2015).

Ayerza et al. (2002) reported that the addition of 10% and 20% chia seeds to Ross 308 chick rations decreased palmitic acid and increased alpha-linolenic acid in breast and thigh meat. Azcona et al. (2008) reported that the polyunsaturated fatty acid composition increased by 157% in thigh meat and 200% in breast meat in a study in which chia seeds were added to broiler rations. Salazar et al. (2009) in a study investigating the use of chia seeds in laying hen rations alpha-linolenic acid increased and palmitic acid decreased in egg yolk. As a result of the data obtained at the end of the experiment, it was observed that chia seed increased oxidative stability due to its antioxidant properties and positively affected meat shelf life. Waszkowiak and Rudzinska (2014) stated in their study that flaxseed is protective against oxidation and can increase the shelf life of products which supports the data in our study.

CONCLUSION

In conclusion numerically better results were obtained in the feed conversion ratio in the group given chia. The linolenic acid level in the meat of the group given chia seeds was statistically higher than the control group. Chia seed is a grain rich in linolenic acid. and this is reflected in the meat analysis results. The shelf life of the meat of the group given chia seed was longer than that of the control. It may be recommended to add chia seeds to meat, especially meats with a risky shelf life, such as white meat.

DECLARATIONS

Ethics approval

The study was conducted with the decision and permission of Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee dated 06.12.2017 and numbered 347.

Conflict of Interest

The authors declare no competing interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: FKO, OC

Data collection and analysis: OC

Drafting of the manuscript: FKO, OC

Critical review: FKO, OC

Data availability

Not applicable.

Acknowledgements

The blood parameters data of this project were presented as a poster at the 4th National Livestock Economics Congress on October 20-23, 2022.The project was presented oral presentation. The 6 th International, Poultry Meat Congress (UBEK-6) on 1-5 March 2023.

REFERENCES

Amela, MI., Feuilles, A., Fernandez, H., Salerno, C., & Kumorkiazwick, L. (2016). Effect of Saccharomyces cerevisiae and chia flour addition in broiler ration. 4. Productive performance. Revista Argentina de Producción Animal, 36(1), 189-293.

AOAC. (2000). OfficialMethodsof Analysisof the Association of Analtytical Chemists..17. Baskı. Gaithersburg: MD. USA. Methods

Ayerza, R. (2008). Chia as a New Source of ω-3 Fatty Acids: Nutritional Comparison with Other Raw Materials and Its Advantages When Producing ω-3 Enriched Eggs. Wild-Type Food in Health Promotion and Disease Prevention: The Columbus Concept, 179-194.

Ayerza, R., Coates, W., & Lauria, M. (2002). Chia seed (Salvia hispanica L.) as an omega-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics. Poultry science, 81(6), 826-837. https://doi.org/10.1093/ps/81.6.826

Azcona, J. O., Schang, M. J., Garcia, P. T., Gallinger, C., Ayerza Jr, R., & Coates, W. (2008). Omega-3 enriched broiler meat: The influence of dietary α-linolenic-ω-3 fatty acid sources on growth, performance and meat fatty acid composition. Canadian journal of animal science, 88(2), 257-269. https://doi.org/10.4141/CJAS07081

Crampton, EW., & Maynard, L. (1938). Therelation of cellulose and lignin content tonutritive value of animal feeds. Journal of Nutrition. 15, 383-395. https://doi.org/10.1093/jn/15.4.383

Fernández, H. T., Arreghi, A., Amela, M. I., Salerno, C. M., & Couto, A. (2018). Effect of hydroxytyrosol and chia flour addition in broiler diet. 1. Productive performance.

Peiretti, P. G., & Meineri, G. (2008). Effects on growth performance, carcass characteristics, and the fat and meat fatty acid profile of rabbits fed diets with chia (Salvia hispanica L.) seed supplements. Meat Science, 80(4), 1116-1121. https://doi.org/10.1016/j.meatsci.2008.05.003

Salazar-Vega, M. I., Rosado-Rubio, J. G., Chel-Guerrero, L. A., Betancur-Ancona, D. A., & Castellanos-Ruelas, A. F. (2009). Composition in alpha linolenic acid (w3) of egg and broiler meat using chia (salvia hispanica l.) In poultry feedstuff. Interciencia, 34(3), 209-213.

Terevinto A., Marta del Puerto, Ayrton da Silva, María Cristina Cabrera and Ali Saadoun. (2023). Effect of chia seeds (Salvia hispanica l.) inclusion in poultry diet on n-3 enrichment and oxidative status of meat during retail display. CYTA – Journal Of 2023, VOL. 21, NO. 1, 93–100 Food. https://doi.org/10.1080/19476337.2022.2162975

Urrutia, O., Soret, B., Insausti, K., Mendizabal, J. A., Purroy, A., & Arana, A. (2015). The effects of linseed or chia seed dietary supplementation on adipose tissue development, fatty acid composition, and lipogenic gene expression in lambs. Small Ruminant Research, 123(2-3), 204-211. https://doi.org/10.1016/j.smallrumres.2014.12.008

Waszkowiak, K., & Rudzińska, M. (2014). Effect of flaxseed meals and extracts on lipid stability in a stored meat product. Journal of the American Oil Chemists' Society, 91, 979-987.

Zeb, A., & Ullah, F. (2016). A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. Journal of analytical methods in chemistry. https://doi.org/10.1155/2016/9412767

Medit Vet J, 9(2): 288-294, 2024

Research Article

Factors associated with the colostrum quality of dairy cows in the Menemen district of İzmir provience

Oktay Genco Kaya[1], Murat Er[1]

[1]Department of Veterinary Medicine, Menemen Vocational School, Izmir Bakırçay University, Izmir, Türkiye

Key Words:
breed
calving season
colostrum quality
dairy cows
herd size

Received : 23 March 2024
Revised : 8 June 2024
Accepted : 11 July 2024
Published : 31 August 2024
Article Code : 1457572

Correspondence:
M. ER
(murat.er@bakircay.edu.tr)

ORCID
OG. KAYA : 0009-0008-3014-61113
M. ER : 0000-0001-6623-1539

This study received the financial support from the Scientific and Technological Research Council of Turkey (TÜBITAK, grant number 1919B012219514).

ABSTRACT

This study aimed to determine the factors affecting the colostrum quality in dairy farms located in the Menemen district of İzmir province. A total of 61 colostrum samples were collected in the first 6 hours after birth from six dairy farms having more than 100 head. The colostrum obtained from each cow was measured for density using a colostrometer. It was found that 40 of the colostrum samples were of good quality, 16 were of medium quality, and 5 were of poor quality. The current study identified a statistically significant effect of herd size ($P<0.05$) on colostrum quality, with higher quality observed in farms with a herd size of less than 250 head. Parity and calving season did not affect colostrum quality in this study. Simmental cows had better colostrum quality than Holstein and Jersey cows. The specific gravity of Jersey colostrum was lower (-23 g/L) in summer compared to other seasons, whereas that of Simmental was higher. Therefore, it can be concluded that Simmental and Holstein cows are more suitable for profitable dairy farming in this region.

INTRODUCTION

The first week after birth is the most vulnerable time for the calves since the fetal period of pregnancy is in a sterile field (Bıyıklı et al., 2017), and the transfer of immunoglobulins from the cow to the fetus does not occur during this period (Koyun and Karaca, 2018). Mortality rates in calves are the highest during the first week of their life (Svensson et al., 2003). Therefore, the survival of calves during this period depends on colostrum feeding that delivers high amounts of immunoglobulins at the right time (Godden, 2008).

Colostrum production begins in the mammary glands 3-4 weeks before parturition. It contains twice the dry matter, 4.5 times the protein, 1.7 times the fat, and more vitamins and minerals than milk (Lopez and Heinrichs, 2022). Salient feature of colostrum is its high concentration of immunoglobulins, also known as maternal antibodies. These antibodies are developed by the dam throughout her life that provide crucial immunity to the calf during its first week of life. In bovine colostrum, immunoglobulins make up 70 to 80% of the total protein content, whereas they only make up 1-2% of the total protein in normal milk (Kozat, 2019).

Quality of colostrum is dependent on several factors such as the breed, age, parity, feeding management, and dry period length of the dairy cows (Wattiaux, 2006). According to Shearer et al. (1992), cows in first lactation produce lower quality colostrum compared to multiparous cows. Sellers (2001) stated that colostrum quality is affected by feeding management during the dry period. Additionally, Selk (2007) found that cows that were malnourished in the last period of pregnancy experienced a significant decrease in colostrum production.

Environmental conditions can also impact colostrum quality. It was reported that high ambient temperature has a detrimental effect on colostrum quality (Coşkun, 2020). In many regions of Turkey, summer average temperatures are at a level (>25 °C) that may cause stress in dairy cows (Er and Özcan, 2023). Menemen district of İzmir province is situated in one of these regions has many dairy farms. However, no studies have been conducted to investigate the quality of colostrum in this area, considering the high ambient temperature and farm practices. Therefore, this study aimed to assess the factors affecting the colostrum quality in dairy farms located in the Menemen district of İzmir province.

MATERIALS and METHODS

Sampling

The study was conducted in the Menemen district of İzmir

To cite this article:
Kaya OG, Er M. Factors associated with the colostrum quality of dairy cows in the Menemen district of İzmir. Medit Vet J. 2024; 9(2): 288-294.

province between May 2023 and March 2024. In the present study, 61 colostrum samples were collected within the first 6 hours after birth from six dairy farms having herd size more than 100 heads. Table 1 presents the size of the herds from which colostrum was collected in the study, along with the number of colostrum samples collected by season.

Table 1. The herd size and number of colostrum samples collected.

Herds no	Size of herds, heads	Numbers of colostrum samples		
		Summer	Other seasons	Total
1	>500	6	4	10
2	250-500	5	4	9
3	250-500	4	6	10
4	250-500	4	6	10
5	<250	5	6	11
6	250-500	6	5	11
Total		30	31	61

Breed, lactation number, calving season, and diet composition in the dry period were recorded from the herd management system. In addition, 500g feed samples were collected from each farm for three consecutive days and then stored at -20°C to determine the chemical compositions of the ration in the dry period.

The average ambient temperature and relative humidity of the Menemen district of İzmir province for the months of summer were obtained from the General Directorate of Meteorology. The temperature-humidity index (THI) was calculated using the following formula of THI (NWSCR, 1976).

THI = (9/5 × temperature + 32) – (11/20 – 11/20 × Humidity) × (temperature – 26)

Colostrum quality and Chemical analyses

The colostrum obtained from each cow after calving was cooled to 20 °C within the first 24 hours. then it was measured for density using a colostrometer (Kerbl, Germany) according to Kaygısız and Köse, (2007). Based on density, colostrum was classified as good (>1045 g/L), medium (1035-1045 g/L), and low quality (<1035 g/L).

For chemical compositions, samples of TMRs were dried to a constant weight in an air-forced oven at 65 °C for 72h. Dried samples were ground to pass through a 2-mm screen. Dry matter (DM), and crude protein (CP) contents of the samples were analyzed according to AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber were assayed according to the methods described by Van Soest et al. (1991.) The ingredients and chemical compositions of the diets are presented in Table 2.

Table 2. The ingredients and chemical compositions of the diets used in farms.

Ingredients	Herds 1	Herds 2	Herds 3	Herds 4	Herds 5	Herds6
Wheat straw	50	45	25	48	50	45
Maize silage	30	20	40	35	30	35
Wheat silage		10				
Concentrate 1	20	15	10	17	20	20
Concentrate 2			25			
Chemical compositions						
Dry matter	68.7	65.5	56.9	61.7	67.1	62.6
Crude protein	9.3	10.1	13.4	9.7	8.8	9.4
NDF	45.0	46.1	33.7	46.9	43.6	46.5

Concentrate 1: Commercial feed for dry period (% 14 crude protein); Commercial 2: Commercial feed for lactatating period (%19 crude protein).

Statistical analyses

The effects of ambient temperature, breed, herd size, lactation number, and calving season on colostrum quality were analyzed using the General Linear Model (GLM) procedures

with Least Squares Mean (LSMS) in SPSS software package (22.0, IBM Corp. Inc., NY, US). Duncan's multiple comparison test was used to compare the differences among the means. P<0.05 was accepted as statistically significant difference among the means.

RESULTS

Figure 1 shows the average records of ambient temperature, relative humidity, and temperature humidity index during the summer season in the study. The minimum and maximum recorded ambient temperatures were 16.6 and 44.1 °C respectively. Relative humidity ranged from 23.4 to 78.1%. Daily average THI was recorded above 70 in summer in the current study.

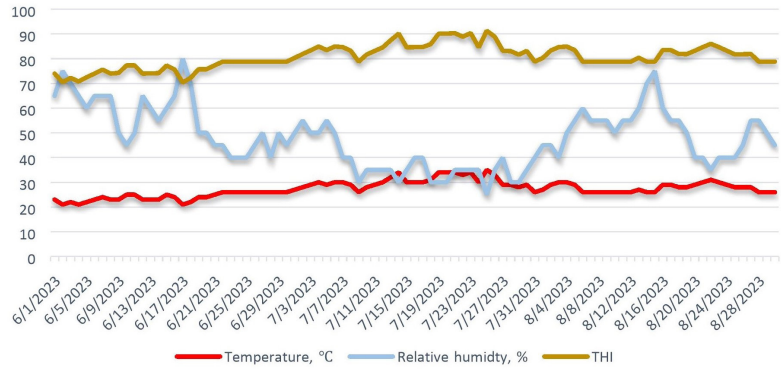

Figure 1. The average records of ambient temperature, relative humidity, and temperature humidity index during the summer season

The quality distribution of colostrum collected during the study is presented in Figure 2. It was found that 40 of the colostrum samples were of good quality, 16 were of medium quality, and 5 were of poor quality.

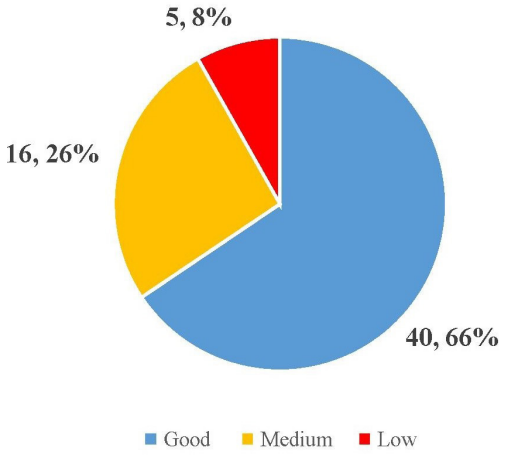

Figure 2. The quality distribution of colostrums collected during the study.

The effects of herd size and parity on the colostrum quality are shown in Table 3. The current study identified a statistically significant effect of herd size (P<0.05) on colostrum quality, with higher quality observed in farms with a herd size of less than 250 head. Meanwhile, parity did not show a notable influence on colostrum quality.

Table 4 shows the effects of calving season and breed on the colostrum quality. In the present study, the colostrum quality was not affected by calving season. However, Simmental cows had better colostrum quality than Holstein and Jersey cows (respectively 1067, 1049, and 1047 g/L) (P<0.05). In addition, we observed an interaction between calving season and breed (P<0.05) (Figure 3). The specific gravity of Jersey colostrum was lower (-33 g/L) in summer compared to other seasons, whereas that of Simmental was higher (15 g/L) (Table 4).

DISCUSSION

Growth and profitability of dairy farms rely on the continuous addition of healthy and young animals to the herd. Sustainable management of dairy farms aims to achieve one calf per cow per year. Therefore, survival and growth performance of newborn calves significantly impact the continuity of the herd. Some critical points such as proper colostrum feeding, and colostrum quality have an important effect on the survival and growth performance of neonatal calves.

The collected colostrums in this study were, in general, good quality (Figure 2). In total, only 8.3% of the colostrum samples in this study contained <1035 g/L specific gravity, which is considered to be an indication of low-quality colostrum (Kaygısız and Köse, 2007). No previous study has investigated the

factors associated with the colostrum quality of dairy cows in İzmir. However, Tatar and Esenbuğa (2022) found that farmers in the Odemis district of İzmir province had sufficient knowledge about animal care and nutrition. Therefore, it can be concluded that the dairy management practices in İzmir province have good results. In addition, the rate of low-quality

Table 3. The effects of herd size, and parity on the colostrum quality.

Item	n	Colostrum specific gravity, g/l			P value
Size of herds, heads		$\overline{X} \pm Sx$	**Minimum**	**Maximum**	
>500	10	1049 ± 5.70^{b}	1038	1061	
250-500	40	1049 ± 2.28^{b}	1043	1054	0.017
<250	11	1067 ± 5.43^{a}	1056	1077	
Number of lactations					
Uniparous	26	1050 ± 3.74	1042	1057	0.436
Multiparous	35	1054 ± 3.22	1047	1060	

->1045 g/l good quality; 1035-1045 g/l; medium quality; <1035 g/l low quality
a, b= Column means within a classification with different superscripts differ ($P < 0.05$).

Table 4. The effects of calving season and breed on the colostrum quality.

Item	n	Colostrum specific gravity, g/l
Calving season		$\overline{X} \pm Sx$
Summer	30	1051 ± 3.4
Other seasons	31	1059 ± 4.1
Breed		
Holstein Friesian	39	1049 ± 2.6^{b}
Jersey	11	1047 ± 4.9^{b}
Simmental	11	1067 ± 5.42^{a}
Summer		
Holstein Friesian	19	1047 ± 3.77
Jersey	6	1032 ± 6.70
Simmental	5	1075 ± 7.34
Other seasons		
Holstein Friesian	20	1052 ± 3.67
Jersey	5	1065 ± 7.34
Simmental	6	1060 ± 6.70
	Calving season	0.131
P value	Breeds	0.007
	Calving season x Breed	0.004

>1045 g/l good quality; 1035-1045 g/l; medium quality; <1035 g/l low quality
a, b=Column means within a classification with different superscripts differ

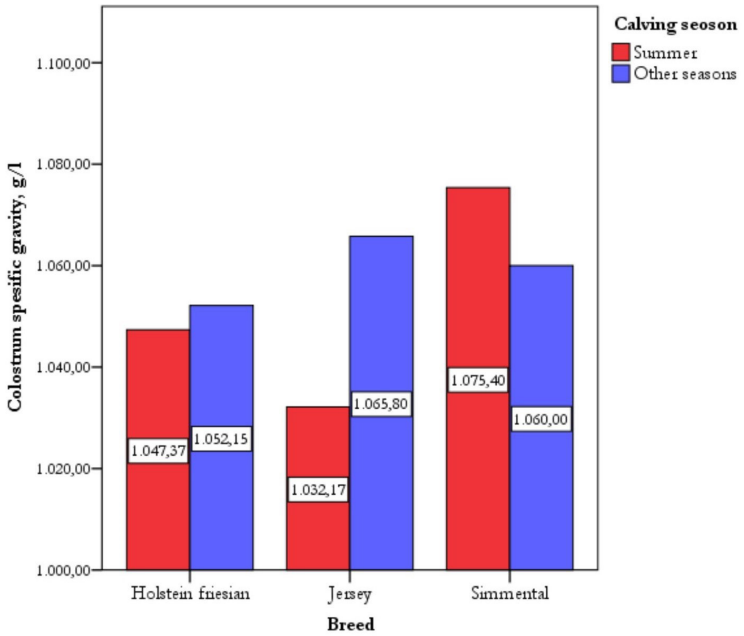

Figure 3. Interaction between calving season and breed for colostrum quality.
->1045 g/l good quality; 1035-1045 g/l; medium quality; <1035 g/l low quality

colostrum was in agreement with the rates reported in other studies; Conneely et al. (2013) reported a rate of 4% in 741 cows, while 13.5% was reported in another study (Göncü and Gökçe, 2015). Contrary to the findings of our study, Shearer et al. (1992) reported that 79.8% of 2045 colostrum samples had low concentrations of Ig (less than 50 mg/mL). As already known, calving interval and colostrum collection is one of the main factors affecting colostrum quality (Kehoe et al., 2011). However, Shearer et al., (1992) collected colostrum samples 12 h after the calving that might have resulted in low quality colostrum.

In the present study, cows produced high quality colostrum (+17.2 g/L specific gravity) when the herd size was above 250 heads (Table 3). There could be two possible reasons for this result. The first one was the breed of the cows which was the Simmental in these herds. As shown in Table 4, Simmental cows produced higher quality colostrum than Holstein and Jersey cows in the current study. The second possible reason was the management of these herds. There is a lack of studies that investigate the effect of herd size on colostrum quality. It can be expected that improved management and nutritional practices, which are more likely to be present in larger herds, would be effective in producing high-quality colostrum (Godden, 2008). However, smaller herds with a higher number of farm workers per 100 dairy cows (+0.6) in the current study might have more effective management.

The parity of cows did not affect colostrum quality (Table 3). Consistent with our findings, Kıyıcı and Sevişoğlu (2022) reported a numerical increase in colostrum quality in multiparous cows. On the other hand, lower colostrum quality in the first lactation was reported in many studies (Erdem and Atasever 2005; Connely et al., 2013). Differences in findings may be associated with environmental conditions. Calving season of multiparous cows in our study occurred primarily during the summer, which may cause stress for dairy cows.

Heat stress is a primary concern for dairy farms in warm regions and it has been reported that the effects of heat stress start to appear in dairy cows when THI is higher than 68 (Collier et al., 2012) or 72 (Armstrong, 1994). Additionally, as colostrum production begins up to 3 weeks before parturition, heat stress during the dry period has a significant impact on colostrum production and quality (Avendaño-Reyes et al., 2023). In the present study, the daily average THI was recorded as above 80 in the summer season. (Figure 1). Therefore, we can say that cows were exposed to heat stress in this period. Although the specific gravity of colostrum was numerically lower in summer (-8 g/L), the calving season did not affect colostrum quality in this study. (Table 4). Thi result contrasts with that of Genç (2015) who reported that cows produced significantly lower quality colostrum in the summer season ($P<0.05$). Although there is a high correlation between colostrum specific gravity and actual Ig concentration, it has been suggested that the scale may not be sufficiently refined to allow the use of Ig concentration as a continuous variable (Shearer et al., 1992). However, Genç (2015) quantified colostrum IgG using an ELISA method, which may provide more accurate results. Furthermore, our results are in line with those of other studies (Morin et al., 2001; Kaygısız and Köse, 2007) that used colostrometers to determine colostrum quality in cows.

In agreement with previous studies (Ontsouka et al., 2003; Kıyıcı and Sevişoğlu; 2022), colostrum quality was affected by breed in our study and Simmental's colostrum quality was higher (+19 g/L) than Holstein and Jersey. In addition, there was a negative correlation between ambient temperature and the specific gravity of Jersey colostrum, whereas there was a

positive correlation for Simmental cows. Heat tolerance, which was reported to be higher in Simmental (Gartner el., 2017a; 2017b) and lower in Jersey (Bianca, 1965), could be a possible explanation of these findings.

CONCLUSION

Findings of the present study indicate that, in general, dairy herds in the Menemen district of İzmir province were well-managed, as only 8.3% of the collected colostrum samples were of low quality. However, management of dairy cows in large herds (>250 heads) was suboptimal. Parity did not affect the colostrum quality in the current study. Due to the use of the similar diets in dairy farms, except for farm 3, and the limited number of colostrum samples, this study does not provide conclusive evidence on the effects of nutritional management on colostrum quality. However, Simmental cows produced higher quality colostrum than Holstein and Jersey cows. Furthermore, Jersey cows were not well adapted to the region and environmental conditions. Therefore, it can be concluded that Simmental and Holstein cows are more suitable for profitable dairy farming in this region.

DECLARATIONS

Ethics Approval

Since the data collected in the study were obtained from routine farm practices, the approval of the ethics committee is not required.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Author contribution

Idea, concept and design: OGK, ME

Data collection and analysis: OGK, ME

Drafting of the manuscript: OGK, ME

Critical review: ME

Data Availability

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

Acknowledgements

This study received the financial support from the Scientific and Technological Research Council of Turkey (TÜBITAK, grant number 1919B012219514).

REFERENCES

AOAC. (1990). Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA

Armstrong, D. V., (1994). Heat stress interaction with shade and cooling Journal of Dairy Science, 77:2044–2050. https://doi.org/10.3168/jds.S0022-0302(94)77149-6.

Avendaño-Reyes, L., Macías-Cruz, U., Sánchez-Castro, M. A., Anzures-Olvera, F., Vicente-Pérez, R., Mellado, M., ... & López-Baca, A. (2024). Effects of parity, seasonal heat stress, and colostrum collection time postpartum on colostrum quality of Holstein cattle in an arid region. International Journal of Biometeorology, 68(3), 427-434. https://doi.org/10.1007/s00484-023-02601-5.

Bıyıklı, E., Azkur, A. K., Tonbak, Ş., & Macun, H. C. (2017). Buzağılarda Schmallenberg Virüs Özgül Maternal Antikor Varlığının İzlenmesi. Firat Universitesi Saglik Bilimleri Veteriner Dergisi, 31(3).

Bianca, W. (1965). Section A. Physiology. Cattle in a hot environment. Journal of Dairy Research, 32(3), 291-345.

Collier, R. J., L. W. Hall, S. Rungruang, and R. B. Zimbleman., (2012). Quantifying heat stress and its impact on metabolism and performance. Pages 74–84 in Proc. 23rd Annual Florida Ruminant Nutrition Symposium, Gainesville. University of Florida, Gainesville

Conneely, M., Berry, D. P., Sayers, R., Murphy, J. P., Lorenz, I., Doherty, M. L., & Kennedy, E. (2013). Factors associated with the concentration of immunoglobulin G in the colostrum of dairy cows. Animal, 7(11), 1824-1832. https://doi.org/10.1017/S1751731113001444

Kaygısız, A. ve Köse, M. 2007. Siyah Alaca ineklerde kolostrum kalitesi ve kolostrum kalitesinin buzağı gelişme özelliklerine etkisi. Tarım Bilimleri Dergisi, 13 (4) 321-325.

Coşkun, B. (2020). Kuru Dönemde Bakım ve Beslemenin Buzağı Sağlığı Üzerine Etkiler. T.C. Sanayi ve Teknoloji Bakanlığı. https://www.kalkinmakutuphanesi.gov.tr/assets/upload/dosyalar/buzagi-kayiplarinin-onlenmesinde-buzagi-sagligi-ve-yetistiriciligi-el-kitabi.pdf

Gantner, V., Bobic, T., Gantner, R., Gregic, M., Kuterovac, K., Novakovic, J., & Potocnik, K. (2017). Differences in response to heat stress due to production level and breed of dairy cows. International journal of biometeorology, 61, 1675-1685. https://doi.org/10.1007/s00484-017-1348-7

Er, M., & Cengiz, Ö. (2023). The effects of ration particle size and live yeast supplementation on dairy cows performance under heat stress conditions. Tropical Animal Health and Production, 55(2), 130. https://doi.org/10.1007/s11250-023-03550-2

Erdem, H., & Atasever, S. (2005). Yeni doğan buzağilarda kolostrumun önemi. Anadolu Tarım Bilimleri Dergisi, 20(2), 79-84. https://doi.org/10.7161/anajas.2005.20.2.79-84

Gantner V, Bobic T, Gregic M, Gantner R, Kuterovac K, Potocnik K. (2017). The differences in heat stress resistance due to dairy cattle breed. Mljekarstvo 67(2):112–122 https://doi.org/10.15567/mljekarstvo.2017.0203

Genç, M. (2015). İsviçre esmeri ve siyah alaca sığırlarda bazı çevresel faktörlerin kolostrum kalitesi ve pasif immunite üzerine etkileri. Doktora tezi. T.C. Atatürk Üniversitesi Sağlik Bilimleri Enstitüsü.

Godden, S. (2008). Colostrum management for dairy calves. Veterinary Clinics of North America: Food Animal Practice,

24(1), 19-39. https://doi.org/10.1016/j.cvfa.2007.10.005

Göncü, S., and Gökçe, G. (2015). Çok ve Tek Doğum Yapmış Siyah Alaca İneklerin Kolostrum İçerik Değişimi. Çukurova Üniversitesi Ziraat Fakültesi Dergisi, 30(1), 9-16

Kehoe, S. I., Heinrichs, A. J., Moody, M. L., Jones, C. M., & Long, M. R. (2011). Comparison of immunoglobulin G concentrations in primiparous and multiparous bovine colostrum. The Professional Animal Scientist, 27(3), 176-180. https://doi.org/10.15232/S1080-7446(15)30471-X

Kıyıcı, J. M., & Sevişoğlu, B. (2022). Farklı Sığır Irklarında Kolostrum Kalitesinin Araştırılması. Hayvan Bilimi ve Ürünleri Dergisi, 5(1), 40-47. https://doi.org/10.51970/jasp.1014836

Koyuncu, M., and Karaca, M. (2018). Buzağılarda Yaşama Gücünün Anahtarı; Kolostrum. Hayvansal Üretim, 59(1), 67-78. https://doi.org/10.29185/hayuretim.359971

Kozat, S. (2019). Yenidoğan buzağılarda kolostrum yönetiminin önemi. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 14(3), 343-353. https://doi.org/10.17094/ataunivbd.541789

Lopez, A. J., and Heinrichs, A. J. (2022). Invited review: The importance of colostrum in the newborn dairy calf. Journal of dairy science. 105, 2733–2749. https://doi.org/10.3168/jds.2020-20114

Morin, D. E., Constable, P. D., Maunsell, F. P., & McCoy, G. C. (2001). Factors associated with colostral specific gravity in dairy cows. Journal of Dairy Science, 84(4), 937-943. https://doi.org/10.3168/jds.S0022-0302(01)74551-1

Ontsouka, C. E., Bruckmaier, R. M., & Blum, J. W. (2003). Fractionized milk composition during removal of colostrum and mature milk. Journal of dairy science, 86(6), 2005-2011. https://doi.org/10.3168/jds.S0022-0302(03)73789-8

Ravagnolo, O., & Misztal, I. (2000). Genetic component of heat stress in dairy cattle, parameter estimation. Journal of dairy science, 83(9), 2126-2130. https://doi.org/10.3168/jds.S0022-0302(00)75095-8

Sellers, R. (2001). A guide to colostrum and colostrum management for dairy calves. Publication of the Bovine Alliance on Management and Nutrition. AFIA, Arlington, VI USA.

Selk, G. E. (2007). Disease Protection for Baby Calves. Oklahoma Cooperative Extension Service.

Shearer, J.H.O., Mohammed, J.S., Brenneman, Tran, T.Q., 1992. Factors Associated With Concentrations Of Immunoglobulins in Colostrum At The First Milking Post-Calving. Preventive Veterinary Medicine. 14 (1-2), 143-154. https://doi.org/10.1016/0167-5877(92)90091-S

SPSS. (2010). SPSS for Windovs, Version 22, Inc. Chicago.

Svensson CK, Lundborg UE, Olsson SO. (2003). Morbidity in Swedish dairy calves from birth to 90 days of age and individual calf-level risk factors for infectious diseases. Preventive Veterinary Medicine. 58: 179-197. https://doi.org/10.1016/S0167-5877(03)00046-1

Tatar, Y. E., and Esenbuğa, N. (2022). İzmir İli Ödemiş İlçesi Sığırcılık İşletmelerinin Sürü Yönetim Tekniklerinin İncelenmesi. Palandöken Journal of Animal Sciences Technology and Economics, 1(1), 16-23.

Wattiaux, M.A. (2006). Heifer Raising Birth to Weaning 28 Importance of Colostrum Feedıng. https://www.infodairy.com/infodairy_upload_files/Cows_heifers_calves/Calves/0188Importance%20of%20colostrum%20feeding-e.pdf

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(10), 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Medit Vet J, 9(2): 295-298, 2024

Research Article

Cats with systemic ınflammatory response syndrome: granulocyte/lymphocyte ratio in hypothermia and hyperthermia conditions

Ramazan Yıldız[1]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
cats
granulocyte-lymphocyte ratio
SIRS

Received : 2 April 2024
Revised : 25 May 2024
Accepted : 27 May 2024
Published : 31 August 2024
Article Code : 1463329

Correspondence:
R. YILDIZ
(ramazanyildiz@mehmetakif.edu.tr)

ORCID
R. YILDIZ : 0000-0001-5772-0891

This study was supported by the Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordination Unit (BAP, Project No: 0619-MP-19).

ABSTRACT

Systemic inflammatory response syndrome (SIRS) is a conceptual framework developed to underscore the significance of systemic inflammation activation in precipitating organ dysfunction in cases of sepsis. This study endeavors to explore the interplay between hyperthermia, hypothermia, and the granulocyte-to-lymphocyte ratio (G/L) in feline patients diagnosed with SIRS. A total of 25 cats positive for SIRS were included in the study. The groups were determined as G1: ≤37.8 °C (n=8), G2: ≥39.7 °C (n=11) and G3: ≥40.0 °C (n=6). The G/L ratio has no statistical difference was found between G1 and G2, but G3 was found to have a statistically higher G/L ratio than G1 and G2. In conclusion, our findings suggest a potential association between temperature dysregulation and altered G/L ratios in feline SIRS cases. These results contribute to our understanding of the inflammatory response in cats and may inform future diagnostic and therapeutic strategies.

INTRODUCTION

Systemic inflammatory response syndrome (SIRS) stands as a pivotal concept acknowledging the widespread activation of inflammation, a significant contributor to the organ failure observed in cases of sepsis. Although commonly associated with sepsis, SIRS encompasses a spectrum of pathological conditions that trigger the release of endogenous mediators, leading to systemic inflammation. These conditions range from trauma and burns to major surgical procedures and pancreatitis, potentially resulting in multiple organ failure, shock, and mortality, depending on the severity of the inflammatory cascade. It is crucial to note that SIRS is characterized as a clinical syndrome rather than a distinct disease entity. The proposed diagnostic criteria involve four clinical parameters: hypothermia or hyperthermia, leukocytosis or leukopenia, tachycardia or bradycardia, and tachypnea. Timely recognition of systemic inflammation and prompt intervention directed at the underlying pathology are critical in managing the diverse manifestations of the inflammatory response (Purvis and Kirby, 1994; Brady et al., 2000).

Complete blood count (CBC) and the ratio values of CBC parameters are widely used in the diagnosis and follow-up of SIRS and sepsis in veterinary field as well as in human medicine (Oncel et al., 2012). The neutrophil-lymphocyte ratio (NLR) serves as an inflammatory biomarker, offering valuable insights into systemic inflammation levels. Derived from the ratio of absolute neutrophil count to absolute lymphocyte count, the NLR offers a simple evaluation without incurring supplementary expenses on standard complete blood count analyses routinely performed in hospital settings. Extensively studied across a spectrum of conditions including cancer, community-acquired pneumonia, and sepsis, NLR has demonstrated utility as a prognostic indicator (de Jager et al., 2010; Lanziotti et al., 2016). In studies on adult humans, NLR is used to determine the presence and severity of sepsis. This ratio is an indicator of inflammation and can be used to predict septicemia (de Jager et al., 2010). The objective of this study was to assess the relation between hyperthermia and hypothermia and the granulocyte-to-lymphocyte ratio (G/L) in cats positive for SIRS, furthermore, our secondary objective is to establish a foundation for subsequent research endeavors in this area.

MATERIALS and METHODS

The study group consisted of 25 cats with systemic inflammatory response syndrome (SIRS). All these groups were admitted to Burdur Mehmet Akif Ersoy University Animal Hospital for different diseases and the hemogram parameters of the cats showing SIRS symptoms were examined during routine examination. Blood samples for hemogram analysis were collected from the patients' cephalic veins during routine clinical examinations. A tube containing ethylenediaminetetraacetic acid (K_3EDTA) was used for hemogram measurements. Granulocytes (GRA) and lymphocytes (LYM) levels were me-

To cite this article:
Yıldız R. Cats with systemic ınflammatory response syndrome: granulocyte/lymphocyte ratio in hypothermia and hyperthermia conditions. Medit Vet J. 2024; 9(2): 295-298.

asured using the Abacus Junior Vet device (Diatron MI Ltd., Hungary). Among these parameters, the ratios of granulocyte count to lymphocyte count (G/L) were calculated and the differences in animals with different body temperatures were revealed. The groups were determined as G1: ≤37.8 °C (n=8), G2: ≥39.7 °C (n=11) and G3: ≥40.0 °C (n=6).

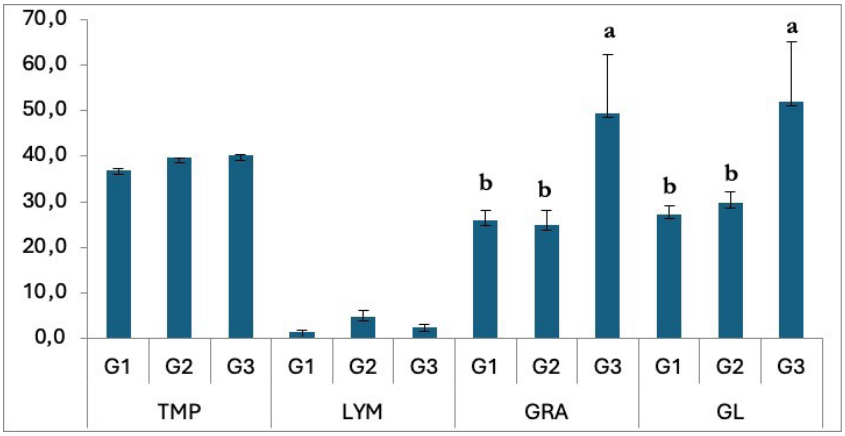

Figure 1. Statistical variations in body temperature, lymphocyte count, granulocyte count, and the granulocyte-to-lymphocyte ratio between the groups (Mean±SEM). TMP (Temperature), LYM (Lymphocyte), GRA (Granulocyte) and GL (Granulocyte/Lymphocyte Ratio), different letters (a, b, c) were set as statistically significant (p <0.05) at graph.

For the inclusion criteria of cats diagnosed with SIRS undergoing hemogram parameter examination, the study encompassed cases exhibiting systemic inflammation along with abnormal body temperature, bradycardia and/or tachycardia, and tachypnea. SIRS criteria, as outlined by Brady et al. (2000), comprised: (1) abnormal body temperature (≤37.8 or ≥39.7 °C); (2) tachycardia (≥225 beats/min) or bradycardia (≤140 beats/min); (3) tachypnea (≥40 respirations/min); and (4) White Blood Cell (WBC) abnormalities (WBC ≥19500 or ≤5000 k/μL or band neutrophils ≥5%).

Statistical analysis

All data were presented as mean and standard errors (mean ± SEM). Normal distribution preconditions were assessed using the Kolmogorov–Smirnov test. One-way ANOVA (Posthoc Tukey) was employed to analyze the statistical differences in parameters between groups using SPSS 22.0 software (USA). A p-value < 0.05 was considered statistically significant.

RESULTS

The study showed that all cats met at least 3 SIRS criteria. Common clinical symptoms included hypothermia or hyperthermia, weakness, depressed appearance, lack of appetite, hyperemic or pale mucous membranes, prolonged capillary refill time and weak pulse, changes in respiratory rate, and increasing heart rate. The body temperature of G1, G2 and G3 were 37.01, 39.71 and 40.22, respectively (Figure 1). The lymphocyte (LYM) level showed no statistically significant difference between the groups. Neither the granulocyte (GRA) count nor the granulocyte-to-lymphocyte (G/L) ratio exhibited any statistical difference between G1 and G2. However, G3 demonstrated a statistically significant higher level (p < 0.05) in both GRA count and G/L ratio compared to G1 and G2 (Figure 1).

DISCUSSION

In response to either infectious or non-infectious stimuli, inflammation initiates a multifaceted interaction involving the humoral and cellular immune response, cytokines, and the complement pathway. This intricate interplay leads to the SIRS when the equilibrium between pro-inflammatory and anti-inflammatory cascades is skewed toward the former (Chakraborty and Burns, 2023). SIRS often triggered by bacterial infection, represents an extensive inflammatory response characterized by the release of multiple cytokines and indicative signs of infection, including fever or hypothermia, elevated heart rate, accelerated respiratory rate, and heightened serum white blood cell count such as the patients in this study. The NLR, a readily available parameter derived from a complete blood count, has emerged as an independent predictor of morbidity and mortality in sepsis (Hwang et al., 2017). In the veterinary field, it is common to use hemogram devices that measure granulocytes and do not distinguish neutrophils. There are not many studies in the veterinary field evaluating G/L and NLR. One of the first studies evaluated dogs with and without septic peritonitis, and no significant difference in NLR was found between dogs that survived and those that died (Hodgson et al., 2018). Gori et al. (2021) discovered that the NLR, in addition to two other leukocyte ratios (BLR: band neutrophil-to-lymphocyte and BNLR: band neutrophil-to-neutrophil-to-lymphocyte) served as robust diagnostic markers, effectively distinguishing between SIRS, septic, and healthy cats. Although the prognostic relevance of BLR and BNLR remains uncertain, the NLR exhibited a correlation with mortality rates in both SIRS and septic feline populations. In the present study, WBC values of all groups were above the normal values for cats. At different body temperatures, the G/L ratio was found to be different, but the highest level (p <0.05) was found in the group with a temperature above 40 degrees (Figure 1). It was determined that the G/L ratio was related to the increase in body temperature indicating the severity of the infection, but did not differ

between hypothermia and moderate body temperature increase. In cats with high G/L ratio in this study, it is observed that there is a pathological process that causes systemic inflammatory response syndrome due to infection or trauma-like condition. As shown in some studies, such as cardiac, pneumonia and chronic renal patients with high NLR, has the potential to have an increased mortality rate (Zahorec, 2020; Huang et al., 2020) however, this study lacks follow-up data on the mortality rate. Moreover, it is important to note that the NLR value is indirectly influenced by circulating concentrations of cortisol and endogenous catecholamines, which have been identified as primary factors (Zahorec, 2020; Buonacera et al., 2022). Elevated blood levels of cortisol can induce neutrophilia and lymphopenia, while fluctuations in catecholamine levels, such as epinephrine, may result in leukocytosis (Margaryan et al., 2017; Chapman, 2018). This pathological mechanism elucidates the elevated G/L ratio observed across all groups and underscores the significant disparity noted in the G3 group with hyperthermia. In a meta-analysis study, NLR levels of complex febrile seizure (FS) patients were found to be significantly higher compared to the simple FS group. It has been pointed out that this result indicates that the level of inflammation is higher in complex FS patients compared to simple FS patients and that inflammation plays a more dominant role in the pathogenesis of complex FS compared to simple FS. This study reported that NLR can be recommended as an inexpensive diagnostic biomarker for FS and may also be useful in differentiating between simple FS and complex FS (Hosseini et al., 2022).

CONCLUSION

In conclusion, it was observed that G/L ratio has the potential to be an important marker in the febrile acute stages of infection. However, it was concluded that studies comparing different body temperature groupings in SIRS, sepsis and septic shock cases would provide more specific and definitive findings.

DECLARATIONS

Ethics Approval

This study was approved by the Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (decision no:2019-575).

Conflict of Interest

Authors declare that there are no conflicts of interest for this study.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: RY

Data and analysis: RY

Drafting of the manuscript: RY

Critical review: RY

Acknowledgments

This study was supported by the Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordination Unit (BAP, Project No: 0619-MP-19). Thanks to Vet.Hek. Fevzi ALPAT for his support.

REFERENCES

Brady, C.A., Otto, C.M., Van, Winkle., T.J., King, L.G. (2000). Severe sepsis in cats: 29 cases (1986–1998). J Journal of the American Veterinary Medical Association. 217(4), 531-535. https://doi.org/10.2460/javma.2000.217.531.

Buonacera, A., Stancanelli, B., Colaci, M., Malatino, L. (2022). Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. International Journal of Molecular Sciences. 23,3636. https://doi.org/10.3390/ijms23073636

Chakraborty, R.K., Burns, B. (2023). Systemic Inflammatory Response Syndrome. (In: StatPearls. Treasure Island (FL): StatPearls Publishing. PMID: 31613449.

Chapman, K.E. Glucocorticoids and immunity. In: Encyclopedia of Endocrine Diseases. Edinburgh: Elsevier; (2018). p. 112–20. 10.1016/B978-0-12-801238-3.66151-2

de Jager, C.P., van Wijk, P.T., Mathoera, R.B., de Jongh-Leuvenink, J., van der Poll, T., Wever, P.C. (2010). Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. Critical care (London, England). (14), 192. 10.1186/cc9309

Gori, E., Pierini, A., Lippi, I., Lubas, G., Marchetti, V. (2021). Leukocytes Ratios in Feline Systemic Inflammatory Response Syndrome and Sepsis: A Retrospective Analysis of 209 Cases. Animals (Basel). 1,11(6),1644. https://doi.org/10.3390/ani11061644.

Hodgson, N., Llewellyn, E.A., Schaeffer, D.J. (2018). Utility and Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Dogs with Septic Peritonitis. Journal of the American Animal Hospital Association. 54(6),351-359. https://doi.org/10.5326/JAAHA-MS-6808

Hosseini, S., Gharedaghi, H., Hassannezhad, S., Sadeghvand, S., Maghari, A., Dastgiri, S., Talebi, M., Khanzadeh, S. (2022). The Impact of Neutrophil-Lymphocyte Ratio in Febrile Seizures: A Systematic Review and Meta-Analysis. BioMed Research International. 11,8472795. https://doi.org/10.1155/2022/8472795.

Huang, Z., Fu, Z., Huang, W., Huang, K. (2020). Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. The American Journal of Emergency Medicine. 38, 641–647. https://doi.org/10.1016/j.ajem.2019.10.023

Hwang, S.Y., Shin, T.G., Jo, I.J., Jeon, K., Suh, G., Lee, T.R., Yoon, H., Cha, W.C., Sim, M.S. (2017). Neutrophil-to-lymphocyte ratio as a prognostic marker in critically-ill septic patients. The American Journal of Emergency Medicine. 35(2),234-239. 10.1016/j.ajem.2016.10.055

Lanziotti, V.S., Póvoa, P., Soares, M., Silva, J.R., Barbosa, A.P., Salluh, J.I. (2016). Use of biomarkers in pediatric sepsis:

literature review. Revista Brasileira de Terapia Intensiva. 28(4), 472–482. https://doi.org/10.5935/0103-507X.20160080

Margaryan, S., Hyusyan, A., Martirosyan, A., Sargsian, S., Manukyan, G. (2017). Differential modulation of innate immune response by epinephrine and estradiol. Revista Brasileira de Terapia Intensiva. 30,1–9. 10.1515/hmbci-2016-0046

Oncel, M.Y., Ozdemir, R., Yurttutan, S., Canpolat, F.E., Erdeve, O., Oguz, S.S., Uras, N., Dilmen, U. (2012). Mean platelet volume in neonatal sepsis. Journal of Clinical Laboratory Analysis. 6(6):493-496. https://doi.org/10.1002/jcla.21552

Purvis, D., Kirby, R. (1994). Systemic inflammatory response syndrome: septic shock. Vet Clin North Veterinary Clinics of North America: Small Animal Practice. (24),1225–1247. https://doi.org/10.1016/s0195-5616(94)50136-0

Zahorec, R. (2021). Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratislava Medical Journal. 122,474–88. https://doi.org/10.4149/BLL_2021_078

Medit Vet J, 9(2): 299-305, 2024

Research Article

Neuroprotective role of n-acetylcysteine (NAC): countering doxorubicin neurotoxicity via TH, Nurr1, and iNOS expression

Tuğçe Anteplioğlu[1], Miyase Çınar[2], Gözde Yaldız[1], Sevgi Betül Kayabaşı[1], Özkan Duru[2], Ruhi Kabakçı[3]

[1]Department of Pathology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Türkiye
[2]Department of Biochemistry, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Türkiye
[3]Department of Physiology, Faculty of Veterinary Medicine, Kirikkale University, Kirikkale, Türkiye

Key Words:
doxorubicin
iNOS
N-acetylcysteine
Nurr1
tyrosine hydroxylase

Received : 17 April 2024
Revised : 20 August 2024
Accepted : 21 August 2024
Published : 31 August 2024
Article Code : 1469812

Correspondence:
T. ANTEPLİOĞLU
(tugceanteplioglu@kku.edu.tr)

ORCID
T. ANTEPLİOĞLU : 0000-0001-7033-0759
M. ÇINAR : 0000-0003-3806-9938
G. YALDIZ : 0000-0002-3833-3201
SB. KAYABAŞI : 0000-0002-6060-8830
Ö. DURU : 0000-0002-8792-442X
R. KABAKÇI : 0000-0001-9131-0933

ABSTRACT

Chemotherapy is an effective treatment for cancer, but it can cause cognitive disorders broadly referred to as "chemobrain." One of the most commonly used chemotherapeutics, doxorubicin (DOX), has been associated with the potential for brain damage and cognitive dysfunction. N-acetylcysteine (NAC) has been identified as a potential brain protector with antiapoptotic, antioxidant, and anti-inflammatory effects. The objective of this study was to investigate the potential protective effect of NAC against DOX-induced brain damage. Female Wistar albino rats were randomly assigned to one of three groups: control, DOX, or NAC prophylaxis. Brain samples were collected for histopathological and immunohistochemical analyses, with a particular focus on regions that are crucial for cognition and memory. The DOX group exhibited significant histopathological changes, including neuronal shrinkage, degeneration, and necrosis in the striatum, hippocampal region, and cerebral cortex. Immunohistochemical analysis revealed the presence of neuroinflammation and neurodegeneration, with an increase in inducible nitric oxide synthetase (iNOS) immunopositivity. Administration of NAC effectively reduced iNOS immunopositivity, neuronal damage, degeneration, and necrosis in the prophylaxis group. Among the brain regions examined, the prophylaxis group demonstrated the most effective protection in the hippocampal region. Therefore, NAC has the potential to protect against or alleviate DOX-induced cognitive impairments.

INTRODUCTION

Cancer constitutes a significant global health concern, with an estimated 16% of global deaths annually attributed to the disease (Sung et al., 2021). Currently, combinations of surgical intervention, chemotherapy, radiotherapy, targeted therapy, and immunotherapy are used to combat cancer. Among these methods, chemotherapy, either alone or in combination with other treatments, is commonly preferred and considered the most effective (El-Hussein et al., 2020). Doxorubicin (DOX), an anthracycline chemotherapy drug, was originally isolated from *Streptomyces peucetius* in the 1970s. Since then, it has become a standard treatment for a range of cancers, including ovarian, breast, stomach, lung, non-Hodgkin and Hodgkin lymphomas, sarcoma, multiple myeloma, and pediatric cancers (Rau, 1992). DOX is frequently utilized in the management of mammary tumors (Hernandez-Aya and Gonzalez-Angulo, 2013). However, despite its effectiveness, some studies have indicated that patients receiving DOX therapy may develop cognitive problems, commonly referred to as "chemobrain" (Raffa et al., 2006; Andryszak et al., 2017). DOX can cause neurotoxicity by crossing the blood-brain barrier directly or by triggering neuroinflammation (Du et al., 2021). It also inhibits DNA topoisomerase II, which releases free radicals, including superoxide and hydrogen peroxide, resulting in tissue damage through oxidative stress (Cheruku et al., 2017).

In contrast, N-acetylcysteine (NAC) is capable of traversing the blood-brain barrier and augmenting the concentration of glutathione (GSH), rendering it an efficacious antioxidant as it diminishes the levels of free oxygen radicals (Dean et al., 2011; Prakash et al., 2014). NAC has been utilized in research related to neurodegenerative disorders, such as Parkinson disease, and in cases where neurotoxic agents are used in chemotherapy to reduce or prevent neuronal damage (Gil-Martínez et al., 2018; Abdel-Wahab et al., 2019; Kitamura et al., 2021).

The dopaminergic system is associated with learning, motor movements, memory, and the reward mechanism in the mammalian brain (Mehta and Riedel, 2006). Nuclear receptor-related protein 1 (Nurr1) and tyrosine hydroxylase (TH) are key players in the development and functionality of dopaminergic neurons (Perlmann and Wallén-Mackenzie, 2004).

The objective of this study is to investigate the histopathological and immunohistochemical effects of NAC on brain damage induced by DOX in key regions involved in cognitive and memory processes, namely the cerebral cortex, hippocampus, substantia nigra (SN), striatum, and ventral tegmental area (VTA). This study also aims to investigate the potential prophylactic effect of NAC. An immunohistochemical evaluation was conducted to assess the expression levels of Nurr1, a crucial factor in dopaminergic neuron development and function, and TH, which is involved in dopamine synthesis

To cite this article:
Anteplioğlu T, Çınar M, Yaldız G, Kayabaşı SB, Duru Ö, Kabakçı R. Neuroprotective role of n-acetylcysteine (NAC): countering doxorubicin neurotoxicity via TH, Nurr1, and iNOS expression. Medit Vet J. 2024; 9(2): 299-305.

by dopaminergic neurons. The presence and expression levels of inducible nitric oxide synthetase (iNOS), which is associated with neuroinflammation and neurodegeneration, were also evaluated.

MATERIALS and METHODS

Animals

All experimental procedures and applications were approved by Kirikkale University Huseyin Aytemiz Experimental Research and Application Center, Experiments Ethics Committee (report number: 23/06/25). The experiments involved the use of 24 female Wistar albino rats with a weight range of 200-250 g. The rats had unrestricted access to water and were fed a standard chow diet. The rats were housed in a facility with a 12-hour light/dark cycle and controlled temperature (25±2 °C) and relative humidity (42±5%).

The 24 rats were randomly allocated to three groups, each comprising eight animals. The control group was administered saline intraperitoneally. The second group received a single intraperitoneal dose of 10 mg/kg DOX (Adriamycin, Deva®, Turkey) after 20 days. The third group received daily intraperitoneal administration of 100 mg/kg NAC for 20 days as prophylaxis, followed by a single dose of 10 mg/kg DOX on day 20.

Tissue Collection and Histopathological Analysis

On the 21st day of the study, all groups were euthanized using a combination of 10 mg/kg xylazine (Xylazinbio 2%, Bioveta®, Czech Republic) and 200 mg/kg ketamine (Ketasol 10%, RichterPharma®, Austria). Following the removal of brain samples, they were fixed in a 10% buffered formaldehyde solution for 72 hours to facilitate histopathological and immunohistochemical examinations. Once the tissue collection was completed, a routine pathological examination was conducted and the tissues were trimmed and embedded in paraffin wax. After obtaining paraffin blocks, serial sections with a thickness of 4-5 µm were taken for both immunoperoxidase tests and histopathological examinations. Hematoxylin and eosin staining was applied to all tissue sections, and subsequent observations were made using a light microscope. Photomicrographs were acquired using an Olympus BX51 microscope (Japan).

Immunohistochemical Examination

Serial tissue sections were obtained and they were labeled using commercial primary antibodies against Nurr1 (Santa Cruz (N-20)/1/100, USA), TH (Santa Cruz (H-196)/1/200, USA), and iNOS (Sigma Aldrich/1/200, USA). Immunohistochemical staining was conducted using a commercial immunoperoxidase kit (Thermo, USA) in accordance with the manufacturer's instructions. Chromogen visualization was achieved using 3-amino-9-ethylcarbazole (AEC), while Mayer's hematoxylin was used as the counterstain. Negative controls were also included, following the same staining procedure, with nonimmunized rat serum replacing the primary antibody.

Following the placement of tissue sections on electrostatic adhesive slides, dewaxing was performed using xylene, followed by hydration using graded alcohols. The sections underwent antigen retrieval with a 30-min microwaving process in citrate buffer (pH 6.0). To prevent endogenous peroxidase activity, a solution of 3% H_2O_2 in methanol was applied for 15 min. Furthermore, nonspecific labeling was prevented by preincubation with normal goat serum for 10 min. The slides underwent a series of treatments with the appropriate primary antibody (Nurr1, TH, or iNOS), followed by the secondary antibody and then streptavidin. Overnight incubation at 4 °C was employed for the primary antibody. This was followed by a 30-min application of the secondary antibody and an additional 30-min incubation with streptavidin. Subsequently, AEC was utilized as the chromogen and the slides were sealed with an aqueous mounting medium. Microphotographs were captured and examined using an Olympus BX51 microscope (Japan).

Data and Statistical Analysis

Utilizing ImageJ (USA) image analysis software, positive staining density was quantified with the aid of a 40× objective. The measurement process involved capturing the integrated optical density of all immunopositive stains, and the subsequent calculation allowed the determination of the average area of Nurr1, TH, or iNOS immunopositivity relative to the total area, employing ImageJ software.

Data were shown as means ± standard deviations (SDs), and comparisons between groups for histopathological and immunohistochemical analyses were performed with the Mann-Whitney U test. GraphPad Prism version 9.0 (GraphPad Software, USA) was employed for all statistical analyses and graph preparations with the significance level set at $p<0.05$.

RESULTS

Histopathology

Comparing the DOX-induced group with the control group, significant histopathological changes were observed. Particularly in the hippocampal region, shrinkage, hyperchromasia, degeneration, and necrosis were found in pyramidal neurons in the dentate gyrus (DG) and cornu ammonis-3 (CA3) regions of the hippocampus, neurons in the frontal region of the cerebral cortex, and neurons in the nucleus accumbens (ACB) and caudoputamen (CP) in the striatum. The nuclei of necrotic neurons were generally pyknotic and the Nissl bodies in the neuronal cytoplasm were lost (Figures 1A, B, and C). In contrast, the control group showed neurons with nuclei located in the center, prominent nucleoli in the nuclei and Nissl granules in the cytoplasm, and basophilic-stained nuclei and eosinophilic-stained cytoplasm (Figures 1D, E, and F). The DOX-induced group exhibited perineuronal edema and satellitosis around the affected neurons (Figures 1A, B and C). Congestion was observed in the blood vessels in the cerebral cortex, striatum, thalamus, and midbrain (Figures 1B and 1C).

The observations indicated that neurons in the hippocampal region exhibited less damage in the NAC prophylaxis group compared to the group induced with DOX. In addition, the pathological changes of DOX toxicity were reduced in the NAC prophylaxis group, including the reduction of neuronal degeneration and necrosis and even the disappearance

of perineuronal edema (Figures 1G, H, and I). In contrast, in the NAC prophylaxis group, vascular changes were similar to those observed in the DOX-induced group and there was no reduction in vascular congestion, although other findings more closely resembled those of the control group.

Immunohistochemistry

Comparing the DOX-induced group to the other groups, it was observed that the iNOS immunopositivity in the DOX-induced group was statistically significant compared to that of the others ($p<0.0001$) (Figures 2A, D, and H; Table 1). iNOS immunopositivity was mainly detected in selected hippocampal regions, such as CA1, CA3, and DG, as well as in the striatum, thalamus, midbrain, SN, and VTA and in glial cells and astrocytes. Furthermore, a notable disparity was identified between the control group and the prophylaxis group ($p<0.05$) (Figures 2A, E, H, and I; Table 1). The prophylaxis group exhibited iNOS immunopositivity closest to that of the control group and no statistically significant difference was noted between these two groups (Figures 2E, H, and I). In the prophylaxis group, iNOS immunopositivity was not prominent in the neurons, especially in the hippocampal neurons; rather, it was observed mainly in glial cells in the cerebral cortex, striatum, SN, and VTA.

The proportion of Nurr1-immunopositive cells was consistently high in both the control and prophylaxis groups. In contrast, it was significantly reduced in the DOX-induced group in comparison to the other two groups ($p<0.001$) (Figures 2B, F, J, and L; Table 1). Nurr1 immunopositivity was observed in neurons and glial cells to varying degrees, mainly in the SN, VTA, striatum, and hippocampus, with neuronal immunopositivity being more prominent (Figure 2B, F, J, and L).

The TH immunopositivity results were found to be quite similar to those for Nurr1 immunopositivity. Accordingly, the expression of TH was markedly decreased in the DOX-induced group compared to both the prophylaxis and control groups ($p<0.001$) (Figures 2C, D, G, and K; Table 1). The intense immunopositivity observed in the control and prophylaxis groups was notable in neurons in the SN, VTA, and striatum.

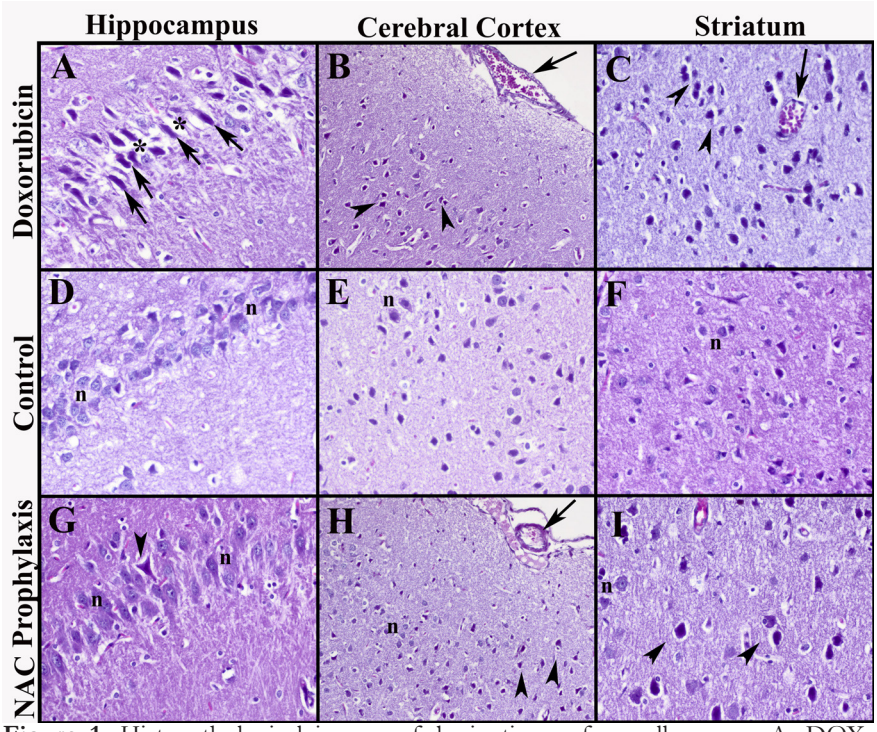

Figure 1. Histopathological images of brain tissues from all groups. A. DOX-induced group. Degeneration and hyperchromasia (arrow) in pyramidal neurons in the hippocampal CA3 region and perineuronal edema (asterisk). Hematoxylin and eosin (H&E) staining; 400× magnification. B-C. DOX-induced group. Shrunken and degenerative neurons (arrowhead) and congested blood vessels (arrow) in the cerebral cortex and striatum, respectively. H&E staining; 100× and 200× magnification, respectively. D-E-F. Control group. Normal brain structure characterized by healthy neurons (n) with central nucleus and normal nuclei and cytoplasmic Nissl bodies in the hippocampal CA3 region, cerebral cortex, and striatum. H&E staining; 400×, 100×, and 200× magnification, respectively. G. NAC prophylaxis group. Normal pyramidal neurons (n) and degenerative, hyperchromatic neurons (arrowhead) in the hippocampal CA3 region. H&E staining; 400× magnification. H-I. NAC prophylaxis group. Degenerative, shrunken neurons (arrowhead) and normal neurons (n) together with congested blood vessels (arrow) in the cerebral cortex and striatum. H&E staining; 100× and 200× magnification, respectively.

Table 1. Statistical data on the immunopositivity of iNOS, Nurr1, and TH

	iNOS		Nurr1		TH		p
	n	Mean±SD	n	Mean±SD	n	Mean±SD	
Control	8	0.089±0.073 ^{A,a}	8	3.191±0.282 ^{B,b}	8	4.042±0.151C ^{C,b}	<0.001
DOX	8	5.437±1.018 ^{c}	8	1.005±0.310 ^{A,a}	8	2.569±0.595 ^{B,a}	<0.001
Prophylaxis	8	1.082±0.208 ^{A,b}	8	3.389±0.209 ^{B,b}	8	3.986±0.112 ^{C,b}	<0.001
p		<0.001		<0.001		<0.001	

A, B, C: There is a statistical difference between groups with different letters in the rows. a, b, c: There is a statistical difference between groups with different letters in the columns.

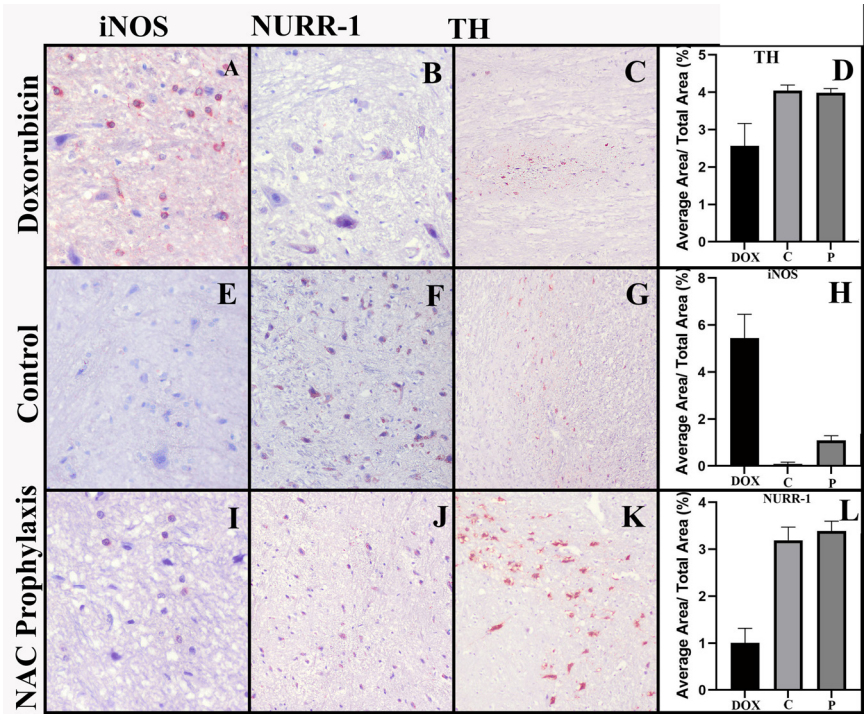

Figure 2. Immunoreactivities of anti-iNOS, anti-Nurr1, and anti-TH in all groups. A-B-C. DOX-induced group. Intense anti-iNOS immunoreactivity in the striatum and mild Nurr1 and TH immunopositivity in the SN and VTA, respectively. IHC. AEC chromogen with hematoxylin counterstain; magnification of 200×, 200×, and 100×, respectively. D. Comparative graph of anti-TH immunoreactivity in all groups. x: Average area of immunopositivity of TH/Total area percentages; y: Groups. E-F-G. Control group. Very mild anti-iNOS immunoreactivity in the striatum and strong Nurr1 and TH immunopositivity in the SN and VTA, respectively. IHC. AEC chromogen with hematoxylin counterstain; magnification of 200×, 200×, and 100×, respectively. H. Comparative graph of anti-iNOS immunoreactivity in all groups. x: Average area of immunopositivity of iNOS/Total area percentages; y: Groups. I-J-K. NAC prophylaxis group. Mild to moderate anti-iNOS immunoreactivity in the striatum and significantly strong Nurr1 and TH immunopositivity in the SN and VTA, respectively. IHC. AEC chromogen with hematoxylin counterstain; magnification of 200×, 200×, and 100×, respectively. L. Comparative graph of anti-Nurr1 immunoreactivity in all groups. x: Average area of immunopositivity of Nurr1/Total area percentages; y: Groups.

DISCUSSION

DOX-induced neurotoxicity arises from a number of mechanisms, including DNA damage, apoptosis induction, reactive oxygen species (ROS) production, neurotransmitter downregulation, and synaptic dysplasia (Tangpong et al., 2011; Kitamura et al., 2014; Habbas et al., 2015; Lim et al., 2016; Keeney et al., 2018). In particular, it has been shown that DOX causes impairment of the mitochondria in the hippocampus, which in turn results in elevated levels of reactive ROS (Park et al., 2018). Furthermore, Kwatra et al. (2016) found a marked reduction in serotonin and dopamine levels in rats treated with DOX, as quantified spectrophotometrically following homogenization

of the hippocampal region. The results of the histopathological analysis revealed significant alterations in the brain tissues of the DOX-induced group in comparison to the control group. These changes were observed to encompass shrinkage, hyperchromasia, degeneration, and necrosis in diverse brain areas, including the striatum, cerebral cortex, and hippocampus. In contrast, the histopathologic changes, and particularly those in the hippocampal region, were less pronounced in the NAC prophylaxis group. The DOX-induced group also exhibited perineuronal edema and vascular congestion and showed significant immunopositivity for iNOS, indicating the presence of neuroinflammation and neurodegeneration.

Chemotherapeutic drugs, especially DOX, can lead to cognitive dysfunction ranging from mild to severe, affecting approximately 75% of cancer patients (Ahles and Saykin, 2007). Such cognitive dysfunction may include memory and attention problems, difficulties in multitasking, and emotional and behavioral changes (Seigers and Fardell, 2011). These clinical findings suggest that regions of the brain, particularly those with dopaminergic neurons, may be affected. In our study, both histopathological and immunohistochemical analyses demonstrated the involvement of pyramidal neurons located in the hippocampal regions of CA1, CA3, and DG, as well as neurons in the striatum, frontal cortex, VTA, and SN in DOX-induced rats.

Cognitive impairment forms the basis of many disorders, including neurodegenerative diseases, mood disorders, anxiety, chronic pain, and psychosis. Studies have directly associated oxidative stress with cognitive impairments (Skvarc et al., 2017). However, it is believed that NAC can reverse cognitive impairments by increasing GSH levels (Cao et al., 2012). In addition, NAC has been demonstrated to modulate a number of biological processes, including oxidative stress, apoptosis, mitochondrial dysfunction, and neurotransmitters such as glutamate and dopamine. These effects have been observed both directly and indirectly (Frye et al., 2018). In this study, NAC administered prior to DOX administration was shown to have no effect on vascular changes in the DOX-treated group. However, it prevented neuronal damage at a high rate. Immunohistochemical analysis further confirmed the protective effects of NAC. Conversely, NAC administration reduced iNOS immunopositivity, particularly in glial cells, suggesting its anti-inflammatory properties. The prophylaxis group had the immunopositivity levels closest to those of the control group, indicating the potential preventive effects of NAC against DOX-induced neuroinflammation.

While studies have investigated the effects of DOX on the nervous system, research on the prophylactic effect of NAC against this damage remains limited. Previous studies were limited to histopathology, the damage was assessed by blood parameters and serum biochemistry, and the damaged dopaminergic neurons and mechanisms of action were not shown in the damaged tissue (Mohammed et al., 2019).

Research has demonstrated that Nurr1 exhibits anti-inflammatory properties and promotes the development of dopaminergic neurons from neural stem cells (Chen et al., 2018). It is also known that the most commonly used markers to demonstrate the presence of dopaminergic neurons immunohistochemically are TH and DAT, and TH is involved in dopamine synthesis (Huot et al., 2007). Furthermore, studies have revealed that NAC plays dual roles in the hippocampal CA1 and DG regions, acting as both an anti-inflammatory and an antiapoptotic agent (Song et al., 2019; Fan et al., 2020). In the present study, the DOX-induced group showed significantly lower immunopositivity for both Nurr1 and TH compared to the control group. Furthermore, the administration of NAC effectively preserved the expression levels of Nurr1 and TH, suggesting its potential in preserving dopaminergic neurons and neurotransmitter synthesis, especially in the VTA, SN, and striatum. Additionally, this study has demonstrated that NAC has prophylactic properties and that its effects are not limited to the hippocampal region but also extend to the striatum, VTA, and SN.

NAC administration also decreased iNOS immunoreactivity, demonstrating its anti-inflammatory effect, and resulted in the dopaminergic system approaching levels similar to those of the control group.

CONCLUSION

The study has demonstrated the favorable impact of NAC on DOX-induced brain damage. NAC administration reduced neuronal damage, degeneration, and necrosis in regions of the brain crucial for cognitive and memory processes. Furthermore, NAC exhibited anti-inflammatory properties, as evidenced by decreased iNOS immunopositivity, and preserved the expression levels of Nurr1 and TH, which are crucial for dopaminergic neuron function. These findings indicate that NAC may have a prophylactic effect in preventing the cognitive impairments associated with DOX treatment. Furthermore, the utilisation of varying doses of DOX in forthcoming studies may prove advantageous in terms of comparative assessment.

DECLARATIONS

Ethics Approval

Kirikkale University Huseyin Aytemiz Experimental Research and Application Center, Experiments Ethics Committee 23/06/25 Number Ethics Committee Decision

Conflict of Interest

The authors declare that they have no competing interests

Consent for Publication

Not applicable

Author contribution

Idea, concept and design: TA, MÇ

Data collection and analysis: GY, SBK, ÖD, RK

Drafting of the manuscript: TA

Critical review: MÇ, GY, SBK, ÖD, RK

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to [reasons such as privacy

concerns]. Data are available from the corresponding author upon reasonable request.

Acknowledgements

We thank Ali Şenol and Dr Evren Üstüner for assistance in data collection.

REFERENCES

Abdel-Wahab, W. M., & Moussa, F. I. (2019). Neuroprotective effect of N-acetylcysteine against cisplatin-induced toxicity in rat brain by modulation of oxidative stress and inflammation. Drug design, development and therapy, 13, 1155-1162. https://doi.org/10.2147/dddt.s191240

Ahles, T. A., & Saykin, A. J. (2007). Candidate mechanisms for chemotherapy-induced cognitive changes. Nature Reviews Cancer, 7(3), 192–201. https://doi.org/10.1038/nrc2073

Andryszak, P., Wiłkość, M., Żurawski, B., & Izdebski, P. (2017). Verbal memory in breast cancer patients treated with chemotherapy with doxorubicin and cyclophosphamide. European Journal of Cancer Care, 27(1), 1-11. https://doi.org/10.1111/ecc.12749

Cao, L., Li, L., & Zuo, Z. (2012). N-acetylcysteine reverses existing cognitive impairment and increased oxidative stress in glutamate transporter type 3 deficient mice. Neuroscience, 220, 85–89. https://doi.org/10.1016/j.neuroscience.2012.06.044

Chen, X., Qian, Y., Wang, X., Tang, Z., Xu, J., Lin, H., Yang, Z., Song, X., Lu, D., Guo, J., Bian, L., Li, Y., Zhou, L., & Deng, X. (2018). Nurr1 promotes neurogenesis of dopaminergic neuron and represses inflammatory factors in the transwell co-culture system of neural stem cells and microglia. CNS Neuroscience & Therapeutics, 24(9), 790–800. https://doi.org/10.1111/cns.12825

Cheruku, S. P., Ramalingayya, G. V., Chamallamudi, M. R., Biswas, S., Nandakumar, K., Nampoothiri, M., Gourishetti, K., & Kumar, N. (2017). Catechin ameliorates doxorubicin-induced neuronal cytotoxicity in in vitro and episodic memory deficit in in vivo in Wistar rats. Cytotechnology, 70(1), 245–259. https://doi.org/10.1007/s10616-017-0138-8

Dean, O., Giorlando, F., & Berk, M. (2011). N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. Journal of Psychiatry & Neuroscience, 36(2), 78–86. https://doi.org/10.1503/jpn.100057

Du, J., Zhang, A., Li, J., Liu, X., Wu, S., Wang, B., Wang, Y., & Jia, H. (2021). Doxorubicin-Induced Cognitive Impairment: The Mechanistic Insights. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.673340

El-Hussein, A., Manoto, S. L., Ombinda-Lemboumba, S., Alrowaili, Z. A., & Mthunzi-Kufa, P. (2020). A Review of Chemotherapy and Photodynamic Therapy for Lung Cancer Treatment. Anti-Cancer Agents in Medicinal Chemistry, 21(2), 149–161. https://doi.org/10.2174/1871520620666200403144945

Fan, C., Long, Y., Wang, L., Liu, X., Liu, Z., Lan, T., Li, Y., & Yu, S. Y. (2020). N-Acetylcysteine Rescues Hippocampal Oxidative Stress-Induced Neuronal Injury via Suppression of p38/JNK Signaling in Depressed Rats. Frontiers in Cellular Neuroscience, 14, 1-11. https://doi.org/10.3389/fncel.2020.554613

Frye, R. E., Andrus, J. P., Lemley, K. V., De Rosa, S. C., Ghezzi, P., Holmgren, A., Jones, D., Jahoor, F., Kopke, R., Cotgreave, I., Bottiglieri, T., Kaplowitz, N., Nakamura, H., Staal, F., Ela, S. W., Atkuri, K. R., Tirouvanziam, R., Heydari, K., Sahaf, B., … Herzenberg, L. A. (2018). Pharmacology, Formulations, and Adverse Effects. The Therapeutic Use of N-Acetylcysteine (NAC) in Medicine, 387–394. https://doi.org/10.1007/978-981-10-5311-5_21

Gil-Martínez, A.L., Cuenca, L., Sánchez, C., Estrada, C., Fernández-Villalba, E., & Herrero, M. T. (2018). Effect of NAC treatment and physical activity on neuroinflammation in subchronic Parkinsonism; is physical activity essential? Journal of Neuroinflammation, 15, 1-13. https://doi.org/10.1186/s12974-018-1357-4

Habbas, S., Santello, M., Becker, D., Stubbe, H., Zappia, G., Liaudet, N., Klaus, F. R., Kollias, G., Fontana, A., Pryce, C. R., Suter, T., & Volterra, A. (2015). Neuroinflammatory TNFα Impairs Memory via Astrocyte Signaling. Cell, 163(7), 1730–1741. https://doi.org/10.1016/j.cell.2015.11.023

Hernandez-Aya, L. F., & Gonzalez-Angulo, A. M. (2013). Adjuvant Systemic Therapies in Breast Cancer. Surgical Clinics of North America, 93(2), 473–491. https://doi.org/10.1016/j.suc.2012.12.002

Huot, P., Levesque, M., & Parent, A. (2006). The fate of striatal dopaminergic neurons in Parkinson's disease and Huntington's chorea. Brain, 130(1), 222–232. https://doi.org/10.1093/brain/awl332

Keeney, J. T. R., Ren, X., Warrier, G., Noel, T., Powell, D. K., Brelsfoard, J. M., Sultana, R., Saatman, K. E., St. Clair, D. K., & Butterfield, D. A. (2018). Doxorubicin-induced elevated oxidative stress and neurochemical alterations in brain and cognitive decline: protection by MESNA and insights into mechanisms of chemotherapy-induced cognitive impairment ("chemobrain"). Oncotarget, 9(54), 30324–30339. https://doi.org/10.18632/oncotarget.25718

Kitamura, H., Tsukamoto, T., Shibata, T., Masumori, N., Fujimoto, H., Hirao, Y., Fujimoto, K., Kitamura, Y., Tomita, Y., Tobisu, K., Niwakawa, M., Naito, S., Eto, M., & Kakehi, Y. (2014). Randomised phase III study of neoadjuvant chemotherapy with methotrexate, doxorubicin, vinblastine and cisplatin followed by radical cystectomy compared with radical cystectomy alone for muscle-invasive bladder cancer: Japan Clinical Oncology Group Study JCOG0209. Annals of Oncology, 25(6), 1192–1198. https://doi.org/10.1093/annonc/mdu126

Kitamura, Y., Ushio, S., Sumiyoshi, Y., Wada, Y., Miyazaki, I., Asanuma, M., & Sendo, T. (2021). N-Acetylcysteine Attenuates the Anxiety-Like Behavior and Spatial Cognition Impairment Induced by Doxorubicin and Cyclophosphamide Com-

bination Treatment in Rats. Pharmacology, 106(5–6), 286–293. https://doi.org/10.1159/000512117

Kwatra, M., Jangra, A., Mishra, M., Sharma, Y., Ahmed, S., Ghosh, P., Kumar, V., Vohora, D., & Khanam, R. (2016). Naringin and Sertraline Ameliorate Doxorubicin-Induced Behavioral Deficits Through Modulation of Serotonin Level and Mitochondrial Complexes Protection Pathway in Rat Hippocampus. Neurochemical Research, 41(9), 2352–2366. https://doi.org/10.1007/s11064-016-1949-2

Lim, I., Joung, H.Y., Yu, A. R., Shim, I., & Kim, J. S. (2016). PET Evidence of the Effect of Donepezil on Cognitive Performance in an Animal Model of Chemobrain. BioMed Research International, 2016, 1–7. https://doi.org/10.1155/2016/6945415

Mehta, M., & Riedel, W. (2006). Dopaminergic Enhancement of Cognitive Function. Current Pharmaceutical Design, 12(20), 2487–2500. https://doi.org/10.2174/138161206777698891

Mohammed, W. I., Radwan, R. A., & Elsayed, H. M. (2019). Prophylactic and Ameliorative Effect of N-Acetylcysteine on Doxorubicin-Induced Neurotoxicity in Wister Rats. Egyptian Journal of Basic and Clinical Pharmacology, 9(14), 1-16. https://doi.org/10.32527/2019/101396

Park, H.S., Kim, C.J., Kwak, H.B., No, M.H., Heo, J.W., & Kim, T.W. (2018). Physical exercise prevents cognitive impairment by enhancing hippocampal neuroplasticity and mitochondrial function in doxorubicin-induced chemobrain. Neuropharmacology, 133, 451–461. https://doi.org/10.1016/j.neuropharm.2018.02.013

Perlmann, T., & Wallén-Mackenzie, Å. (2004). Nurr1, an orphan nuclear receptor with essential functions in developing dopamine cells. Cell and Tissue Research, 318(1), 45–52. https://doi.org/10.1007/s00441-004-0974-7

Prakash, A., Kalra, J. K., & Kumar, A. (2014). Neuroprotective effect of N-acetyl cysteine against streptozotocin-induced memory dysfunction and oxidative damage in rats. Journal of Basic and Clinical Physiology and Pharmacology, 26(1), 13–23. https://doi.org/10.1515/jbcpp-2013-0150

Raffa, R. B., Duong, P. V., Finney, J., Garber, D. A., Lam, L. M., Mathew, S. S., Patel, N. N., Plaskett, K. C., Shah, M., & Jen Weng, H.-F. (2006). Is "chemo-fog'/'chemo-brain" caused by cancer chemotherapy? Journal of Clinical Pharmacy and Therapeutics, 31(2), 129–138. https://doi.org/10.1111/j.1365-2710.2006.00726.x

Rau, W. C. (1992). The Good Sociology Departments: Will We Ever Find Them? Will We Even Try? Teaching Sociology, 20(2), 165-170. https://doi.org/10.2307/1317402

Seigers, R., & Fardell, J. E. (2011). Neurobiological basis of chemotherapy-induced cognitive impairment: A review of rodent research. Neuroscience & Biobehavioral Reviews, 35(3), 729–741. https://doi.org/10.1016/j.neubiorev.2010.09.006

Skvarc, D. R., Dean, O. M., Byrne, L. K., Gray, L., Lane, S., Lewis, M., Fernandes, B. S., Berk, M., & Marriott, A. (2017). The effect of N-acetylcysteine (NAC) on human cognition – A systematic review. Neuroscience & Biobehavioral Reviews, 78, 44–56. https://doi.org/10.1016/j.neubiorev.2017.04.013

Song, Q., Feng, Y., Wang, L., Shen, J., Li, Y., Fan, C., Wang, P., & Yu, S. Y. (2019). COX-2 inhibition rescues depression-like behaviors via suppressing glial activation, oxidative stress and neuronal apoptosis in rats. Neuropharmacology, 160, 107779. https://doi.org/10.1016/j.neuropharm.2019.107779

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

Tangpong, J., Miriyala, S., Noel, T., Sinthupibulyakit, C., Jungsuwadee, P., & St. Clair, D. K. (2011). Doxorubicin-induced central nervous system toxicity and protection by xanthone derivative of Garcinia Mangostana. Neuroscience, 175, 292–299. https://doi.org/10.1016/j.neuroscience.2010.11.007

Medit Vet J, 9(2): 306-310, 2024

Research Article

Estimating the carbon footprint of dairy cattle in the district of Karapınar, in the province of Konya

Onur Erzurum[1]

[1]Department of Veterinary, Vocational School of Karapınar Aydoganlar, Selcuk University, Konya, Türkiye

Key Words:
carbon dioxide
carbon footprint
dairy cattle
global warming
methane

Received : 22 April 2024
Revised : 14 August 2024
Accepted : 19 August 2024
Published : 31 August 2024
Article Code : 1472090

Correspondence:
O. ERZURUM
(onurerzurum@selcuk.edu.tr)

ORCID
O. ERZURUM : 0000-0001-7074-8573

ABSTRACT

Global warming refers to the increase in the amount of heat resulting from the release of greenhouse gases into the atmosphere. One of the prominent strategies to mitigate global warming in the face of an increasing world population is to regulate the livestock sector. The effect of cattle on global warming is through the release of greenhouse gases (CO2, CH4). The carbon footprint can be defined as the damage caused to the environment by the amount of greenhouse gases in the form of carbon dioxide resulting from the activities of living beings. One of the places where dairy farming is performed intensively in Türkiye is the district of Karapınar in the province of Konya. According to the data from the Turkish Statistical Institute, the total number of dairy cattle in the district of Karapınar was 28,186 heads in 2018 and 32,405 heads in 2019. The difference of 4,219 heads is estimated to raise the carbon footprint potential by 426.4 kg CO2e year-1 in 2019 compared to 2018. When the increase per animal was calculated, the result was 0.01 kg CO2e year-1. This calculation was made utilising the Tier-1 method, using the equations provided in the Intergovernmental Panel on Climate Change (IPCC) 2006 guide. In the guide, the methods Tier-2 and Tier-3 are also specified, and more detailed methods are planned for future studies.

INTRODUCTION

It is stated that the world population reached approximately 8 billion people in 2023. According to the United Nations, this increasing number is expected to reach 9.7 billion in 2050 and 10.4 billion in 2100. According to the data from the Turkish Statistical Institute (TUIK), Türkiye's population reached 85.2 million in 2023, ranking 18th in the world. This number is estimated to reach 93 million in 2050.

The increasing population also increases the need for food for the continuity of life. In order to meet the increasing food demand, new techniques in agriculture and animal husbandry must become widespread, and the number of products produced per unit area must increase (Kitani, 1999; Özpınar, 2023). For this reason, there has been an increase in the use of intensive farming methods for dairy farming. Increasing the production in dairy farming can be achieved by improving the productivity of animals or by increasing the number of animals. As a result of the activities carried out to achieve the mentioned increases, the emission of greenhouse gases into the atmosphere is increasing. With the increase in the concentrations of gases released into the atmosphere, an increase in the earth's temperature, referred to as "global warming", is observed (Köknaroğlu and Akünal, 2010). While Bayraç (2010) defines global warming as a systematic increase in the temperature worldwide, Doğan et al. (2010) define it as the increase in the temperature on the earth due to the increase in gas concentrations in the atmosphere.

As a result of the increase in the number of dairy animals and various activities carried out to enhance productivity, the amount of greenhouse gases released into the atmosphere will also increase. The term carbon footprint is used to track the outputs of these activities at the production and consumption stages. The increase in greenhouse gases in the atmosphere due to livestock farming is caused by the effect of carbon dioxide (CO_2) and methane (CH_4) gases (Türkeş, 2000). 35-40% of worldwide methane emissions originate from enteric fermentation and manure management in livestock (Steinfeld et al., 2006). It is stated that approximately 65% of the total emissions of greenhouse gases resulting from livestock activities are caused by cattle. In most developing countries such as Türkiye, 39% of greenhouse gas emissions from the livestock sector originate from enteric fermentation and 26% from manure management (Herrero et al., 2013). This reveals that most of the greenhouse gases released from activities in the livestock sector originate from cattle (Koyuncu and Akgün, 2017).

Cattle have a unique digestive system compared to other animals, allowing them to digest materials rich in poor-quality cellulose. As a result of this digestion, they have an important place in the production of methane and greenhouse gases. While it can be seen that cattle, when considered individually, produce a small amount of methane gas (80-110 kg year^{-1}), this amount is quite high when considering the cattle population (Koyuncu and Akgün, 2017). Greenhouse gases resulting from milk production in cattle account for approximately 20% of the total emissions (Gerber et al., 2013). 2-12% of the gross energy ingested with the diet is lost by being converted into methane (CH_4) during microbial digestion in the rumen. This

To cite this article:
Erzurum O. Estimating the carbon footprint of dairy cattle in the district of Karapınar, in the province of Konya. Medit Vet J. 2024; 9(2): 306-310.

induces a negative impact on global warming (Öztürk, 2007). It is also stated that the energy required to produce feed raw materials constitutes approximately 10% of the total carbon dioxide emissions, and the effect of the amount of energy consumed for milk production on emissions is considered insignificant (Koyuncu and Akgün, 2017). Among the greenhouse gases that cause global warming, methane gas (CH_4) ranks second after carbon dioxide (CO_2) (Çetin et al., 2020). However, it is also stated that the global warming potential of methane might be 21 times that of carbon dioxide over a period of approximately 100 years (Köknaroğlu and Akünal, 2010).

The concept of carbon footprint in global warming is used to indicate the impact of the activities performed to meet the needs of living beings. Different methods are used to measure this impact, and online programs are even designed to calculate the results (Güven ve İlker, 2016).

In light of this information, the study aimed to evaluate the impact of dairy cattle on global warming in the district of Karapınar, located in the province of Konya, an important location for dairy farming. Both carbon dioxide and methane gases were focused on in order to calculate this effect.

MATERIALS and METHODS

Karapınar, the region chosen to conduct the research, is a district of the province of Konya, one of the prominent provinces in Türkiye regarding dairy cattle breeding. According to TUIK data, Karapınar district ranked first among the districts of Konya in 2018 and second in 2019 regarding the number of dairy cattle. Among the dairy cattle bred in the district, the number of milking animals was 28,186 heads in 2018 and 32,405 in 2019 (TUIK, 2023). The carbon footprint of dairy cattle in the Karapınar district was calculated by matching the data received from TUIK with the calculation tables in the Intergovernmental Panel on Climate Change (IPCC, 2006) guide. For the values to be used in the calculations to be made in the study, Türkiye was classified as part of the "Eastern Europe" region in the tables specified in the IPCC guide. No living or non-living animal material was used within the scope of the study.

Calculation of the carbon footprint

Tier (Tier 1, Tier 2, Tier 3) methods determined by the IPCC are used to calculate carbon footprints. The Tier 1 method was selected due to its ease of use. This method works based on prediction (IPCC, 2006). Emission factor values in the IPCC guide are organised based on animal species and climate zones (temperatures). In the IPCC guide (IPCC, 2006), equation 10.22 was used to calculate the carbon footprint resulting from manure management (Equation 1).

$$CH_{4(manure)} = \sum_{(T)} \frac{EF_{(T)} \times N_{(T)}}{10^6}$$

(Eq. 1)

In this equation, $CH_{4(Manure)}$ refers to methane emissions from manure management (Gg CH_4 $year^{-1}$), $EF_{(T)}$ is the defined emission factor for the livestock sector (kg CH_4 $head^{-1}$ $year^{-1}$), $N_{(T)}$ is the total number of animals (head) in the population, T is the animal species or category.

For the $EF_{(T)}$ value in the equation, the values from Table 10.14 of the IPCC (IPCC, 2006) guide (Table 1) were used. In the related table, the levels of development of the countries, differences in manure management systems and temperature conditions of the region are taken into account. Türkiye has the status of a developing country. The average temperature for the district of Karapınar in the province of Konya in 2018 and 2019 was 11.1°C (MGM, 2023). The values provided in Table 10.14 of the IPCC (IPCC, 2006) guide will be used for the calculations and are given in Table 1.

Table 1. Methane emission factor from manure management for cattle according to temperature (IPCC, 2006)

	Cold					Mild											Hot		
	≤10°C	11°C	12°C	13°C	14°C	15°C	16°C	17°C	18°C	19°C	20°C	21°C	22°C	23°C	24°C	25°C	26°C	27°C	≥28°C
Dairy cattle	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Other cattle	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Manure-derived methane emission factors that should be obtained according to annual average temperatures

The methane gas emission factor resulting from manure management was selected from the values in Table 1. Since the average temperature of Karapınar in 2018 and 2019 was 11.1°C, and based on the 11°C value in the table, the methane emission factor resulting from manure management for dairy cattle will be 2 kg CH_4 $head^{-1}$ per year.

The Tier 1 method in the IPCC (IPCC, 2006) guide is a simplified method used for estimating emissions by selecting emission values according to animal species and subcategories, feeding patterns and annual average milk yield. The IPCC (IPCC, 2006) guide states that Equation 10.19 (Equation 2) should be used to calculate CH_4 emissions from enteric fermentation.

$$\text{Emissions} = EF_{(T)} \times \frac{N_{(T)}}{10^6}$$

(Eq. 2)

In this equation, emissions refers to the methane emissions from enteric fermentation (10^3 tonne CH_4 $year^{-1}$), $EF_{(T)}$ refers to the emission factor defined for livestock sectors (kg CH_4 $head^{-1}$ $year^{-1}$), $N_{(T)}$ refers to total number of animals in the population, T refers to the animal species.

For the $EF_{(T)}$ value in the concerning equation, the emission factor used was specified in Table 10.11 within the subcategories of the IPCC (IPCC, 2006) guide. In this table, enteric fermentation emission factor values originating from cattle calculated by the Tier 1 method are evaluated, as well as the status of development of the countries, differences in feed composition and annual milk amount (head) (Table 2). In this table, since Türkiye is located in Eastern Europe, the value taken as a basis for dairy cattle is 99 (the annual milk yield of the cows selected is 2550 kg head^{-1}). In the case that the annual milk yield does not meet the specified value in the country category, the corresponding emission factor should be selected. For example, if the annual milk yield is 2000 kg head^{-1}, 90 should be selected as the emission factor value for dairy cattle.

For the year 2019; $CH_{4(manure)} = \sum(T) \frac{2x\ 32405}{10^6}$

- According to Equation 2, methane emission values from enteric fermentation were 2.790 Gg CH_4 year^{-1} for 2018 and 3.208 Gg CH_4 year^{-1} for 2019. The increase in one year was 0.418 Gg CH_4 year^{-1}.

For the year 2018; $Emissions = 99\ x\ \frac{28186}{10^6}$

For the year 2019; $Emissions = 99\ x\ \frac{32405}{10^6}$

Table 2. Enteric fermentation emission factors for cattle according to the Tier 1 method (IPCC, 2006)

Region	Type of Cattle	Emission Factor (kg CH_4 head^{-1} year^{-1})	Explanation (Other)
North America	Dairy cattle	128	Annual milk production 8.400 kg head^{-1}
	Others	53	
West Europe	Dairy cattle	117	Annual milk production 6.000 kg head^{-1}
	Others	57	
East Europe	Dairy cattle	99	Annual milk production 2.550 kg head^{-1}
	Others	58	
Australia	Dairy cattle	90	Annual milk production 2.200 kg head^{-1}
	Others	60	
South America	Dairy cattle	72	Annual milk production 800 kg head^{-1}
	Others	56	
Asia	Dairy cattle	68	Annual milk production 1.650 kg head^{-1}
	Others	47	
Middle Asia/Africa	Dairy cattle	46	Annual milk production 500 kg head^{-1}
	Others	31	
India	Dairy cattle	58	Annual milk production 900 kg head^{-1}
	Others	27	

Emission factor values to be taken according to regions

In the IPCC (IPCC, 2006) guide, it is stated that, when converting methane emissions to a carbon footprint value as CO_2e, it should be multiplied by 25.

RESULTS

When the values corresponding to the formulas in the equations are inserted, the results are presented below and shown in Table 3.

- According to Equation 1, the manure-derived methane emission value found was 0.0564 Gg CH_4 year^{-1} for 2018, while it was 0.0648 Gg CH_4 year^{-1} for 2019. Methane emissions from manure increased in 2019 compared to 2018. The value of this increase is 0.008 Gg CH_4 year^{-1}.

For the year 2018; $CH_{4(manure)} = \sum(T) \frac{2x\ 28186}{10^6}$

In order to express methane emission values as global warming potential, it is stated that the value found for CO_2 equivalence is multiplied by 25 (IPCC, 2006; Crosson et al., 2011). The CO_2 equivalence of emission values are also given in Table 3. The CH_4 emission value per animal was 0.1 kg CH_4 head^{-1} in both years.

Ersoy (2017) calculated the greenhouse gas emission values of cattle in the province of Konya according to 2015 and found that the methane emission value from manure was 1560 tonnes CH_4 year^{-1}, the methane emission value from enteric fermentation was 70040 tonnes CH_4 year^{-1} and the total emission value was 71600 tonnes CH_4 year^{-1}. According to the findings of the study, methane emissions from enteric fermentation in the province of Konya constituted approximately 3.9% in 2018 and approximately 4.5% in 2019. Manure-derived methane emission values constituted approximately 3.62% in 2018 and 4.15%

Table 3. Enteric fermentation emission factors for cattle according to the Tier 1 method (IPCC, 2006)

Years	$CH_{4(manure)}$ (tonne CH_4 $year^{-1}$)	Emissions (tonne CH_4 $year^{-1}$)	CO_2e $year^{-1}$	
			$CH_{4(manure)}$	Emissions
2018	56,4	2790	1410	1743,75
2019	64,8	3208	1620	2005

Emission values and CO2e equivalents by year

in 2019. When Türkiye's ranking is observed in the respective study, the values of Aydın province, which ranks 16th, are approximately similar to those found for the district of Karapınar.

In the study conducted by Yaylı and Kılıç (2020) on dairy cattle farms, the enteric fermentation-derived methane emission value was found to be 659.3 Gg CH_4 $year^{-1}$ for Türkiye, 8.4 Gg CH_4 $year^{-1}$ for the province of Bursa, and manure-derived methane emission value was found to be 99.9 Gg CH_4 $year^{-1}$ for Türkiye and 1.7 Gg CH_4 $year^{-1}$ for Bursa province. The values found for Karapınar correspond to approximately 1/3 of those found for Bursa province. The enteric fermentation-derived methane emission value of Karapınar is approximately 2% of that of Türkiye, and the manure-derived methane emission value is approximately 1.66%.

Ceyhan et al. (2020), in their study in Niğde on 2000 heads of Awassi sheep, found that the CH_4 emission produced by enteric fermentation was 0.016 Gg CH_4 $year^{-1}$, while the CH_4 emission from manure was 0.0002 Gg CH_4 $year^{-1}$. Compared to the values we calculated for cattle, the values found for sheep are relatively low. This is due to the difference in $EF_{(I)}$ value. For sheep, this value is taken as 8 for enteric fermentation and 0.10 for methane emission from manure.

In their study, Kara et al. (2019) found that the CH_4 emission value per animal in autochthonous, imported and hybrid cattle breeds in Konya in 2017 was 21.6 kg The value per animal found in Karapınar in 2018 and 2019 were significantly lower than this value.

In some studies, it has been stated that the largest contribution to greenhouse gas emissions is CH_4 resulting from enteric fermentation (Robertson et al., 2015; Buratti et al., 2017; Kılıç and Amet, 2017; Kiggundu et al., 2019). In the calculated values, enteric fermentation accounted for approximately 55% of the total emissions in 2018, while it represented approximately 44% in 2019.

DISCUSSION

Agriculture and animal husbandry are the biggest sectors expected to be affected by climate change. There is an increasing need for food due to the rise in population. Animal products, which are essantial for maintaining a healthy lifestyle, are insufficient to meet the requirements. Therefore, increasing the production of animal products is possible through methods such as increasing the number of animals and improving productivity. There is a direct relationship between this situation and global warming, and an increase in greenhouse gas emissions is inevitable.

In the study, Tier 2 method is selected for the calculation of greenhouse gas emissions. This method provides more reliable results as it includes more comprehensive calculations compared to Tier 1. Reducing the emission of greenhouse gases is essential in the battle against climate change. Karapınar district is a prominent area not only in animal husbandry but also in the agricultural sector. In order to reduce greenhouse gas emissions caused by dairy cattle in the Karapınar district, farms should be provided with training on manure management and enteric fermentation. Breeders should be made aware of environmentally friendly practices. A correct plan should be created and implemented to address these issues. For example, arrangements should be made for feed rations, and support should be provided for the installation of biogas facilities.

CONCLUSION

As a result, a contribution has been made to the literature on carbon footprint, which is stated to have a significant impact on gases emitted from animals. In this context, the extent to which dairy cows affect the carbon footprint has been revealed. Some suggestions for reducing this effect have been presented, and a basis for future studies has been established. The number of studies on this subject in Türkiye is insufficient, and more research is needed. It is intended to establish a basis with this study, and it is also planned to use the Tier 2 method in future studies and to calculate the carbon footprint of dairy cattle nationwide.

DECLARATIONS

Conflict of Interest

The author declared that there is no conflict of interest.

Author contribution

Idea, concept and design: OE

Data collection and analysis: OE

Drafting of the manuscript: OE

Critical review: OE

Data Availability

The data and calculation methods used in the study are available from TurkStat and IPCC guidelines.

Acknowledgements

Not applicable

REFERENCES

Bayraç, H.N. (2010). Enerji kullanımının küresel ısınmaya etkisi ve önleyici politikalar. Eskişehir Osmangazi Üniversitesi

Sosyal Bilimler Dergisi, 11 (2): 229-259.

Buratti, C., Fantozzi, F., Barbanera, M., Lascaro, E., Chiorri, M., & Cecchini, L. (2017). Carbon footprint of conventional and organic beef production systems: An Italian case study. Science of the Total Environment 576 (2017): 129-137. https://doi.org/10.1016/j.scitotenv.2016.10.075

Ceyhan, A., Akyol, E., Ünalan, A., Çınar, S., & Ali, W. (2020). Estimation of the carbon footprint in dairy sheep farm. Iranian Journal of Applied Animal Science, 10(4), 639-645.

Crosson, P., Shalloo, L., O'brien, D., Lanigan, G., Foley, P., Boland, T., & Kenny, D. (2011). A review of whole farm systems models of greenhouse gas emissions from beef and dairy cattle production systems. Animal Feed Science and Technology, 166: 29-45. https://doi.org/10.1016/j.anifeedsci.2011.04.001

Çetin, M., Saygın, S., & Demir, H. (2020). Tarım sektörünün çevre kirliliği üzerindeki etkisi: Türkiye ekonomisi için bir eşbütünleşme ve nedensellik analizi. Tekirdağ Ziraat Fakültesi Dergisi 17 (3): 329-345. https://doi.org/10.33462/jotaf.678764

Doğan, S., Özçelik, S., Dolu, Ö., & Erman, O. (2010). Küresel ısınma ve biyolojik çeşitlilik. İklim Değişikliği ve Çevre 3 (1): 63-88.

Ersoy, A.E. (2017). Türkiye'nin hayvansal gübre kaynaklı sera gazı emisyonları durumu ve biyogaz enerjisi potansiyeli. (Yüksek Lisans Tezi) Hacettepe Üniversitesi Fen Bilimleri Enstitiüsü, Ankara.

Emissions, F. A. O. (2000). Due to Agriculture. Global, Regional and Country Trends, 2018.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). Tackling Climate Change Through Livestock, A Global Assessment of Emissions and Mitigation Opportunities, (FAO), Rome.

Güven, G., & İlker, A. (2016). Food footprint in daily life: opinions about the consumption of convenience food. Journal of Theory & Practice in Education 12 (2): 403-426.

Herrero, M., Havlík, P., Valin, H., Notenbaert, A., Rufino, M. C., Thornton, P. K., Blümmel, M., Weiss, F., Grace, D., & Obersteiner, M. (2013). Biomass use, production, feed efficiencies, and greenhouse gas emissions from global livestock systems. Proceedings of the National Academy of Sciences, 110(52), 20888-20893. https://doi.org/10.1073/pnas.1308149110

IPCC, (2006). Guidelines for National Greenhouse Gas Inventories. Intergovernmental Panel on Climate Change, NGGIP Publications, IGES, Japan.

Kara, G., Yalınız, İ., & Sayar, M. (2019). Konya ili hayvansal gübre kaynaklı sera gazı emisyonları durumu. Ulusal Çevre Bilimleri Araştırma Dergisi, 2(2), 57-60.

Kılıç, İ., & Amet, B. (2017). Bir süt sığırı işletmesinin karbon ayak izinin tahminlenmesi: Bursa örneği. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi, 34 (Eksayı): 134-142. https://doi.org/10.13002/jafag4415

Kiggundu, N., Ddungu, S.P., Wanyama, J., Cherotich, S., Mpairwe, D., Zziwa, E., Mutebi, F., & Falcucci, A. (2019). Greenhouse gas emissions from Uganda's cattle corridor farming systems. Agricultural Systems, 176 (4): 102649. https://doi.org/10.1016/j.agsy.2019.102649

Kitani, O. (1999). Energy and Biomass Engineering. In: CIGR Handbook of Agricultural Engineering. ASAE, USA.

Koyuncu, M., & Akgün, H. (2017). Çiftlik hayvanları ve küresel iklim değişikliği arasındaki etkileşim. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 32 (1): 154-161.

Köknaroğlu, H., & Akünal, T. (2010). Küresel ısınmada hayvancılığın payı ve zooteknist olarak bizim rolümüz. Ziraat Fakültesi Dergisi, 5(1), 67-75.

MGM, Meteoroloji Genel Müdürlüğü (2023, September 1). https://www.mgm.gov.tr/eng/forecast-cities.aspx

Özpinar, S. (2023). Analysis of energy use efficiency and greenhouse gas emission in rainfed canola production (Case study: Çanakkale province, Turkey). Tekirdağ Ziraat Fakültesi Dergisi, 20 (1): 197-210. https://doi.org/10.33462/jotaf.1121863

Öztürk, H. (2007). Küresel ısınmada ruminantların rolü. Veteriner Hekimler Derneği Dergisi, 78 (1): 17-22.

Robertson, K., Symes, W., & Garnham, M. (2015). Carbon footprint of dairy goat milk production in New Zealand. Journal of Dairy Science, 98 (7): 4279-4293. https://doi.org/10.3168/jds.2014-9104

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., & de Haan, C. (2006). Livestock's long shadow: environmental issues and options. FAO, Rome, Italy.

TUIK, Türkiye İstatistik Kurumu (2023, September 9). https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr

Türkeş, M. (2000). Hava, iklim, şiddetli hava olayları ve küresel ısınma. T.C. Başbakanlık Devlet Meteoroloji İşleri Genel Müdürlüğü: 187-205.

Yaylı, B., & Kılıç, İ. (2020). Süt sığırı işletmelerinin küresel ısınma potansiyelinin Tier-1 yöntemi ile tahminlenmesi. Uluslararası Biyosistem Mühendisliği Dergisi, 1 (2): 79-86.

Medit Vet J, 9(2): 311-316, 2024

Research Article

Intramammary ozone therapy in Candida mastitis

Afşin Köker[1]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words:
cattle
fungus
mastitis
ozone
yeast

Received : 8 May 2024
Revised : 4 July 2024
Accepted : 12 July 2024
Published : 31 August 2024
Article Code : 1480764

Correspondence:
A.KÖKER
(afsinkoker@mehmetakif.edu.tr)

ORCID
A.KÖKER : 0000-0001-6580-1309

ABSTRACT

This study investigated the effect of intramammary ozonated distilled water on treatment success rates for mastitis caused by Candida spp., which resulted from intensive antibiotic use. The study material involved 60 Holstein udder quarters infected with Candida spp., which were divided into an ozone treatment group (n=30) and a control group (n=30). To conduct the study, 25 µg/ml of ozonated distilled water was applied intramammary to each udder quarter from which the causative agent had been isolated. Milk samples were collected on days 6 and 18 after application, and microbiological and mycological analyses were performed on them. The analysis results showed no statistically significant difference in recovery rates between days 0-6 after treatment, but a significant difference emerged between days 6-18 ($P<0.05$). A significant statistical difference also existed in the overall recovery rates between the ozone and control groups ($P<0.05$). Consequently, the conclusion drawn was that utilizing ozonated distilled water may prove effective for treating mastitis caused by Candida spp. and may increase recovery rates.

INTRODUCTION

Mastitis denotes an inflammatory reaction occurring in mammary tissue, typically triggered by microorganisms or physical trauma. It is widely acknowledged as a prevalent issue in dairy farming, leading to notable economic losses attributed to diminished milk yield and quality (Cheng & Han, 2020). Regarding the etiology of udder infections in cattle, bacteria stand as the primary causative agents, with viruses, mycoplasma, algae, yeast, and fungi being less frequently implicated (Costa et al., 2012). While bacterial infections are most commonly associated with mastitis, studies have shown that the prevalence of yeast-induced mastitis varies from 1% to 12%. However, in specific herds, this prevalence may exceed 50% (Kirk et al., 1986).

In several research studies, mycotic infections of the mammary gland induced by yeast are predominantly attributed to Candida spp. Instances of mastitis infections resulting from Candida spp. have been documented in animals over an extended period. The earliest documentation of mycotic mastitis caused by Candida spp. dates back to Fleischer in 1930 (Du Pereez, 2000; Sartori et al., 2014). Additionally, yeast species such as Cryptococcus, Rhodotorula, and Trichosporum have also been implicated in mastitis among dairy cattle (Costa et al., 2012).

Yeasts are commonly found in humid environments abundant in organic materials and can be easily identified on teats and milking equipment (Akdouche et al., 2014). Prolonged use of antibiotics can lead to a decrease in vitamin A levels, consequently compromising the integrity of the mammary epithelium. This, in turn, facilitates the invasion of fungi and yeasts into the mammary glands (JasmMohammed and Yassein, 2020; Kalinska et al., 2017). Additionally, inadequate sanitation in the milking parlor and with milking equipment, improper handling of the cannulas used for intramammary treatment, and insufficient hygiene practices can result in severe yeast and fungal infections (Abd-El Razik et al., 2011).

While spontaneous recovery is possible in mastitis caused by fungi and yeasts, persistent yeast or fungal infections can endure for 6 to 12 months (Bourtzi-Hatzopoulou et al., 2003). Antifungal medications and supportive treatment approaches are typically utilized to manage mastitis resulting from yeast and fungi. Moreover, implementing management protocols and culling chronically infected animals can aid in preventing outbreaks (da Costa et al., 2012).

Diverse alternative therapeutic modalities have been employed for the treatment of both clinical and subclinical mastitis. These modalities encompass ozone therapy, acupuncture, saline, glyoxylate, homeopathic remedies, and the use of lactobacilli (probiotics). Among these options, ozone therapy has emerged as one of the more favored approaches. Ozone possesses bactericidal, fungicidal, and virucidal properties due to its oxidative effects on microorganisms (Sciorsci et al., 2020). However, it is noteworthy that yeasts and fungi exhibit greater resistance to ozone compared to bacteria (Varga & Szigeti, 2016).

To cite this article:
Köker A. Intramammary ozone therapy in Candida mastitis. Medit Vet J. 2024; 9(2): 311-316.

The present study aims to investigate the efficacy of intramammary administration of ozonated distilled water in the treatment of Candida spp.-induced mastitis in dairy cows with a history of mastitis due to intensive antibiotic usage. The study will evaluate the therapeutic outcomes based on California Mastitis Test (CMT) scores and mastitis type following the ozone therapy intervention.

MATERIALS and METHODS

Animal

The study was conducted on 240 Simmental cows aged 2-7 years in 2012-2014 province, Burdur. Medical records indicated the intensive use of systemic and intramammary antibiotic therapy for the treatment of clinical mastitis cases, with an average of 45 days elapsing between the antibiotic treatment and the commencement of the present study. Subsequent to bacteriological and mycological analyses, the animal material comprised 60 udder quarters (from 38 Simmental cows) exhibiting clinical or subclinical mastitis.

Collection of milk samples

Prior to sample collection, the teats were cleaned with 70% ethanol solution. After discarding the initial few streams of milk into a strip cup, milk samples were aseptically collected into sterile 10 ml syringes. All milk samples were properly labelled and transferred to the laboratory for microbiological and mycological analyses under cold chain conditions.

Mastitis determination and California Mastitis Test

The presence of clinical mastitis was assessed based on symptoms such as redness, edema, pain, induration in the udder, and the presence of visually abnormal milk. Udder quarters exhibiting any of these signs were recorded as clinical mastitis cases. Two ml of milk was sampled from each udder quarter. The California Mastitis Test (CMT) was performed according to the standard procedure, and results were scored as +1, +2, +3, +4, or +5 following the Scandinavian system (Klastrup, 1975).

Microbiological and mycological analyzes

Udder quarters milk samples with clinical mastitis, CMT>+1 score and Candida spp. growth were included in the study. Each milk sample was inoculated onto sheep blood agar and MacConkey agar and incubated at 37°C for 48 hours. Standard biochemical methods and tests were employed for the isolation and identification of bacterial isolates (Quinn, 2011). For mycological culture, each milk sample was inoculated in duplicate onto Sabouraud's dextrose agar containing 0.05 mg/ml chloramphenicol and incubated at 37°C and 25°C for 3-5 days. Yeast isolates were identified based on morphological and microbiological characteristics. The standard procedure was followed for the isolation and identification of Candida spp. (Jasm Mohammed & Yassein, 2020).

Groups and application of intramammary ozonated distilled water

The study comprised two separate groups: the ozone treatment group (n=30 udder quarters) and the control group (n=30 udder quarters). In both groups, the mammary quarters were completely milked out prior to the treatment applications. For the ozone group, 25 µg/ml ozonated distilled water (100 ml) was administered intramammarily at 12-hour intervals for a duration of 3 days. Similarly, in the control group, only non-ozonated distilled water (100 ml) was administered intramammarily.

Ozone production

A mobile ozone generator (Medozon Compact, Germany) was transported to the study site on the scheduled treatment days. Subsequently, 5 liters of distilled water were added to the bubbler accessory of the device, and the ozone generator was activated. The distilled water was ozonated at a concentration of 100 µg/ml for a total duration of 5 minutes under manometer control. As a result of the ozonation process, a gas/liquid mixture was prepared at a concentration of 25 µg/ml (Vertini, 2004). The intramammary administration of ozonated water was completed within an average of 15 minutes.

Statistical analyzes

Statistical analyses were performed using the Minitab 16 software package. The chi-square test was employed to evaluate differences in CMT scores and recovery rates between the groups. Statistical significance was set at the cut-off point as $P<0.05$.

RESULTS

Microbiological and mycological analysis

According to the results of microbiological and mycological analyses of CMT-positive milk samples, Candida spp. was isolated in combination with coagulase-negative staphylococci (CNS), S. aureus, or Streptococcus spp. in 7 udder quarters. In the remaining 60 mammary quarters, pure Candida spp. was isolated. The study focused solely on the 60 mammary quarters from which Candida spp. was isolated.

CMT scores and clinical presentation

The CMT score of +1 was considered negative for mastitis, while scores of +2, +3, +4, and +5 were considered positive. Subclinical Candida spp. mastitis was identified in 26 isolated mammary quarters, and clinical mastitis was present in 34 mammary quarters. Mammary quarters with clinical symptoms and CMT score +4 and higher were considered as clinical mastitis. The distribution of Candida spp.-isolated mammary quarters according to CMT scores is presented in Table 1. Mild redness, increased body temperature, and coagulation-like structures were observed in the udder quarters diagnosed with clinical mastitis. There was no significant difference in the incidence of subclinical and clinical mastitis based on CMT scores ($P>0.05$).

Recovery rates

The gold standard diagnostic method for mastitis is microbiological analysis. For this purpose, milk samples that yielded negative results in the microbiological analysis were considered indicative of recovery. Recovery rates increased at both

sampling time points in the ozone and control groups. However, no statistical difference in recovery rates was observed between the two groups after 6 days and 18 days of treatment. group. However, according to the data obtained at the end of the study, a statistically significant difference in recovery rates was found in the group with the CMT score of +5 (P<0.05).

Table 1. Distribution of mastitis according to CMT scores

	CMT score	Number of isolated udder quarters
Subclinical mastitis[a]	2	10
	3	16
Clinical mastitis[a]	4	15
	5	19

Statistical analysis was performed within the columns only. Different letters indicate statistically significant difference.

Table 2. A total number of healed udder quarters on the 0-6. and 6-18 days and at the end of the study by groups

Groups	Number of negative udder quarters on the 6. day after treatment	Number of negative udder quarters on the 18. day after treatment	Total number of negative udder quarters (Healing rate)
Ozone group	14[a]	9[a]	23[a] (%76,67)
Control group	8[a]	3[b]	11[b] (%36,67)

Statistical analysis was performed within the columns only. Different letters indicate statistically significant difference.

Table 3. Number of microorganism negative udder quarters on days 6 and 18 according to groups and mastitis scores

Distribution of groups according to CMT scores	CMT scores	Pre-treatment Candida (+)	0-6. days Candida (-)	6-18 days Candida (-)	Total Candida (-)
Ozone group	2	6	3	2	5
Control group		4	0	1	1
Ozone group	3	9	4	2	6
Control group		7	3	0	3
Ozone group	4	6	1	2	3
Control group		9	1	1	2
Ozone group	5	9	6	3	9[a]
Control group		10	4	1	5[b]

Statistical analysis was performed within the columns only. Different letters indicate statistically significant difference.

There was a significant difference in recovery rates between the two sampling time points (P<0.05). Additionally, a significant statistical difference was found in the overall recovery rates between the ozone and control groups (P<0.05) (Table 2). Recovery rates according to treatment groups and are presented in Table 3. When evaluating recovery rates based on CMT scores, the causative pathogen was not detected in almost all udder quarters across the groups. In fact, during the study period of 0-6 days and 6-18 days, complete recovery was not observed in either the ozone-treated group or the control

DISCUSSION

Yeasts are considered opportunistic pathogens of the mammary tissue. The types of mastitis caused by yeasts are closely related to environmental hygiene (Akdouche et al., 2014). The excessive use of antibacterial drugs (Crawshaw et al., 2005), contaminated antibiotic solutions, infected syringes, or other materials used in intramammary treatment may predispose the mammary tissue to yeast proliferation (Zaragoza et al., 2011). Additionally, the mammary defense mechanisms may be compromised due to potential immunosuppressive effects (Bekele

et al., 2019). Mycotic mastitis is classified into primary mycotic mastitis and secondary mycotic mastitis based on its mechanism of occurrence (Akdouche et al., 2014). In the present study, the yeast infections were defined as secondary mycotic mastitis. It was postulated that the resulting mastitis could have occurred due to the prolonged duration of antibiotic treatment or as a consequence of inappropriate manipulations within the mammary canal.

While bacteria are frequently reported as causative agents in mastitis cases, infections caused by yeasts and fungi are less common. However, these prevalence rates can vary according to farm conditions (Tel et al., 2009). In a study investigating the microorganisms responsible for mastitis, bacteria were identified in 64.10%, a combination of bacteria and yeast in 34.62%, and yeast alone in 1.28% of the 78 CMT-positive milk samples collected from 400 cows. In a separate study, Candida spp. was detected in 29.35% of a different herd experiencing a yeast mastitis outbreak due to inadequate hygiene conditions.

Onwuhafua et al. (2018) identified only 12.3% fungal isolates from 300 cows with subclinical mastitis across 26 dairy farms in Nigeria. As can be inferred from these studies, the prevalence of mastitis caused by yeasts and fungi can vary substantially depending on farm and hygiene conditions. The rates of yeast or fungal isolates reported in these studies are quite low compared to the findings of our study. This discrepancy can be attributed to the concurrent bacterial factors causing a relative decrease in the rate of yeast mastitis in those herds. In our study, the etiology of yeast-induced mastitis was associated with long-term antibiotic usage. Consequently, almost all of the isolated microorganisms consisted of Candida spp. Infections can sometimes occur as mixed infections originating from both yeasts and bacteria, while at other times, pure yeast colonies are observed (Crawshaw et al., 2005; Costa et al., 1993; Dudko et al., 2010; Zaragoza et al., 2011).

Candida spp. is the most frequently isolated species from mycotic isolates in different studies (Bourtzi-Hatzopoulou et al., 2003; Czernomysy-Furowicz et al., 2008). However, it is possible to encounter diverse yeast and fungal species in dairy herds (JasmMohammed and Yassein 2020). Various yeast species, such as Candida, Cryptococcus, Rhodotorula, and Trichosporon spp., have been associated with mastitis in dairy cows (Akdouche et al., 2014). Du et al. (2018) collected 482 milk samples from cows with clinical mastitis across 4 different herds, and microorganisms were isolated in 256 of these samples. A total of 60 isolates belonging to nine different Candida species were detected in 23.44% of these samples. In our study, all the yeast agents isolated were identified as Candida spp. The reason for this predominance is that Candida was likely transmitted throughout the herd via milking equipment, udder tubes used for intramammary applications, and other environmental contaminants during the long-term antibiotic treatment period.

Milanov et al. (2014) identified the presence of bacteria (73.49%) and yeasts (6.02%) in cows exhibiting both clinical and subclinical mastitis. Costa et al. (2012) reported the detection of Candida yeasts at a rate of 29.35% in a farm undergoing a yeast mastitis outbreak in Brazil and noted that the mastitis type comprised 6.8% clinical and 30.2% subclinical cases when considering all causative factors. Indeed, the manifestation of clinical symptoms in yeast and fungal mastitis might be linked to the causative agents or their virulence factors (Sukumar and James, 2012). Similarly, in the present study, both clinical and subclinical yeast mastitis were observed, yet no statistically difference was discerned between the two mastitis types. In most clinical instances, mastitis induced by yeasts and yeast-like fungi presents with udder induration and watery or coagulated milk (Sukumar and James, 2012; Şeker, 2010). In certain cases, systemic symptoms may also manifest (Bourtzi-Hatzopoulou et al., 2003). In our investigation, akin symptoms were observed in clinical cases, albeit no systemic issues were noted. The extensive antibiotic and supportive treatment administered 45 days prior to the study commencement is presumed to have mitigated more severe clinical disorders in the udder and milk.

Antifungal medications are commonly employed in the treatment of yeast mastitis. Furthermore, the utilization of probiotics and bioactive natural compounds in therapies has shown promising outcomes (Abd-El Razik et al., 2011). It is recognized that yeasts exhibit varying degrees of sensitivity and resistance to antifungal drugs, including miconazole, ketoconazole, amphotericin B, itraconazole, nystatin, fluconazole, fusidic acid, and voriconazole. Studies have indicated that intramammary administration of miconazole at a dosage of 100 mg/50 ml can yield effective results; however, systemic administration may lead to hepatic and cardiovascular side effects (Asfour et al., 2021). Nonetheless, numerous antifungal drugs can be toxic to udder tissue (Du Preez, 2010). In contrast, the ozone employed in this study did not induce any toxic or systemic adverse effects in the animals.

The medical application of ozone relies on its antioxidant, immunostimulant, and antimicrobial properties. Particularly, when ozone is administered to exert its antibacterial, antiviral, antifungal, anti-yeast, and antiprotozoal effects, no residue is left in tissues and biological fluids post-application (Sciorsci et al., 2020). Ozone has demonstrated effectiveness in treating various forms of mastitis in recent years. Studies have aimed to enhance healing rates, reduce bacterial load, improve clinical symptoms, and decrease the number of somatic cells in bacterial mastitis cases (Ogata and Nagahata, 2000; Jo et al., 2005). However, there is a scarcity of studies investigating the use of ozone in treating yeast mastitis. Research concerning yeast and contamination primarily focuses on food products and storage conditions.

Numerous studies have explored ozone application using various methods and doses. Jo et al. (2005) utilized ozonated oil in cows with chronic mastitis, observing a decrease in somatic cell counts in the ozonated groups, although no statistical difference was found compared to the control group. Conversely, Sertkol et al. (2018) investigated the efficacy of intramammary ozone gas application on bacterial mastitis, concluding that it had no curative effect in acute clinical mastitis caused by Streptococcus spp. and yeast. Enginler et al. (2015) assessed the effectiveness of different doses of ozone gas in combination with antibiotics in cows with clinical

mastitis. They found that ozone insufflation was effective in treating clinical mastitis, with the best results achieved in the group using high-dose ozone (70 µg/mL) alongside antibiotic treatment. In our study, we administered a volume of 100 ml of ozonated water into the mammary lobe, aiming to cover the majority of the glandular area in the udder and thereby achieve effective treatment. The ineffectiveness of ozone on yeast forms observed in other studies may be attributed to the ozone mixture administered in gas form failing to reach a sufficient concentration in all glandular tissues of the udder. Hassan et al. (2016) conducted ozone fumigation on yeast and fungal isolates collected from buffaloes with mycotic mastitis. They demonstrated that low-dose (20ppm) ozone application against Fusarium and Candida spp among fungal isolates effectively reduced fungal growth in a short time. Similar results were obtained in our study concerning mycological recovery against Candida spp. The homogeneous distribution of ozonated water in the udder is presumed to have contributed to this outcome. It can be inferred that an adequate amount of ozone was reached in the isolate in our study, conducted in both the culture medium and tissue.

Recovery rates in yeast mastitis are closely intertwined with the type of causative agent. Yeasts inherently exhibit greater resistance to ozone compared to bacteria, owing to their thicker cell walls. They are also more susceptible to ozone than fungi (Varga and Szigeti, 2016). Moreover, varying sensitivities between yeast species have been reported. Debaryomyces spp. are noted to be more prevalent than other species such as (Vallone and Stella, 2014; Varga and Szigeti, 2016). The isolation of Candida spp. in the study conferred an advantage in treatment.

CONCLUSION

In conclusion, intramammary application of ozonated distilled water did not impact Candida spp. It has been deduced that this method is effective in treating mastitis as it enhances recovery rates and presents itself as an alternative to antibiotic use. Additionally, ozone does not cause harm to intramammary structures, rendering it safe for intramammary applications.

DECLARATIONS

Ethics Approval

The experimental procedures were approved by the Committee of Animal Experiments of Burdur Mehmet Akif Ersoy University. Approval number:MAKU-HADYEK 2013/35

Conflict of Interest

The author declare that they have no conflict of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: A.K.

Data collection and analysis: A.K.

Drafting of the manuscript: A.K.

Critical review: A.K.

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

Acknowledgements

Not applicable.

REFERENCES

Abd El-Razik, K.A., Abdelrahman, K.A., Abd El-Moez, S., & Danial, E.N. (2011). New approach in diagnosis and treatment of bovine mycotic mastitis in Egypt. African Journal of Microbiology Research, 5(31), 5725-5732. https://doi.org/10.5897/AJMR11.1200

Akdouche, L., Aissi, M., Zeina, S., & Saadi, A. (2014). Importance of yeast in the mammary infection of the cattle in the Region of Sidi M'Hamed Ben Ali, Wilaya of Relizane, Algeria. Journal of Veterinary Science Technology, 5(2), 1-4. https://doi.org/10.5897/JMA2014.0300

Asfour, H.A.E., Eid, R.H., El-Wakeel, S.A., Behour, T.S., & Darwish, S.F. (2021). Phenotypic and genotypic investigation of yeast species associated with bovine subclinical mastitis with a special reference to their virulence characteristics. International Journal of Veterinary Science, 10.47278/journal.ijvs/2021.071

Barasch, A., & Griffin, A.V. (2008). Miconazole revisited: new evidence of antifungal efficacy from laboratory and clinical trials. Future Microbiology,3(3), 265-269. https://doi.org/10.2217/17460913.3.3.265

Bekele, T., Lakew, M., Terefe, G., Koran, T., Olani, A., Yimesgen, L., Tamiru, M., & Demissie, T. (2019). Study on bovine mastitis with isolation of bacterial and fungal causal agents and assessing antimicrobial resistance patterns of isolated Staphylococcus species in and around Sebeta town, Ethiopia. African Journal of Microbiology Research, 13(1), 23-32. https://doi.org/10.5897/AJMR2018.8909

Bourtzi-Hatzopoulou, E., Zdragas, A., Petridou, E., & Filiousis, G. (2003). Yeast as a causative agent of bovine mastitis in Greece. Journal Of The Hellenic Veterinary Medical Society, 54(2), 105-110. https://doi.org/10.12681/jhvms.15257

Cheng, W.N., & Han, S.G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments- A review. Asian-Australasian Journal of Animal Sciences, 33(11), 1699-1713. https://doi.org/10.5713/ajas.20.0156

Costa, E.O., Gandra, C.R., Pires, M.F., Coutinho, S.D., Castilho, W., & Teixeira, C.M. (1993). Survey of bovine mycotic in dairy herds in the State of Sao Paulo, Brazil. Mycopathologia, 124(1), 13-17. https://doi.org/10.1007/BF01103051

Crawshaw, W.M., MacDonald, N.R., & Duncan, G. (2005). Outbreak of Candida rugosa mastitis in a dairy herd after intramammary antibiotic treatment. The Veterinary Record, 156(25), 812-813. https://doi.org/10.1136/vr.156.25.812

Czernomysy-Furowicz, D., Karakulska, J., & Silecka, A. (2008). Etiological agents of mastitis in dairy cows on farm

in the west Pomeranian region. Acta Scientiarum Polonorum Zootechnica, 7(1), 3-10.

da Costa, G.M., de Padua Pereira, U., Souza-Dias, M.A.G., & da Silva, N. (2012). Yeast mastitis outbreak in a Brazilian dairy herd. Brazilian Journal of Veterinary Research and Animal Science, 49(3), 239-243. https://doi.org/10.11606/issn.1678-4456.v49i3p239-243

Du, J., Wang, X., Luo, H., Wang, Y., Liu, X., & Zhou, X. (2018). Epidemiological investigation of non-albicans Candida species recovered from mycotic mastitis of cows in Yinchuan, Ningxia of China. BMC Veterinary Research, 14(1), 1-9. https://doi.org/10.1186/s12917-018-1564-3

Du Preez, J.H. (2000). Bovine mastitis therapy and why it fails. Journal of the South African Veterinary Association, 71(3), 201-208. https://doi.org/10.4102/jsava.v71i3.714

Dudko, P., Kostro, K., & Kurpisz, M. (2010). Adaptation of microstix®-candida slide-test for diagnosis of bovine mastitis due to anascogenic yeasts. Acta Veterinaria Brno, 79(1), 113–120. https://doi.org/10.2754/avb201079010113

Enginler, S.Ö., Sabuncu, A., Kahraman, B.B., Koçak, Ö., Yıldar, E., & Güzel, Ö. (2015). Comparison of intramammary ozone administration doses in dairy cows with clinical mastitis. Acta Scientiae Veterinariae, 43, 1-7.

Hassan, A.A. (2016). Comparative Effect of Ozone and Traditional Antimycotic Drugs on the Growth of Some Fungal Causes of Recurrent Mastitis in Egyptian Buffaloes. International Journal of Current Microbiology and Applied Sciences, 5(12), 819-834. https://doi.org/10.20546/ijcmas.2016.512.091

JasmMohammed, S., & Yassein, S.N. (2020). Characterization of some virulence factors of candida albicans isolated froom subclinical bovine mastitis. Plant Archives, 20(1), 238-242.

Jo, S.N., Liu, J., Lee, S.E., Hong, M.S., Kim, D.H., Kim, M.C., Cho, S.W., & Jun, M.H. (2005). A Therapeutic Effect of Ozonated Oil on Bovine Mastitis. Journal of Veterinary Clinics, 22(4), 318-321.

Kalinska, A., Gołebiewski, M., & Wójcik, A. (2017). Mastitis pathogens in dairy cattle – a review. World Scientific News, 89(2), 22-31.

Kirk, J.H., Bartlett, P.C., & Newman, J.P. (1986). Candida mastitis in a dairy herd. Comp Cont Ed Pract Vet, 8:F150-F152.

Klastrup, O. (1975). Scandinavian recommendations on examination of quarter milk samples. In: Dodd, F.H. (Ed.), Proc. IDF Seminar on Mastitis Control. Int. Dairy Fed. document 85, Brussels 49-52.

Milanov, D., & Velhner, M. (2014). Bojkovski JA. Diagnosis of yeast mastitis ın dairy cows. Lucrări Ştiinţifice Medicină Veterinară, 47(1):56-63.

Ogata, A., & Nagahata, H. (2000). Intramammary Application of Ozone Therapy to Acute Clinical Mastitis in Dairy Cows. Journal of Veterinary Medical Science, 62(7), 681-686. https://doi.org/10.1292/jvms.62.681

Onwuhafua, E.U., Kwaga, P.J.K.P., & Bale, J. (2018). Diversity of yeasts associated with bovine subclinical mastitis in periurban dairy farms in Kaduna metropolis, Kaduna state, Nigeria. International Journal of Infectious Diseases, 73, 285. https://doi.org/10.1016/j.ijid.2018.04.4066

Quinn, P.J., Markey, B.K., Leonard, F.C., Fitzpatrick, E.S., Fanning, S., & Hartigan, P.J. (2011). Veterinary Microbiology And Microbial Disease. 2nd ed. West Sussex: Wiley-Blackwell.

Sartori, L.C.A., Santos, R.C., & Marin, J.M. (2014). Identification of Candida species isolated from cows suffering mastitis in four Brazilian states. Arquivo Brasilerio de Medicina Veterinaria e Zootecnia, 66(5), 1615-1617. https://doi.org/10.1590/1678-7576

Sciorsci, R.L., Lillo, E., Occhiogrosso, L., & Rizzo, A. (2020). Ozone therapy in veterinary medicine: A review. Research in Veterinary Science, 130, 240-246. https://doi.org/10.1016/j.rvsc.2020.03.026

Seker, E. (2010). Identification of Candida species isolated from bovine mastitic milk and their in vitro hemolytic activity in Western Turkey. Mycopathologia, 169, 303–308. https://doi.org/10.1007/s11046-009-9255-z

Sertkol, C., Sarıbay, M.K., & Cantekin, Z. (2018). Recovery Effect of Intramammary Ozone Therapy for Acute Clinical Mastitis in Dairy Cows. Fırat Üniversitesi Sağlık Bilimleri Dergisi, 32(3), 285-190.

Sukumar, K., & James, P.C. (2012). Incidence of Fungal Mastitis in Cattle. Tamilnadu Journal of Veterinary and Animal Sciences, 8(6), 356-359.

Tel, O.Y., Keskin, O., Zonturlu, A.K., & Arserim Kaya, N.B. (2009). Şanlıurfa Yöresinde Subklinik Mastitislerin Görülme Oranı, Aerobik Bakteri İzolasyonu ve Duyarlı Antibiyotiklerin Belirlenmesi. Fırat Üniversitesi Sağlık Bilimleri Dergisi, 23(2), 101-106.

Vallone, L., & Stella, S. (2014). Evaluation of antifungal effect of gaseous ozone in a meat processing plant. Italian Journal of Food Safety, 3(2), 1680, 111-113. https://doi.org/10.4081/ijfs.2014.1680

Varga, L., & Szigeti, J. (2016). Use of ozone in the dairy industry: A review. Society of Dairy Technology, 69(2), 157-168. https://doi.org/10.1111/1471-0307.12302

Vertini, F. (2004). Efficia terapeutica dell'ossigeno-ozonoterapia per il trattamento della mastite bovina. Rivista Italiana di Ossigeno-Ozonoterapia, 3, 81-86.

Zaragoza, C.S., Olivares, R.A.C., Watty, A.E.D., de la Pena Moctezuma, A., & Tanaca, L.V. (2011). Yeasts isolation from bovine mammary glands under different mastitis status in the Mexican High Plateu. Revista Iberoamericana de Micología, 28(2), 79-82. https://doi.org/10.1016/j.riam.2011.01.002

Medit Vet J, 9(2): 317-325, 2024

Research Article

Investigation of seroprevalence of small ruminant lentivirus infections in Erzurum province of Türkiye and determination of individual and environmental variables

Mehmet Özkan Timurkan[1], Aykut Erol[2], Hayrunnisa Bostan Yörü[3], Yasin Kalay[3,4], Sebahattin Akça[3,5]

[1]Department of Virology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Türkiye
[2]Faculty of Veterinary Medicine, Atatürk University, Erzurum, Türkiye
[3]Department of Virology, Health Science Institute, Atatürk University, Erzurum, Türkiye
[4]Department of Laboratory and Veterinary Health, Horasan Vocational College, Atatürk University, Erzurum, Türkiye
[5]Erzurum Veterinary Control Institute, Ministry of Agriculture And Forestry, Republic of Türkiye, Erzurum, Türkiye

Key Words:
ELISA
epidemiology
seroprevalence
small ruminant lentiviruses
Türkiye

Received : 01 June 2024
Revised : 10 July 2024
Accepted : 05 August 2024
Published : 31 August 2024
Article Code : 1494117

Correspondence:
MO. TİMURKAN
(motimurkan@atauni.edu.tr)

ORCID
MO. TİMURKAN : 0000-0002-0458-7887
A. EROL : 0009-0004-5370-0286
H. BOSTAN YÖRÜ : 0009-0006-7631-6601
Y. KALAY : 0000-0003-1245-1450
S. AKÇA : 0009-0008-1632-6060

ABSTRACT

Small ruminant lentiviruses (SRLVs) are chronic, incurable, and vaccine-free viral diseases that cause respiratory problems and nervous disorders and yield losses in sheep and goats. Caprine arthritis encephalitis virus in goats and maedi-visna virus in sheep have been named as SRLVs. This study aimed to determine the epidemiological status of SRLV infection in Erzurum province and to evaluate the risk factors of the disease based on breed, age, and sex. For this purpose, 204 animals including 184 sheep (Akkaraman, Morkaraman, and Hemşin breeds) and 20 goats (Anatolian Black goats) from 8 districts of Erzurum province (Aşkale, Hınıs, Horasan, Karaçoban, Palandöken, Pasinler, Pazaryolu, and Tekman) were included in the sample. Commercial antibody-ELISA kit was used to determine the seroprevalence of SRLV and 15.12% seropositivity was detected. In terms of SRLV, 14.67% of females and 20% of males were positive. In terms of breed, 20%, 13.76%, 0%, and 15% seropositivity was determined in Akkaraman, Morkaraman and Hemşin breed sheep and Anatolian Black goats, respectively. Although there was no statistically significant difference in terms of breed groups and sex, the detection rates in the districts were significant. In conclusion, the prevalence of SRLV infection was investigated in 8 locations of Erzurum province, which is one of the important centers of animal breeding and where small ruminant breeding is at a high level, and significant findings were obtained at the district level. With this study, updated data on seroprevalence of SRLV in the region were obtained and a broader perspective was tried to be provided by comparing with other SRLV studies in Türkiye and the world. These findings are important in terms of evaluating the prevalence and transmission risks of SRLV infections in the region and will shed light on future control and prevention strategies.

INTRODUCTION

Sheep farming plays a strategic role for the economy and agricultural sector of many countries as one of the main sources of meat and milk production on a global scale. Compared to other species raised for economic purposes, sheep breeding comes to the forefront due to some reasons such as being content with variables such as feed, environment, etc., being resistant to adverse environmental conditions, and being able to utilize meadows and pastures with low yields better than cattle (Gunes and Akin, 2017). Sheep breeding is a continuous activity that provides various products such as meat, milk, fleece, leather, fertilizer, etc. (Karadas, 2018; Yılmaz, 2019). While pork and beef have a significant share in red meat production in the world, red meat production and consumption in Türkiye is mainly based on beef and mutton (FAO 2023). Türkiye ranks first in Europe and seventh in the world with 44 million sheep and 11 million goats (FAO 2023; TUIK 2023). Erzurum is an important center for sheep breeding in Türkiye and is one of the provinces with the highest small ruminant population in the country with approximately 877 thousand sheep and goats (TUIK 2023).

As in every sector, there are some problems in small ruminant breeding. These problems are largely related to breeding and health. Among the health problems, especially infections have an important place. In small ruminants, especially infectious diseases (bacterial, parasitic, viral infections, etc.) increase their importance due to reasons such as the ease of transmission, herd problems, and lack of treatment for most of them. Among the viral diseases of small ruminants; bluetongue, border disease, sheep-goat pox, and small ruminant lentivirus (SRLV) infections are species-specific infections, while foot and mouth, rift valley fever, akabane, Schmallenberg virus infections are important viral infections that cause infections in both small and large ruminants. Among these infections, SRLV infections are distinguished from other viral infections since they do not have a vaccine, cause slow infections and remain persistent in the host for life (de Andrés et al., 2005).

According to the previous taxonomic nomenclature, SRLV infections included Maedi-Visna (MVV) and Caprine Arthritis Encephalitis viruses (CAEV). In 2023, in the new taxonomic classification, the viruses in the genus *Lentivirus* in the *Retroviridae* family were named as Lentivirus capartenc, the causative agent of the disease formerly known as CAEV, and Lentivirus ovivismae, the causative agent of the disease formerly known as MVV (ICTV, 2023). These viruses are known as SRLVs due to their structural, genetic and pathogenic similarities. Both viruses can infect sheep and goats. However, MVV infection

To cite this article:
Timurkan MO, Erol A, Bostan Yörü H, Kalay Y, Akça S. Investigation of seroprevalence of small ruminant lentivirus infections in Erzurum province of Türkiye and determination of individual and environmental variables. Medit Vet J. 2024; 9(2): 317-325.

is more common in sheep; whereas, CAEV infection is more common in goats (Blacklaws, 2012). The respiratory form of MVV is called as "Maedi" and its nervous form is called as "Visna". Maedi form is more common (Gomez-Lucia et al., 2018). The disease affecting the joints and nervous system in goats is known as CAEV and it causes encephalitis especially in kids and mastitis and arthritis in adult goats (Blacklaws, 2012). Small ruminant lentiviruses contain a positive single-stranded RNA chain with a diameter of 80 - 100 nm. And they contain nucleocapsid, capsid, matrix and envelope proteins from the inside out (Minguijón et al., 2015).

SRLV has a long incubation period in sheep and causes a lifelong infection without clinical signs (Pépin et al., 1998). Clinical signs include pneumonia, mastitis, arthritis and encephalitis (Kalogianni et al., 2023). Infection can lead to weight loss and death in some cases (Straub, 2004). The virus is transmitted to colostrum and milk via macrophages in the mammary gland, and lambs are infected via colostrum (Blacklaws, 2012; Gomez-Lucia et al., 2018). SRLV are slowly progressive infections and although humoral and cellular responses develop against the virus, the disease is difficult to control and eradicate because it is lifelong persistent (Oguma et al., 2014; Peterhans et al., 2004). There is no treatment or commercial vaccine for SRLV. Therefore, accurate diagnosis is fundamental to establish an optimal control program of the infection and reduce its prevalence (Straub, 2004). Currently, the prevalence of SRLVs is investigated based on cross-sectional epidemiological studies. Serological diagnosis of viruses in the family *Retroviridae* also indicates the presence of antigen.

Seroprevalence values vary between countries, animal breeds and age groups and are largely dependent on the laboratory tests used. Various tests such as indirect immunofluorescence (IIF), complement fixation test (CFT), agar gel immunodiffusion (AGID), Western blot, indirect ELISA and radio-immunoassay (RIA) are used for serological diagnosis (de Andrés et al., 2005). AGID and ELISA are recommended as the most suitable methods to detect infected animals; ELISA has higher sensitivity but lower specificity than AGID (Straub, 2004).

Due to the significant socio-economic impact of SRLVs, the World Organization for Animal Health (OIE) has included this disease in the list of notifiable animal diseases (OIE 2023). SRLV puts in danger the sustainability of sheep farms by affecting the health and welfare of animals, causing significant economic losses due to mortality and reduced milk production and lamb growth (Echeverría et al., 2020; Lipecka et al., 2010).

Looking at the national studies, it is seen that among SRLV infections, especially Maedi-Visna virus (MVV) infection has been investigated serologically more (Burgu, 1990; Ün et al., 2018). Although these infections have been studied at the geographical region and province level, a limited number of studies have been conducted in Erzurum region, which has an important share in sheep breeding. The aim of this study was to investigate SRLV infection in small ruminant farms in Erzurum, one of the provinces where sheep breeding is intensively practiced in the Eastern Anatolia Region of Türkiye, and to evaluate the epidemiology of the infection in a broader framework and to make recommendations for combating the infection. For this purpose, blood samples were collected from sheep and goat farms in various districts of Erzurum province, the disease was analyzed by ELISA method and the epidemiology of the infection was evaluated.

MATERIALS and METHODS

Study Area

Erzurum is one of the provinces with the highest altitude in Türkiye and has a harsh continental climate. This climate causes heavy snowfall in the winter months and hot and dry weather in the summer months. Erzurum is covered with snow for about 150 days of the year and this snow feeds the rivers in the region. These climatic characteristics of Erzurum create a very favorable environment for animal husbandry. Winter's heavy snowfall keeps the large plateaus and pastures supplied and ensures an abundance of the plants used as sheep feed. Erzurum's harsh continental climate supports sheep breeding activities and makes a significant contribution to the regional economy.

Materials

A total of 204 blood samples were collected from 15 sheep and goat flocks in Erzurum (Figure 1) by random sampling method. The sampled population consisted of 184 sheep (167 ewes and 17 rams) and 20 goats (17 goats and 3 bucks). In this study, individual (age, gender, breed) and environmental (Erzurum province and districts) factors of the sampled animals were also recorded. Blood samples to be used in serological tests were collected in sterile vacuum tubes and centrifuged at 3000 rpm. The sera obtained were collected in stock tubes and stored at -20 °C until testing.

Detection of SRLV antibodies by ELISA

A commercially available Pourquier ELISA Maedi-Visna/CAEV Screening (Cat. No: P00303-10, Institute Porquier, France) test kit was used for the detection of SRLV-specific antibodies. The ELISA test was performed according to the protocol recommended by the manufacturer. To obtain Optical Density (OD) data, the measurement was performed at 450 nm and OD data were calculated.

Statistical Analysis

Chi-square ($\chi 2$) test was used in IBM SPSS 29.0 statistical program for statistical evaluation of the data obtained in the study. Differences between the groups in terms of the analyzed parameters were considered as significant at level of $P<0.05$.

RESULTS

ELISA Analysis

Of the 204 sheep and goat blood sera tested, 15.12% were positive for SRLV antibodies. When analysed for two species, SRLV antibodies were detected in 28 of 184 sheep (15.2%) and 3 of 20 goats(15%). The seroprevalence of infection was 14.67% in females and 20% in males. When analysed by gender for two species, this rate was 14.37% (24/167) in ewes,

23.53% (4/17) in rams and 17.65% (3/17) in goats. No positivity was detected in bucks (0/3). Among the sheep breeds included in the study, 20% positivity was found in Akkaraman and 13.76% positivity was found in Morkaraman. The presence of specific antibodies was not observed in the Hemşin breed. The positivity rate in Anatolian Black goat was determined as 15% (Figure 1).

No positivity was detected in Pazaryolu and Hınıs districts. Table 1 shows detailed results of the distribution of the variables such as sex, breed, and district.

Statistical Analysis

When the chi-square test was performed for breed and sex variables, p values were found to be 0.32 and 0.52, respectively.

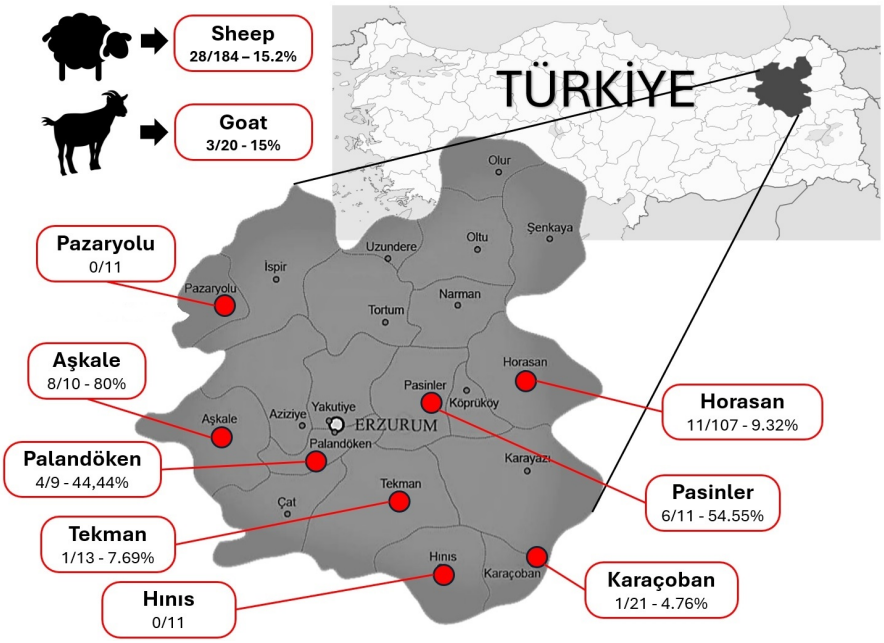

Figure 1. Geographical locations of Erzurum province and its districts in Türkiye. Red circle: The related districts and SRLV positivity rates.

Table 1. Number and positivity rate of sheep and goats by district and gender.

Districts	Sheep Positivity Rate		Goats Positivity Rate	
	Female (n=167)	**Male** (n=17)	**Female** (n=17)	**Male** (n=3)
Aşkale (n=10)	8/10 (%80)	-	-	-
Horasan (n=118)	9/98 (%9,18)	1/10 (%10)	1/9 (%11,11)	0/1
Pazaryolu (n=11)	0/11	-	-	-
Palandöken (n=9)	2/7 (%28,57)	2/2 (%100)	-	-
Hınıs (n=11)	0/10	0/1	-	-
Tekman (n=13)	1/12 (%8,33)	0/1	-	-
Karaçoban (n=21)	0/11	0/2	1/6 (%16,67)	0/2
Pasinler (n=11)	4/8 (%50)	1/1 (%100)	1/2 (%50)	-

When the districts were evaluated in terms of the presence of SRLV specific antibodies, Aşkale ranked first with 80%, followed by Palandöken and Pasinler districts with a positivity rate of 54.55%. The positivity rate was 9.32% in Horasan and 7.69% and 4.76% in Tekman and Karaçoban, respectively.

No significant correlation was found between breed and sex and SRLV antibody positivity rates. The p value was found to be 0.0001 as a result of the chi-square test for the district variable. P<0.05 indicates that there is a significant correlation between the district variable and SRLV antibody positivity.

According to the statistical results in Table 2, no significant correlation was found between seropositivity and breed and sex ($p>0.05$). However, a significant correlation was found between the district variable and seropositivity ($p<0.05$). In ad-dition, Table 2 shows the number and percentage of positive and negative animals according to individual (gender, breed) and environmental (Erzurum province and districts) variables of the sampled animals.

Table 2. Seroprevalence of SRLV according to breed, gender and districts.

Factor	Positive	Negative	Positive %	P value
Location				
Aşkale (n=10)	8	2	80.00	0,0001
Horasan(n=118)	11	107	9.32	
Pazaryolu (n=11)	0	11	0.00	
Palandöken (n=9)	4	5	44.44	
Hınıs (n=11)	0	11	0.00	
Tekman (n=13)	1	12	7.69	
Karaçoban (n=21)	1	20	4.76	
Pasinler (n=11)	6	5	54.55	
Breed				
Akkaraman(n=64)	13	51	20%	0.32
Morkaraman(n=109)	15	94	13.76%	
Hemşin (n=11)	0	11	0.00%	
Anatolian Black goat (n=20)	3	17	15%	
Gender				
Female (n=184)	27	157	14.67%	0.52
Male (n=20)	4	16	20.00%	

DISCUSSION

Small Ruminant Lentivirus (SRLV) infection occurs in most of sheep farming countries of the world except for Australia and New Zealand and causes significant economic losses (Tan and Alkan, 2002). In the case of small ruminants, economic losses are caused by decreased reproductive activity, low milk yield, postpartum litter mortality and low growth rates in seropositive sheep and goats (Arsenault et al., 2003).

In studies conducted to determine the seroprevalence of the disease in the world, different prevalence rates were found. Fevereiro (1995) found 34.3% seropositivity in 1.912 samples in Portugal, Dawson and Clarkson (1995) found 0.39% seropositivity in 14.675 blood serum samples in England, Giangaspero et al. (1993) found 6% seropositivity in 1.445 small ruminants in Syria and Cutlip et al. (1992) found 26% seropositivity in 16.827 blood serum in the USA. In Iraq, seropositivity rates were reported to be 21.82% and 5.88% in 110 blood serum samples and 68 milk serum samples obtained from sheep and goats, respectively (Mosa et al., 2024). Cana et al. (2020), obtained seropositivity rates of 34.8% for sheep and 15.6% for goats in blood serum samples collected from 5.272 sheep and 435 goats in Kosovo. In Italy, where small ruminant farming is very common, Cirone et al. (2019) reported a seropositivity rate of 18.64% in 4800 goats. In studies conducted in Greece (Kalogianni et al., 2023) and Spain (Lago et al., 2012) to determine seroprevalence, 66.5% and 24.8% positivity rates were obtained in sheep, respectively. The differences in the results of these studies may have varied depending on addition of new animals into the facilities, care strategies, hygiene and sanitation practices, and the number of animals included in the sample. Seropositivity rate was 40.9% in Europe and ranged between 16.7% and 21.8% in Africa, Asia and North America (de Miguel et al., 2021). Although SRLV infections have different detection rates around the world, the high seroprevalence values may be a result of the persistence mechanism of the virus. Due to asymptomatic and carrier sheep-goats, the virus persists in the herd and can be transmitted to new generations. The virus may be transmitted between countries through the trade of animals that have not been serologically tested. As shown in this study, the prevalence of SRLV in Türkiye is below the average of the Asian and European continents, but it is high for an infection without a vaccine. Imports are limited in the ovine livestock sector in Türkiye. Breeding is mostly done with domestic breeds and herds are not culled for seropositive animals. In this sense, seronegative animals can be imported by looking at the results of the necessary serological tests, and it can be aimed to reduce the current positivity in Türkiye and to obtain purified herds.

Many serological and virological studies on SRLVs have been conducted in Türkiye (Eroksuz et al., 2022; Karapınar et al., 2016; Muz et al., 2012). The infection was first reported

in sheep in Türkiye by Alibaşoğlu and Arda (1975) and was detected in 1984 by Girgin et al. (1987) in two imported rams through clinical, pathological and serological analysis. In the following years, studies on the presence of infection in both public and state-owned small ruminant enterprises were carried out. Burgu (1990) detected 23.9% seropositivity in 1.099 samples in 12 sheep farms across the country and found positivity in 10 of the 12 flocks they screened. In a serological study conducted by Muz et al. (2012) with blood serum samples collected from 911 sheep from 3 flocks in different regions of Türkiye, they recorded seropositivity in the flocks as 58.65%. Karapınar et al. (2016) conducted a serological examination for the presence of CAEV infection in 435 blood samples and 285 milk samples collected from goats in 4 regions (Central Anatolia, Aegean, Mediterranean, Marmara) between 2007-2010 and found its prevalence rate as 8.5% and 4.9%, respectively. SRLV was reported in all of 7 regions in Türkiye. In Erzurum in Eastern Anatolia Region, Schreuder et al. (1988) found 1.5% seropositivity in sheep. In Van, Ağrı, Hakkâri and Kars provinces, seropositivity rate was 19.76%, 16.66%, 10.5% and 16%, respectively (Ameen and Karapınar, 2018; Gezer et al., 2021). In two studies conducted in the Southeastern Anatolia region, 21.1% seropositivity was detected in Şanlıurfa (Gürçay and Parmaksız, 2013), but seropositivity was not detected in Siirt (Çelik et al., 2018). In a study conducted in the Central Anatolia region, seroprevalence rate was found to be 2.90% in Konya (Yavru et al., 2012). In the study conducted in Afyonkarahisar province in the Aegean region (Arik et al., 2015), a positivity rate of 5.70% was detected. In the Marmara region, Yilmaz et al. (2002) found a seroprevalence of 1.2% in Istanbul. In the Black Sea region, 23% positivity was determined in a study conducted in Samsun, Sinop, Ordu, Trabzon, Rize, Amasya, Tokat, and Giresun provinces (Albayrak et al., 2012). In the Mediterranean region, Doğan et al. (2021) found 9.43% seropositivity in Hatay, Kahramanmaraş and Osmaniye and Kale (2020) found 1.60% seropositivity in Burdur. In the light of all these studies, rates ranging between zero and 58.65% can be mentioned for SRLV seroprevalence in Türkiye. In this study, using the total number of sheep and goats in Erzurum province in 2023 (n=877.000) reported by TUIK, the minimum sample size was determined as 166 at confidence interval of 99% and a margin of error of 10% and a total of 204 samples from 8 districts of Erzurum were studied. The high margin of error can be attributed to the fact that some of the animals were in the barn and some in the pasture, a homogeneous sample could not be achieved from the whole herd and sampling was hardly performed from only 8 of the 20 districts in Erzurum province. SRLV seroprevalence was found to be 15.12% in the present study. In a study conducted in Erzurum province in 1988 (Schreuder et al., 1988), a seroprevalence value of 1.5% was found. Compared to a very old study, the present study both updated the epidemiologic data and determined the current prevalence of the infection. In addition, the correlation of Erzurum with studies in the geographically neighboring provinces (Rize, Trabzon, Van, Ağrı, Kars) is close to the values found in seroprevalence studies. While the highest seroprevalence value was found in Aşkale district (80%), the lowest value (0%) was found in Pazaryolu and Hınıs districts. The reason for the regional high seroprevalence of SRLV in the eastern provinces may be the seasonal migration of small ruminants and the contact of herds with more than one herd in several cities and the grazing of many herds on the same pasture. In addition, the fact that small ruminant breeders in the region do not renew the herd and positive animals live in herds for many years, lambs and kids are added to the herd and fed from the mother may also be an important factor. In order to reduce the current seroprevalence in the region and Erzurum province and to obtain pure herds, precautions such as reforming seropositive animals, adding new animals to the herd after serological controls, bottle feeding lambs and kids in separate sections, selecting breeding animals used in ram and billygoat from seronegative ones can be taken.

Studies on SRLV infection have also mentioned about breed susceptibility. A study conducted in Northern Ireland reported that Texel sheep breed was more susceptible than local breeds (Adair, 1986). In Iceland, Karakul sheep imported from Germany in 1933 for the breeding of local breeds did not show clear clinical signs of the disease, while local breeds were observed to be more susceptible (Tan and Alkan, 2002). Studies conducted in Türkiye have revealed that cultivated sheep are more susceptible to infection compared to local breeds (Yavru et al., 2002). Burgu (1990) compared the susceptibility of domestic sheep breeds to the disease and found seropositivity rates of 2.6%, 3.1% and 3.1% in Akkaraman, Morkaraman and Karakaya sheep breeds, respectively, while high rates of seropositivity of 40.5%, 64.7% and 32.5% were found in Sakız, Dağlıç and Kıvırcık breeds, respectively. In their study, Yavru et al. (2002) found seropositivity rates of 2.36%, 1.42% and 6.45% in Akkaraman, Morkaraman and Merino sheep, respectively. Kalaycı et al. (2023) also found that SRLV prevalence was higher in Pirlak sheep and Saanen goats compared to the other breeds in the study. Albayrak et al. (2012) found in their study that Amasya Herik breed sheep were more susceptible than Karakaya breed sheep, while Muz et al. (2012) reported that seropositivity was higher in Ivesi, Sakız, Dağlıç, Kıvırcık and Merino breeds. In this study, Akkaraman, Morkaraman and Hemşin breed sheep and Anatolian Black goats were studied and seroprevalence was found to be zero in Hemşin sheep. The fact that Hemşin sheep are the only sheep flock in the district where they are raised and they are not combined with other flocks in the pasture may explain the seronegativity. In the present study, seroprevalence values were found to be 20% and 13.76% in Akkaraman and Morkaraman breeds, respectively. When compared with previous studies conducted with these breeds in Türkiye, higher positivity was found. In previous studies, it was evaluated that these breeds may be resistant, but the high positivity rates determined in this study indicate that the presence of a breed resistance is unlikely to be the case, and this situation can be clarified in future studies with a larger sample size and a broader perspective that will include other provinces in the region. In addition, the difference in the regions where the study was conducted and the high rate of breeding and circulation of these two breeds in the Eastern Anatolia region are also important for the value determined. As far as known, there are no studies on the presence of the disease in Anatolian Black goats. Therefore, the present study is the first study in Anatolian Black goats at the breed level. The seroprevalence value in Anatolian Black goats was found

to be 15%. In this study, the seroprevalence value in Anatolian Black goats in Erzurum province was higher than that in sheep. Although no significant inference can be made in terms of breed disposition due to the small number of samples, the first detection of the disease in Anatolian Black goats is an important epidemiological data.

Studies conducted in terms of SRLV have investigated age-related changes in seroprevalence. Gezer et al. (2021) sampled 2- and 3-year-old sheep and found that the seropositivity rate increased with increasing age and interpreted the reason for this increase as increased exposure to the agent with increasing age. In their study, Cana et al. (2020) reported that age did not make a significant statistical difference in sheep, while the seroprevalence value in goats younger than 2 years of age was lower than that in goats younger than 4 years of age. Kalaycı et al. (2023) also obtained data supporting that infection increased with increasing age. Kalogianni et al. (2023) also reported that seroprevalence increased with increasing age. In this study, the animals included in the study were generally over 2 years old. Retroviruses, which are the causative agents of infection, cause slow persistent infection by nature. The high positivity value we found in the present study also supports the correlation with the age factor. This result supports that the seroprevalence of the disease increases proportionally with age in parallel with previous studies.

SRLV can affect both sexes in sheep and goats (Hasegawa et al., 2016). In the studies conducted by Mosa et al. (2024) in Iraq and Gezer et al. (2021) in Türkiye, they showed that sex had no effect on seroprevalence values. In this study, antibody screening of 20 male and 184 female animals yielded positivity rates of 20% and 14.67%, respectively. The presence of SRLVs in male animals is important and should be monitored regularly as it may affect the entire herd if used as breeding stock.

CONCLUSION

It also plays a significant role because Türkiye's meadows and pastures are more appropriate for sheep and goats, small ruminant meat, milk and milk products are produced there, their wool is used, they provide raw materials for industries, and the breeding of sheep and goats increases employment (Benli and Kandemir, 2019; Yazgan et al., 2018). SRLV infections, which are common in small ruminant herds all over the world and in Türkiye, are important due to the econom-ic losses they cause (Carrozza et al., 2023). Europe has been determined as the continent with the highest seroprevalence rate for SRLV and Türkiye acts as a bridge between Asia and Europe due to its location (de Miguel et al., 2021). In Türkiye's neighboring countries (Greece, Iraq, Syria and Iran), approximately 22% positivity for these diseases was reported (Dousti et al., 2020; Giangaspero et al., 1993; Kalogianni et al., 2023; Mosa et al., 2024). However, the disease has been reported in most regions of Türkiye (Albayrak et al., 2012; Kalaycı et al., 2023; Sait and İnce, 2022). SRLV infections are infections that need to be taken into consideration due to the lack of vaccine and treatment options and the fact that they are persistent infections and can be transmitted in many ways (Minguijón et al., 2015). Due to the nature of their persistence mechanism (Trojan horse mechanism), retroviruses can persist in the infected organism for many years and therefore serologic detection can also prove their virologic presence (Bouzalas et al., 2024). For this reason, ELISA antibody test was selected as the appropriate detection method for this study and was deemed sufficient. This study, together with the seropositive sheep and goat data of SRLV infection in Erzurum province, is thought to shed light on new studies to be planned and conducted and eradication measures to be taken. Based on this study conducted in Erzurum, up-to-date data on the presence of infection in flocks were obtained. It is thought that the infection can be eliminated to a significant extent by informing animal owners and veterinarians, identifying persistently infected animals with eradication programs to be planned, and removing persistently infected sheep and goats from the herd.

DECLARATIONS

Ethics Approval

All procedures were approved by the Unit Ethics Committee, Veterinary Faculty, Atatürk University, Türkiye (Date: 27.03.2019 / No:2019/04).

Conflict of Interest

Authors do not have any conflict of interests.

Consent for Publication

Not applicable.

Competing Interest

The authors declare that they have no competing interests

Author contribution

Idea, concept and design: MOT, AE.

Data collection and analysis: AE, HBY, YK, SA.

Drafting of the manuscript: AE, HBY, YK.

Critical review: MOT.

Data Availability

Not applicable.

Acknowledgements

Not applicable.

REFERENCES

Adair, B. M. (1986). Serological surveillance for maedi-visna virus and caprine arthritis-encephalitis virus in Northern Ireland. The Veterinary Record, 118(15), 422-423. https://doi.org/10.1136/vr.118.15.422

Albayrak, H., Yazici, Z., Okur-Gumusova, S., & Ozan, E. (2012). Maedi-visna virus infection in Karayaka and Amasya Herik breed sheep from provinces in northern Turkey. Tropical Animal Health and Production, 44(5), 939-941. https://doi.org/10.1007/s11250-011-9996-9

Alibaşoğlu, M., & Arda, M. (1975). Koyun pulmoner adenomatozis'inin Türkiye'de durumu ile patolojisi ve etiyolojisinin araştırılması. Tubitak Veteriner ve Hayvancılık Araştırma Grubu Yayınları. 274 (3), 111-120.

Ameen, P. M., & Karapınar, Z. (2018). Seroprevalence of Visna-Maedi Virus (VMV) and Border Disease Virus (BDV) in Van province and around. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 70(4), 1029-1035. https://doi.org/10.1590/1678-4162-10005

Arik, C., Acar, A., & Gür, S. (2015). Afyonkarahisar İli ve Çevresinde Maedi- Visna Virus Enfeksiyonunun Klinik ve Serolojik Olarak Araştırılması. Kocaeli Veteriner Dergisi, 8(1), 39-44.

Arsenault, J., Girard, C., Dubreuil, P., Daignault, D., Galarneau, J. R., Boisclair, J., Simard, C., & Bélanger, D. (2003). Prevalence of and carcass condemnation from maedi-visna, paratuberculosis and caseous lymphadenitis in culled sheep from Quebec, Canada. Preventive Veterinary Medicine, 59(1-2), 67-81. https://doi.org/10.1016/s0167-5877(03)00060-6

Benli, T., & Kandemir, O. (2019). Kırsal Kalkınma ve Kırmızı Et Açığını Önlemede Küçükbaş Hayvancılığın Önemi: Kastamonu İli Örneğinde Sorunlar ve Kümelenmenin Uygulanabilirliği. Türk Tarım-Gıda Bilim ve Teknoloji Dergisi, 7(4), 672-683. https://doi.org/10.24925/turjaf.v7i4.672-683.2408

Blacklaws, B. A. (2012). Small ruminant lentiviruses: Immunopathogenesis of visna-maedi and caprine arthritis and encephalitis virus. Comparative Immunology, Microbiology and Infectious Diseases, 35(3), 259-269. https://doi.org/10.1016/j.cimid.2011.12.003

Bouzalas, I., Apostolidi, E. D., Scalas, D., Davidopoulou, E., Chassalevris, T., Rosati, S., & Colitti, B. (2024). A Combined Approach for the Characterization of Small Ruminant Lentivirus Strains Circulating in the Islands and Mainland of Greece. Animals, 14(7), 1119. https://doi.org/10.3390/ani14071119

Burgu, İ. (1990). Türkiye'de Vısna-Maedı Enfeksiyonunu Serolojik Olarak Araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 37(03). https://doi.org/10.1501/Vetfak_0000001331

Cana, A., Taylor, N., Honhold, N., Gjinovci, V., Osmani, A., Nardelli, S., Murati, B., Spahiu, J., Mehmetukaj, D., & Alishani, M. (2020). Sero-Prevalence Survey of Small Ruminant Lentivirus (SRLV) Infections in Kosovo. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 26(1), 1-8. https://doi.org/10.9775/kvfd.2019.22022

Carrozza, M.-L., Niewiadomska, A.-M., Mazzei, M., Abi-Said, M. R., Hué, S., Hughes, J., Gatseva, A., & Gifford, R. J. (2023). Emergence and pandemic spread of small ruminant lentiviruses. Virus Evolution, 9(1), vead005. https://doi.org/10.1093/ve/vead005

Cirone, F., Maggiolino, A., Cirilli, M., Sposato, A., De Palo, P., Ciappetta, G., & Pratelli, A. (2019). Small ruminant lentiviruses in goats in southern Italy: Serological evidence, risk factors and implementation of control programs. Veterinary Microbiology, 228, 143-146. https://doi.org/10.1016/j.vetmic.2018.11.023

Cutlip, R. C., Lehmkuhl, H. D., Sacks, J. M., & Weaver, A. L. (1992). Seroprevalence of ovine progressive pneumonia virus in sheep in the United States as assessed by analyses of voluntarily submitted samples. American Journal of Veterinary Research, 53(6), 976-979. https://doi.org/10.2460/ajvr.1992.53.06.976

Çelik, Ö. Y., Akgül, G., & İrak, K. (2018). Investigation of Seroprevalence of Maedi-Visna and Caprine Arthritis Encephalitis in Sheep and Goats in Siirt Province. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 13(3), 274 - 277. https://doi.org/10.17094/ataunivbd.348533

Dawson, M., & Clarkson. (1995). Maedi-visna: An update. Proceedings of the Sheep Veterinary Society, 18, 49-51.

de Andrés, D., Klein, D., Watt, N. J., Berriatua, E., Torsteinsdottir, S., Blacklaws, B. A., & Harkiss, G. D. (2005). Diagnostic tests for small ruminant lentiviruses. Veterinary Microbiology, 107(1-2), 49-62. https://doi.org/10.1016/j.vetmic.2005.01.012

de Miguel, R., Arrieta, M., Rodríguez-Largo, A., Echeverría, I., Resendiz, R., Pérez, E., Ruiz, H., Pérez, M., de Andrés, D., Reina, R., de Blas, I., & Luján, L. (2021). Worldwide Prevalence of Small Ruminant Lentiviruses in Sheep: A Systematic Review and Meta-Analysis. Animals, 11(3), 784. https://doi.org/10.3390/ani11030784

Doğan F, Ataseven V, Bilge Dağalp S, Ergun Y (2021). Doğu Akdeniz bölgesinde küçük ruminant lentivirus enfeksiyonlarının serolojik ve moleküler yöntemlerle araştırılması. Eurasian Journal of Veterinary Sciences, 37(3), 193 – 201.

Dousti, M., Sayyari, M., & Esmailnejad, A. (2020). Histopathological, serological, molecular and electron microscopy detection of Maedi-Visna infection in sheep population in the West of Iran. Iranian Journal of Veterinary Research, 21(2), 103-108.

Echeverría, I., De Miguel, R., De Pablo-Maiso, L., Glaria, I., Benito, A. A., De Blas, I., De Andrés, D., Luján, L., & Reina, R. (2020). Multi-Platform Detection of Small Ruminant Lentivirus Antibodies and Provirus as Biomarkers of Production Losses. Frontiers in Veterinary Science, 7, 182. https://doi.org/10.3389/fvets.2020.00182

Eroksuz, Y., Timurkan, M. O., Karabulut, B., Incili, C. A., Baydar, E., Cevik, A., & Eroksuz, H. (2022). Clinical, Pathological and Molecular Findings in Caprine Arthritis – Encephalitis Virus Infection in Damascus Goats. Acta Veterinaria, 72(3), 324-333. https://doi.org/10.2478/acve-2022-0026

Food and Agriculture Organization of the United Nations. (2023). Animal wealth rankings of countries. https://www.fao.org/Türkiye/tr/

Fevereiro, M. (1995). Prevalence of maedi-visna infection in sheep in Portugal. Revista Portuguesa de Ciências Veterinárias, 90, 66-70.

International Committee on Taxonomy of Viruses. (2024). Lentivirus Genus Taxonomy. https://ictv.global/report/chapter/retroviridae/retroviridae/ lentivirus

Gezer, T., Akyüz, E., & Gökçe, G. (2021). Investigation of Seroprevalence of Maedi-Visna Infection in some Sheep Flocks in Kars Province, Turkey. Dicle Üniversitesi Veteriner Fakültesi Dergisi, 14(1), 48-51. https://doi.org/10.47027/duvetfd.881430

Giangaspero, M., Tabbaa, D., Nishikawa, H., & Vanopdenbosh, E. (1993). Epidemiological survey of the Maedi Visna (MV) virus in Syrian Awassi sheep. Revue D'elevage Et De Medecine Veterinaire Des Pays Tropicaux, 46(3), 431-434.

Girgin, H., Aydın, N., Yonguç, A. D., Aksoy, E., & Çorak, R. (1987). Ve Şimdi Koyunların Viral Maedi-Visna 'sı Türkiye'de. Etlik Veteriner Mikrobiyoloji Dergisi, 6(1), 9-22.

Gomez-Lucia, E., Barquero, N., & Domenech, A. (2018). Maedi-Visna virus: Current perspectives. Veterinary Medicine, 9, 11-21. https://doi.org/10.2147/VMRR.S136705

Güneş, H., & Akin, P. D. (2017). Koyun Barınakları. Türkiye Klinikleri Veteriner Bilimleri-İç Hastalıkları- Özel Konular, 3(1), 1-5.

Gürçay, M., & Parmaksız, A. (2013). An investigation of Visna-Maedivirus Infection in Şanlıurfa Province, Southeast Anatolia, Turkey. Adana Veteriner Kontrol Enstitüsü Müdürlüğü Dergisi, 3(1), 46-50.

Hasegawa, M. Y., Meira, E. B. S., Lara, M. C. C. S. H., Castro, R. S., Rodrigues, J. N. M., Araújo, J., Keller, L. W., Brandão, P. E., Rizzo, H., Barbosa, M. L., Gaeta, N. C., Rossi, R. S., Durigon, E. L., & Gregory, L. (2016). Small ruminant lentivirus variants and related clinical features in goats from southeastern Brazil. Small Ruminant Research, 140, 32-36. https://doi.org/10.1016/j.smallrumres.2016.05.019

Kalaycı, G., Kaplan, M., Özkan, B., Pekmez, K., Çağırgan, A. A., Türkmen, R., & Tunalıgil, S. (2023). Epizootiological serosurvey on small ruminant lentiviruses: Prevalence, age and breed effects. Acta Veterinaria Hungarica, 71(2), 128-135. https://doi.org/10.1556/004.2023.00833

Kale, M. (2020). Honamlı keçi ırkında Caprine Arthritis Encephalitis Virus (CAEV) enfeksiyonunun araştırılması. Veterinary Journal of Mehmet Akif Ersoy University, 5(2), 58-63. https://doi.org/10.24880/maeuvfd.682590

Kalogianni, A. I., Bouzalas, I., Bossis, I., & Gelasakis, A. I. (2023). Seroepidemiology of Maedi-Visna in Intensively Reared Dairy Sheep: A Two-Year Prospective Study. Animals, 13(14), 2273. https://doi.org/10.3390/ani13142273

Karadaş, K. (2018). Koyunculuk İşletmelerinin Sosyo-Ekonomik Durumu Hakkâri İli Örneği. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 49(1), 29-35. https://doi.org/10.17097/ataunizfd.322684

Karapınar, Z., Chebloune, Y., & Burgu, İ. (2016). First Molecular Characterization of Caprine Arthritis Encephalitis Virus in Blood and Milk Samples from Goats in Turkey Based on Gag Gene Sequence Analysis. International Journal of Veterinary Science, 5(1), 10-18.

Lago, N., López, C., Panadero, R., Cienfuegos, S., Pato, J., Prieto, A., Díaz, P., Mourazos, N., & Fernández, G. (2012). Seroprevalence and risk factors associated with Visna/Maedi virus in semi-intensive lamb-producing flocks in northwestern Spain. Preventive Veterinary Medicine, 103(2), 163-169. https://doi.org/10.1016/j.prevetmed.2011.09.019

Lipecka, C., Szymanowska, A., Szymanowski, M., Junkuszew, A., Gruszecki, T. M., Kuzmak, J., & Olech, M. (2010). Milk yield and quality in sheep with maedi-visna virus. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, 6, 51-61.

Minguijón, E., Reina, R., Pérez, M., Polledo, L., Villoria, M., Ramírez, H., Leginagoikoa, I., Badiola, J. J., García-Marín, J. F., de Andrés, D., Luján, L., Amorena, B., & Juste, R. A. (2015). Small ruminant lentivirus infections and diseases. Veterinary Microbiology, 181(1), 75-89. https://doi.org/10.1016/j.vetmic.2015.08.007

Mosa, A. H., Aljabory, H. A. H., & Abady, N. (2024). Serologıcal detectıon of small rumınant lentıvırus ınfectıon ın babylon governorate, Iraq. Bulgarian Journal of Veterinary Medicine, https://doi.org/10.15547/bjvm.2023-0105

Muz, D., Oğuzoğlu, T. Ç., Rosati, S., Reina, R., Bertolotti, L., & Burgu, İ. (2012). First molecular characterization of visna/maedi viruses from naturally infected sheep in Turkey, Archives of Virology. 158(3), 559-570. https://doi.org/10.1007/s00705-012-1518-1

Oguma, K., Tanaka, C., Harasawa, R., Kımura, A., Sasakı, J., Goryo, M., & Sentsuı, H. (2014). Isolation of Maedi/Visna Virus from a Sheep in Japan. The Journal of Veterinary Medical Science, 76(2), 211-218. https://doi.org/10.1292/jvms.13-0269

Pépin, M., Vitu, C., Russo, P., Mornex, J. F., & Peterhans, E. (1998). Maedi-visna virus infection in sheep: A review. Veterinary Research, 29(3-4), 341-367.

Peterhans, E., Greenland, T., Badiola, J., Harkiss, G., Bertoni, G., Amorena, B., Eliaszewicz, M., Juste, R. A., Krassnig, R., Lafont, J.-P., Lenihan, P., Pétursson, G., Pritchard, G., Thorley, J., Vitu, C., Mornex, J.-F., & Pépin, M. (2004). Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Veterinary Research, 35(3), 257-274. https://doi.org/10.1051/vetres:2004014

Sait, A., & İnce, O. B. (2022). Investigation of the Epidemiology of Small Ruminant Lentivirus Infections southeast part of the Marmara region of Turkey, Hellenic Veterinary Medical Society, 73(4), 5053-5060 https://doi.org/10.12681/jhvms.29095

Schreuder, R. E. C., Yonguç, A. D., Girgin, H., & Akçora, A. (1988). Antibodies to maedi-virus in indigenous sheep in eastern Turkey. Etlik Veteriner Mikrobiyoloji Dergisi, 6(3), 47-53.

Straub, O. C. (2004). Maedi-Visna virus infection in sheep. History and present knowledge. Comparative Immunology, Microbiology and Infectious Diseases, 27(1), 1-5. https://doi.org/10.1016/S0147-9571(02)00078-4

Tan, M. T., & Alkan, F. (2002). Türkiye'de maedi-visna enfeksiyonunun seroepidemiyolojisi ve virus izolasyonu. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 49(1), 45-50. https://doi.org/10.1501/Vetfak_0000001646

World Organisation for Animal Health. (2023). Terrestrial Animal Health Code, https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/

Türkiye İstatistik Kurumu. (2024). Erzurum ili küçükbaş hayvan sayısı, https://biruni.TUIK.gov.tr/ilgosterge/?locale=tr

Ün, H., Özgünlük, İ., & Çabalar, M. (2018). Şanlıurfa Yöresinde Maedi-Visna Virus (MVV) Enfeksiyonunun Serolojik Olarak Araştırılması. Harran Üniversitesi Veteriner Fakültesi Dergisi, 7(2), 144-148. https://doi.org/10.31196/huvfd.508962

Yavru, S., Şimşek, A., Bulut, O., & Kale, M. (2002). Koyunlarda maedi-visna virus (MVV) enfeksiyonu üzerine serolojik araştırma. 5. Ulusal Vet Mikrobiyoloji Kongresi, 24-26.

Yazgan, E., Saygılı, M., & Çokaklı, B. (2018). Osmaniye İlinde Yetiştirilen Yerli Kıl Keçilerinden Elde Edilen Çiğ Sütlerde Biyokimyasal Parametrelerin Türk Standartlarına Uygunluklarının Belirlenmesi. International Journal of Eastern Mediterranean Agricultural Research, 1(1), 12-19.

Yılmaz, Ş. G. (2019). Küçükbaş Hayvan İşletmelerinin Sosyo-Ekonomik Analizi ve Etkinliği: Batı Akdeniz Bölgesi Örneği. PhD Thesis, Isparta Uygulamalı Bilimler Üniversitesi. Fen Bilimleri Enstitüsü. Isparta.

Yilmaz, H., Gurel, A., Turan, N., Bilal, T., Kuscu, B., Dawson, M. M., & Morgan, K. L. (2002). Abattoir study of maedi-visna virus infection in Turkey. The Veterinary Record, 151(12), 358-360. https://doi.org/10.1136/vr.151.12.358

Medit Vet J, 9(2): 326-332, 2024

Research Article

Evaluation of estrus detection by cervical monitoring and pregnancy rates in ovsynch and co-synch treated Anatolian buffalo heifers

İlktan Baştan[1], Fırat Korkmaz[1], Derya Şahin[2], Seher Şimşek[2], Mehmet Ali Yılmaz[2]

[1]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2]International Center for Livestock Research and Training, Department of Biotechnology, Ankara Burdur, Türkiye

Key Words:
artificial insemination
Bubalus bubalis
estrus response
monitoring of cervix uteri
synchronization

Received : 05 June 2024
Revised : 04 August 2024
Accepted : 04 August 2024
Published : 31 August 2024
Article Code : 1496233

Correspondence:
İ. BAŞTAN
(ibastan@mehmetakif.edu.tr)

ORCID
İ. BAŞTAN : 0000-0001-8155-1960
F. KORKMAZ : 0000-0002-9600-908X
D. ŞAHİN : 0000-0003-1265-3618
S. ŞİMŞEK : 0000-0002-5468-0844
MA. YILMAZ : 0000-0002-4497-2894

ABSTRACT

The aim of this study was to assess the relationship between the occurrence and intensity of estrous expression and the success of artificial insemination in Ovsynch and Co-synch treated Anatolian buffalo heifers, employing a novel approach utilizing smartphone-based endoscopic inspection of the cervix uteri. Twenty-seven Anatolian buffalo heifers (n=27) were utilized for the study. In the Ovsynch synchronization protocol (n=15), GnRH (buserelin acetate, 12 µg) was administered on day 0, PGF2α (cloprostenol, 500 µg) on day 7, and GnRH (buserelin acetate, 12 µg) on day 9, with artificial insemination conducted 16-20 hours after the second GnRH application. For the Co-synch protocol (n=12), GnRH (buserelin acetate, 12 µg) was administered on day 0, PGF2α (cloprostenol, 500 µg) on day 7, and GnRH (buserelin acetate, 12 µg) along with artificial insemination on day 10. At the time of artificial insemination, estrous expression was categorized as intense, moderate, weak, or negative. Pregnancies were confirmed between 40 to 70 days after artificial insemination using either pregnancy-related glycoprotein or ultrasonography examinations.Intense, moderate, weak, and negative estrus intensities were observed at rates of 20%, 20%, 26.7%, and 33.3%, respectively, in the Ovsynch group and 41.7%, 58.3%, 0%, and 0%, respectively, in the Co-synch group ($P<0.05$). Pregnancy rates were 30% and 75% in groups exhibiting solely intense and moderate estrus expressions, respectively, in both the Ovsynch and Co-synch groups ($P<0.05$).In conclusion, utilizing a smartphone-based endoscopy apparatus for capturing images of the cervix uteri could serve as a viable alternative for classifying the intensity of estrus and determining the optimal time for artificial insemination. Moreover, in Anatolian buffalo heifers, it is recommended to employ the Co-synch protocol instead of Ovsynch.

INTRODUCTION

The Anatolian buffalo (*Bubalus bubalis*), reared in Türkiye, has 50 chromosomes, typically has a dark coat color, and originates from the Mediterranean subgroup of river buffalos (Ünal et al., 2020). In recent years, it has been established as a state policy to increase the population of Anatolian buffalo, which fell below 100,000 in 2010, through assisted reproductive technologies. (Bastan et al., 2021). The most important of these reproductive technologies is artificial insemination (AI), which is widely used worldwide. The success of AI is a very parametric phenomenon that is influenced by many factors, with the most important being the accurate timing of AI and the synchronization of estrus (Roelofs et al., 2010). A number of estrus synchronization protocols have been developed in buffaloes and applied. These protocols are based on the administration of gonadotropin-releasing hormone (GnRH), prostaglandin, and progesterone hormones. However, these protocols for synchronizing estrus in buffaloes are based on those developed for cattle (Ambarcıoglu et al., 2023; Pursley et al., 1995).

The duration of the estrus phase in buffaloes exhibits considerable variation, spanning from 5 to 72 hours. Ovulation typically transpires between 26 and 33 hours following the initiation of estrus. Consequently, both the duration of estrus and the timing of ovulation are extended in buffaloes relative to cattle breeds. This variability in estrus and ovulation times may result in suboptimal synchronization outcomes and reduced pregnancy rates in buffaloes, including the Anatolian buffalo (Küçükkebabcı and Aslan, 2002; Purohit and Rao, 2018). Additionally, the identification of estrus in buffaloes poses challenges due to the infrequency of homosexual behaviors such as mounting or standing, as well as the anatomical characteristics of the vulva that make external detection of cervical mucus difficult. This presents a substantial obstacle in pinpointing the optimal timing for artificial insemination (Neglia et al., 2020; Peralta-Torres et al., 2020).

Recent research suggests that the success of artificial insemination in cattle is closely related to the intensity of estrus expression than the synchronization protocol used (Ferraz et al., 2017; Madureira et al., 2021; Saini et al., 2023). Although many studies have shown varying pregnancy outcomes in buffaloes subjected to different synchronization protocols, few have specifically examined the relationship between the intensity of estrus expression and pregnancy outcomes (Akhtar et al., 2013; Baruselli et al., 2010; Du et al., 2021). In addition, the criteria used to define the intensity of estrus expression may vary between studies. In this study, the intensity of estrus

To cite this article:
Baştan İ, Korkmaz F, Şahin D, Şimşek S, Yılmaz MA. Evaluation of estrus detection by cervical monitoring and pregnancy rates in ovsynch and co-synch treated Anatolian buffalo heifers. Medit Vet J. 2024; 9(2): 326-332.

expression was defined by evaluating the visual appearance of estrus symptoms such as cervical mucus, vulvar edema, and vulvar hyperemia. Additionally, a smartphone-based endoscopic inspection of cervix uteri was also used to determine the intensity of estrus. This methodological approach differs from conventional techniques used in previous studies. This study aims to evaluate the relationship between the occurrence and intensity of estrous expression and the success of artificial insemination in Ovsynch and Co-synch treated Anatolian buffalo heifers, using a smartphone-based endoscopic inspection of the cervix uteri.

MATERIALS and METHODS

Animal Material

For this study, 27 Anatolian buffalo heifers (Bubalus bubalis), each at least 22 months old, were selected under field conditions (n = 27). These heifers were identified by the Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies, and the National Anatolian Buffalo Breeding Project Technical Staff. Comprehensive health examinations were performed before and during the study to ensure the inclusion of only healthy animals.

Synchronization Protocols

The absence of pregnancy in the animals was confirmed using an ultrasonography examination (7,5 MHz Linear prob, Hasvet® 838, Türkiye) before initiation of the synchronization protocol. For the Ovsynch synchronization protocol (n=15), GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, intramuscular [im]) was administered on day 0, PGF2α (cloprostenol, 500 µg, Estrumate® 2 ml, im) on day 7, and GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) on day 9, with AI performed 16-20 hours after the second GnRH application (Neglia et al., 2016). For the Co-synch protocol (n=12), GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) was administered on day 0, PGF2α (cloprostenol, 500 µg, Estrumate® 2 ml im) on day 7, and GnRH (buserelin acetate, 12 µg, Receptal® 3 ml, im) along with AI application on day 10 (Fig. 1) (Akhtar et al., 2013).

Classification of Estrous Expression

Classification of estrus expression was performed based on vulva edema and vulva hyperemia on external examination. Additionally, the cervix uteri was monitored using a smartphone-based endoscopic inspection apparatus to determine whether the ostium externum uteri was open or closed and the presence of cervical mucus. Estrus intensity was classified as follows: (Baştan 2019; Bulut 2012; Kaurav et al. 2019; Da Silva et al. 2023):

Intense: The presence of vulvar hyperemia, vulvar edema, cervical mucus, and open cervix uteri characterized intense estrus (Fig. 2).

Moderate: Moderate estrus was identified by vulvar hyperemia and vulvar edema, accompanied by open cervix uteri. However, cervical mucus was not observed.

Weak: The vulvar hyperemia and vulvar edema were observed to be mild, with an open cervix uteri noted. However, cervical mucus was not present.

Negative: Negative estrus was characterized by the absence of vulvar hyperemia, vulvar edema, and cervical mucus, along with a closed cervix uteri.

Artificial Insemination (AI)

Following the estrus examination, AI was performed using Anatolian buffalo semen on the buffalo heifers that did not have negative estrus intensity. The study utilized frozen semen in the same straw batch from Anatolian buffalo that had appropriate spermatological values (at least 15 million motile spermatozoa/straw) according to legal regulations (Bastan et al., 2021).

Pregnancy Diagnosis

The pregnancy diagnosis was performed using ultrasonography (7,5 MHz Linear prob, Hasvet® 838, Türkiye) or measuring pregnancy-associated glycoprotein in blood serum with the enzyme-linked immunosorbent assay method using the

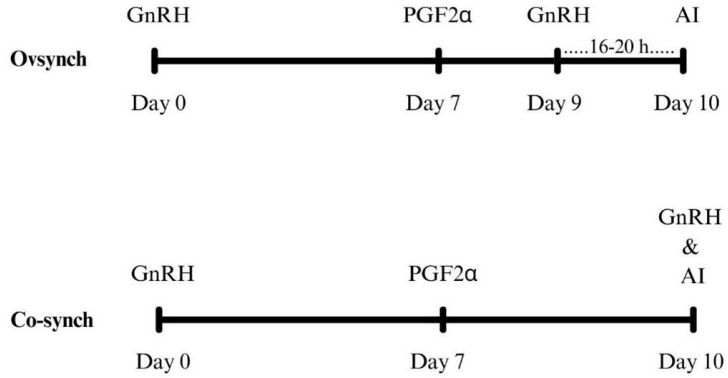

Figure 1. Ovsynch and Co-synch synchronization protocols.
GnRH: Gonadotropin-releasing hormone (buserelin acetate, 12 µg, Receptal® 3 ml), PGF2α: Prostaglandin F2-alpha (cloprostenol, 500 µg, Estrumate® 2 ml), AI: artificial insemination with frozen-thawed semen.

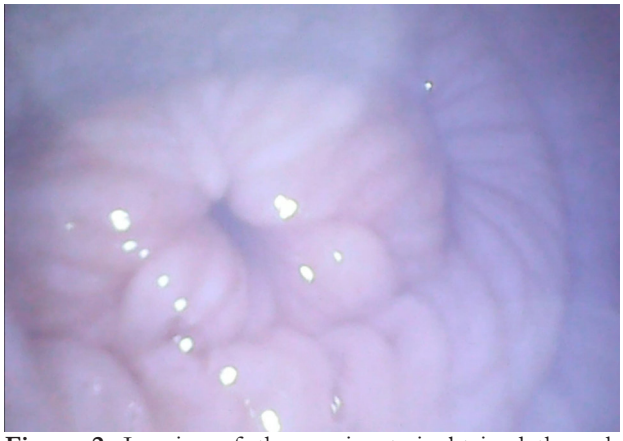

Figure 2. Imaging of the cervix uteri obtained through a smartphone-based endoscopic apparatus in Anatolian buffalo heifers during intense estrus.

IDEXX® Rapid Visual Pregnancy Test kit, 40 to 70 days after artificial insemination. (Arshad et al., 2022).

Statistical Analysis

Descriptive statistics were presented as percentages and numbers for qualitative variables. The relationship between estrus intensity, pregnancy rates, and the synchronization protocols used was analyzed using Fisher's exact probability test. Statistical analyses were conducted using Stata SE 15.1 statistical software, with a significance level of $P < 0.05$.

RESULTS

Table 1 presents the estrus expression of Anatolian buffalo heifers at the time of artificial insemination (AI), along with their corresponding pregnancy rates within 40 to 70 days post-AI, categorized by the synchronization protocol utilized. In the Co-synch group, the highest rate of intense estrus was observed at 41.7%. Meanwhile, buffaloes that exhibited weak and negative intensity of estrus had rates of 26.7% and 33.3%, respectively. These estrus expressions were observed only in the Ovsynch group ($P < 0.05$). The pregnancy rates for buffalo heifers in the Co-synch group were 80% for those with intense

Table 1. Estrus intensities and pregnancy rates of the ovsynch and co-synch groups.

Synchronization protocol	n	Estrus intensity (%)				Number of AIs	Number of pregnancies	P/AI (%)
		Intense	Moderate	Weak	Negative			
Ovsynch	15	20 (3/15)	20 (3/15)	26.7 (4/15)	33.3 (5/15)	10	3	30[a]
Co-synch	12	41.7 (5/12)	58.3 (7/12)	0 (0/12)	0 (0/12)	12	9	75[b]

AI: artificial insemination, P/AI: pregnancy per AI.
a,b Different letters in the same column indicate statistically significant differences ($P < 0.05$).

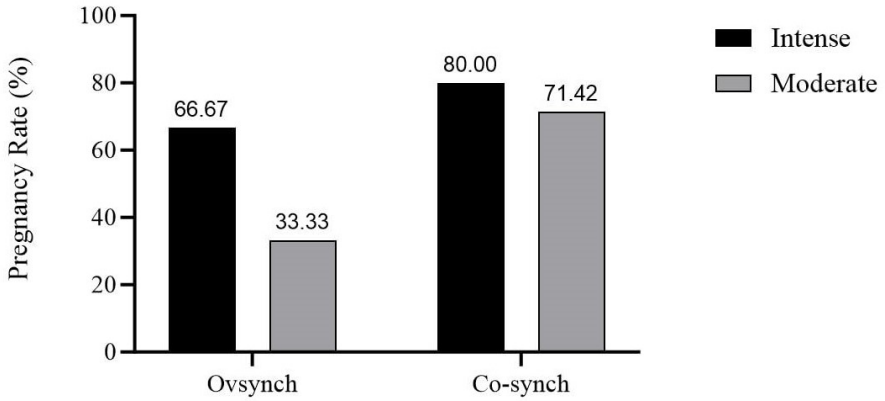

Figure 3. Pregnancy rates of the buffalo heifers with intense and moderate estrus intensities in the Ovsynch and Co-synch group ($P<0.05$).*
*: In the group exhibiting negative estrus, artificial insemination was not performed, and pregnancy was not achieved in the group displaying weak estrus.

estrus and 71.42% for those with moderate estrus (Fig. 3). In contrast, none of the four buffaloes that exhibited a weak estrus response were found to be pregnant in the Ovsynch group, while the pregnancy rates were 66.67% for buffalo heifers with intense estrus and 33.33% for those with moderate estrus. Statistical analysis revealed a significant difference ($P < 0.05$), as depicted in Figure 3.

DISCUSSION

In this study, we detected the occurrence and intensity of estrus using a novel approach that image of the cervix uteri with a smartphone-based endoscopy apparatus. Previous studies have employed various methods to assess estrus occurrence, including the observation of different behaviors (standing to be mounted, mounting, bellowing, walking fence line, increased nervousness and activity, etc), and, in part, the measurement of estradiol (E_2) and progesterone (P_4) concentrations in plasma. Several studies on artificial insemination and even embryo transfer in cattle have shown a positive relationship between the intensity of estrus and pregnancy rates (Burnett et al., 2022; Madureira et al., 2015; Madureira et al., 2022: Tippenhauer et al., 2021) Maduriera et al. (2015) reported that high physical activity during estrus was associated with the success of pregnancy per artificial insemination (P/AI) in Holstein cows.

The study investigated the relationship between estrus intensity, assessed through physical activity, and pregnancy outcomes. Findings revealed that calving ease (unassisted vs. assisted) and postpartum diseases (such as retained fetal membrane, displaced abomasum, and hyperketonemia) had adverse effects on both the intensity and duration of estrus in lactating cows (Madureira et al., 2023). For this reason, it is thought that evaluating estrus intensity through cervix uteri monitoring will provide a more objective assessment of both estrus intensity and conditions such as postpartum cervicitis and metritis, compared to determining estrus findings solely based on behavioral observations or plasma hormone (P_4 and E_2) levels.

There is limited research in the literature on the effects of the occurrence and intensity of estrus expression on the success of AI in buffaloes. Gayke et al. (2022) reported that after the Ovsynch synchronization protocol in buffalo heifers, the rates of intense, intermediate, and weak estrus were 33.3%, 41.7, and 25.00%, respectively, and the pregnancy rate was 33.33%. Sharma et al (2021) conducted a study on the Murrah buffalo and found that the Co-synch synchronization protocol resulted in 33.3% intense, 41.7% moderate, and 25% weak estrus intensities. The progesterone-supported Co-synch protocol resulted in rates of 46.7%, 33.3%, and 20% for intense, moderate, and weak intensities, respectively. These results are consistent with the estrus intensity rates observed in the current study. In Sharma et al. (2021) study, the pregnancy rate was 33.3% in the Co-synch group and 46.6% in the progesterone-supported Co-synch group. It is worth noting that hCG was used in the last stage of their synchronization protocols, unlike in the current study where GnRH was used. Kumar et al. (2016) conducted a study on Murrah buffalo and found that 38.4% exhibited intense estrus, 46.1% exhibited moderate estrus, and 15.3% exhibited weak estrus following the Co-synch protocol. The overall conception rate was 62.5%. During the study, pregnancy outcomes were observed in buffalo heifers that exhibited intense and moderate estrus expressions in both synchronization groups. The relationship between estrus intensity and pregnancy outcomes outlined in these studies aligns with the findings of the current study, despite variations in the behavioral signs used to evaluate estrus expressions. In a study examining the administration of oestradiol benzoate (OEB) on Nili-Ravi buffalo, there was no significant relation between estrous intensity and pregnancy rate (Yousuf et al., 2015). It is thought that this circumstance is due to the weak behavioral signs of estrus in buffaloes.

The efficacy of estrus synchronization protocols is closely related to the dynamics of follicular waves. In contrast to cattle breeds, buffaloes typically exhibit a two-phase pattern of follicular waves. (Jan et al., 2020; Abulaiti et al., 2022; Chaudhari et al., 2022; Manasa et al., 2022). Research on the follicular dynamics of Anatolian buffalo is limited. Additionally, while it is generally observed that Anatolian buffalo exhibit three follicular waves, there is research suggesting that two-phase follicular waves are also common in this breed. (Aksoy et al., 2002; Uçar et al., 2004; Yilmaz et al., 2014; Yilmaz et al.,7 2021). Therefore, their estrus cycle is shorter than that of buffaloes with three follicular waves, supporting the possibility of earlier ovulation after administering a second GnRH in the Ovsynch protocol. For this reason, it is thought that estrus intensity and pregnancy rate were lower in the Ovsynch group. Since biphasic follicular growth is commonly observed in bovine heifers, it is recommended to utilize the Co-synch synchronization protocol either alone or in combination with progesterone hormone supplementation. Pregnancy rates in Ovsynch and Co-synch treated buffaloes are highly variable when estrus findings are not assessed during artificial insemination. Hussein et al. (2016) achieved a 40% pregnancy rate in Egyptian buffalo heifers following the Ovsynch synchronization protocol. Chaikhun et al. (2010) stated that 15% and 42.9% pregnancy rates were obtained from treated Ovsynch swamp buffalo heifers and cows, respectively. In a study conducted by Biradar et al. (2016) on repeat breeder buffaloes aged over 10 years, the Ovsynch group exhibited a 50% pregnancy rate, whereas the Co-synch group showed a 37.5% pregnancy rate.

In the present study, significantly higher pregnancy rates were achieved in the group of Co-synch treated buffalo heifers compared to the Ovsynch group. In the Co-Synch protocol, the second GnRH application is administered during artificial insemination, distinguishing it from the Ovsynch protocol. Literature suggests that in buffaloes, the second follicular wave results in the development of a secondary follicle that is comparable in size to the dominant follicle. Additionally, it has been observed that this secondary follicle can induce simultaneous or interval ovulations with the administration of GnRH stimulation. (Wagas et al., 2016; Neglia et al., 2020). In this context, it is considered that fertilization occurs depending on the quality of the sperm used. Moreover, natural estrus and ovulation periods have been reported to be longer in buffaloes than in cattle (Neglia et al., 2020). Synthetic GnRH analogs have a longer-lasting effect than natural GnRH. On average, natural GnRH release has a plasma half-life of four minutes.

The buserelin acetate preparation utilized in this study exerts an effect that is 20-170 times stronger than natural GnRH. (Kumar et al., 2022). Therefore, the above-average pregnancy per artificial insemination (P/AI) outcomes observed in the Co-synch group could be attributed to the unique characteristics of follicular wave dynamics in Anatolian buffalo heifers and their responsiveness to exogenous GnRH treatments.

CONCLUSION

Presently, Anatolian buffaloes account for only 1% of the total population compared to cattle breeds in Türkiye. This low percentage can be attributed primarily to the difficulties associated with the care, feeding, and reproduction of Anatolian buffaloes, which are often characterized as semi-domesticated. Hence, ovarian dynamics could not be monitored using ultrasonography in this study. In conclusion, however, the study suggests that using a smartphone-based endoscopy apparatus to capture images of the cervix uteri could be a viable alternative to ultrasound applications for classifying the intensity of estrus and determining the optimal time for artificial insemination. Further studies on larger buffalo populations are needed to validate this model. Moreover, to avoid early ovulation in Anatolian buffalo heifers, it is recommended to use the Co-synch protocol instead of Ovsynch.

DECLARATIONS

Ethics Approval

This study was approved by International Center for Livestock Research and Training, Animal Experiments Local Ethics Committee at the meeting dated 20.12.2019 with the number of 66091008/1310 decisions.

Conflict of Interest

The authors have no conflicts of interest.

Author contribution

Idea, concept and design: İB

Data collection and analysis: İB, FK, DŞ, SŞ, MAY

Drafting of the manuscript: İB, FK, DŞ, SŞ

Critical review: İB, FK, DŞ, SŞ

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

Acknowledgments

We would like to thank the Ministry of Agriculture and Forestry, the General Directorate of Agricultural Research and Policies, and the National Anatolian Buffalo Breeding Project Technical Staff for their contribution to this study.

REFERENCES

Abulaiti, A., Riaz, U., Naseer, Z., Ahmed, Z., Hua, G., & Yang, L. (2022). Follicular Dynamics during Estrous Cycle of Pubertal, Mature and Postpartum Crossbred (Nili Ravi Jianghan) Buffaloes. Animals 2022, 12, 1208. https://doi.org/10.3390/ani12091208

Akhtar M. S., Ullah S., Farooq A. A., Mazhar M., & Murtaza S. (2013). Pregnancy rate in lactating buffaloes treated with or without estradiol after estrus synchronization protocols at timed AI. Buffalo Bull, 32, 366-369

Arshad, B., Shabir, A., Sagheer, M., Arshad, U., Yousuf, M. R., & Riaz, A. (2022). Validation of pregnancy associated glycoproteins-based ELISA kits to determine early pregnancy status in lactating Nili-Ravi buffaloes. Reproduction in Domestic Animals, 57(10), 1267-1276. https://doi.org/10.1111/rda.14202

Baruselli, P.S., Carvalho, N.A.T., Gimenes, L.U., & Crepaldi, G.A., (2010). Fixed-Time Artificial Insemination In Buffalo. Italian Journal of Animal Science, 6(2s), 107-118.

Badrakh, D., Yanagawa, Y., Nagano, M., & Katagiri, S. (2020). Effect of seminal plasma infusion into the vagina on the normalization of endometrial epidermal growth factor concentrations and fertility in repeat breeder dairy cows. Journal of Reproduction and Development, 66(2), 149-154. https://doi.org/10.1262/jrd.2019-148

Baştan, İ. (2019). A video-assisted intrauterine artificial insemination device for Anatolian buffalo and local cattle breed. Turkish Patent and Trademark Office. Application Number of Patent: 2019/21012.

Bastan, İ., Korkmaz, F., Şahin, D., Şimşek, S., & Satılmış, M. (2021). First Commercial Semen Cryopreservation and Main Spermatological Features of Anatolian Buffalo. Livestock studies, 61(2), 60-65. https://doi.org/10.46897/livestockstudies.610203

Biradar, S., Tandle, M. K., Suranagi, M. D., Usturge, S. M., Patil, N. A., & Babu, Y. H. (2016). Study on efficacy of cosynch and ovsynch protocols on fertility in repeat breeder buffaloes. Buffalo Bulletin, 35(4), 737-743.

Bulut, G. (2012). The ultrasonographic evaluation of ovarian function and the effects of PRID implementation on fertility parameters in dairy cattle without postpartum reproductive problems. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 52(2), 1-14.

Burnett, T. A., Madureira, A. M., Bauer, J. W., & Cerri, R. L. (2022). Impact of gonadotropin-releasing hormone administration at the time of artificial insemination on conception risk and its association with estrous expression. Journal of Dairy Science, 105(2), 1743-1753. https://doi.org/10.3168/jds.2021-20156

Chaikhun, T., Tharasanit, T., Rattanatep, J., De Rensis, F., & Techakumphu, M. (2010). Fertility of swamp buffalo following the synchronization of ovulation by the sequential administration of GnRH and PGF2alpha combined with fixed-timed artificial insemination. Theriogenology, 74(8), 1371-1376. https://doi.org/10.1016/j.theriogenology.2010.06.007

Chaudhari, D. V., Panchal, M. T., Dhami, A. J., Sarvaiya, N. P., Pathan, M. M., Hadiya, K. K., & Patel, J. A. (2022). Follicular Dynamics and Endocrine Profile during Nor-

mal Estrous Cycle and Early Pregnancy in Surti Buffaloes. Indian Journal of Veterinary Sciences & Biotechnology, 18(5), 1-8.

Da Silva, W. A. L., da Costa e Silva, E. V., Ferreira, M. G. C. R., Gheller, J. M., Poehland, R., & Melo-Sterza, F. D. A. (2023). Effect of shading on the physiological and reproductive parameters in Nelore cows. Tropical Animal Health and Production, 55(6), 374. https://doi.org/10.1007/s11250-023-03774-2

Du, C., Nan, L., Sabek, A., Wang, H., Luo, X., Hua, G., & Zhang, S. (2021). Evaluation of Ovsynch versus modified Ovsynch program on pregnancy rate in water buffaloes: A meta-analysis. Tropical Animal Health and Production, 53, 1-12. https://doi.org/10.1007/s11250-021-02828-7

Ferraz, P. A., Loiola, M. V. G., Rodrigues, A. S., Lima, M. C. C., Bittencourt, T. C. B. D. S. C. D., & Ribeiro, A. D. L. (2017). The effect of the intensity of estrus expression on the follicular diameter and fertility of Nellore cows managed under a FTAI program. Ciência Animal Brasileira, 18. https://doi.org/10.1590/1089-6891v18e-37643

Gayke, P., Razzaque, W. A. A., Patil, A., Ramteke, S. S., Gaikwad, N. Z., Ali, S. S., & Jadhav, S. (2022). Inclusion of Antioxidants in Ovsynch Protocol for Improving Fertility in Pubertal Anestrus Buffalo Heifers. Journal of Animal Research, 12(2), 273-277. 10.30954/2277-940X.02.2022.17

Hussein, H. A., Mohamed, R. H., Hossam, M., & Wehrend, A. (2016). Ovarian response and conception rate following oestrus synchronization using three protocols in Egyptian buffalo heifers. Tierärztliche Praxis Ausgabe G: Großtiere/Nutztiere, 44(05), 287-294. 10.15653/TPG-160214

Jan, M. H., Kumar, H., Kumar, S., Sharma, R. K., Gupta, A., & Mehrara, K. L. (2020). Effect of progesterone administration during growing phase of first dominant follicle on follicular wave pattern in buffalo heifers. Tropical animal health and production, 52, 1395-1402. https://doi.org/10.1007/s11250-019-02143-2

Kaurav, P. S., Shukla, S. P., Bajaj, N. K., Manjhi, V. K., & Thakur, R. K. (2019). Incidence of Postpartum Anoestrus and Oestrus Behavior and Gynecological Symptoms in Hormonal Treated Postpartum Anoestrus Buffaloes. Journal of Animal Research, 9(1), 153-157. 10.30954/2277-940X.01.2019.21

Kumar, L., Phogat, J. B., Pandey, A. K., Phulia, S. K., Kumar, S., & Dalal, J. (2016). Estrus induction and fertility response following different treatment protocols in Murrah buffaloes under field conditions. Veterinary world, 9(12), 1466. 10.14202/vetworld.2016.1466-1470

Küçükkebabçı M., & Aslan S., (2002). Evcil Dişi Mandaların Üreme Özellikleri. Lalahan Hay. Araşt. Enst. Derg. 42(2): 55-63.

Manasa Varra, G. K. V., Ramesh, H. S., Suchitra, B. R., Sudha, G., & Pooja, C. H. (2022). Cellular and molecular pathways associated with ovarian physiology and estrus cycle in buffaloes. The Pharma Innovation Journal.

Madureira, A. M., Burnett, T. A., Borchardt, S., Heuwieser, W., Baes, C. F., Vasconcelos, J. L., & Cerri, R. L. (2021). Plasma concentrations of progesterone in the preceding estrous cycle are associated with the intensity of estrus and fertility of Holstein cows. PLoS One, 16(8), e0248453. https://doi.org/10.1371/journal.pone.0248453

Madureira, A. M. L., Burnett, T. A., Marques, J. C. S., Moore, A. L., Borchardt, S., Heuwieser, W., & Cerri, R. L. A. (2022). Occurrence and greater intensity of estrus in recipient lactating dairy cows improve pregnancy per embryo transfer. Journal of Dairy Science, 105(1), 877-888. https://doi.org/10.3168/jds.2021-20437

Madureira, A. M. L., Silper, B. F., Burnett, T. A., Polsky, L., Cruppe, L. H., Veira, D. M., & Cerri, R. L. A. (2015). Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. Journal of Dairy Science, 98(10), 7003-7014. https://doi.org/10.3168/jds.2015-9672

Neglia, G., de Nicola, D., Esposito, L., Salzano, A., D'Occhio, M. J., & Fatone, G. (2020). Reproductive management in buffalo by artificial insemination. Theriogenology, 150, 166-172. https://doi.org/10.1016/j.theriogenology.2020.01.016

Neglia, G., Gasparrini, B., Salzano, A., Vecchio, D., De Carlo, E., Cimmino, R., & Campanile, G. (2016). Relationship between the ovarian follicular response at the start of an Ovsynch–TAI program and pregnancy outcome in the Mediterranean river buffalo. Theriogenology, 86(9), 2328-2333. https://doi.org/10.1016/j.theriogenology.2016.07.027

Peralta-Torres, J. A., Torres-Chablé, O. M., Segura-Correa, J. C., Ojeda-Robertos, N. F., Chay-Canul, A. J., Luna-Palomera, C., & Aké-Villanueva, J. R. (2020). Ovarian dynamics of buffalo (Bubalus bubalis) synchronized with different hormonal protocols. Tropical Animal Health and Production, 52, 3475-3480. https://doi.org/10.1007/s11250-020-02381-9

Purohit, G. N., & Rao, T. K. (2018). Estrus detection in buffaloes. International Veterinary Information Service, Ithaca NY (www. ivis. org), Last updated.

Pursley, J.,R., Mee, M.,O., & Wiltbank, M.,C., (1995). Synchronization of Ovulation in Dairy Cows Using PGF2 Alpha and GnRH. Theriogenology, 44, 915-923. https://doi.org/10.1016/0093-691X(95)00279-H

Roelofs, J., Lopez-Gatius, F., Hunter, R. H. F., Van Eerdenburg, F. J. C. M., & Hanzen, C. H. (2010). When is a cow in estrus? Clinical and practical aspects. Theriogenology, 74(3), 327-344. https://doi.org/10.1016/j.theriogenology.2010.02.016

Saini, G., Kumar, S., Pandey, A. K., Yadav, V., & Sharma, S. (2023). Intensity of estrus expression–valuable obvious determinant of fertility in Bos indicus cows. Animal Biotechnology, 1-10. https://doi.org/10.1080/10495398.2023.2234961

Sharma, K., Phogat, J. B., Pandey, A. K., Dhaka, A. P., Singh, S., & Ghadwal, S. (2021). Role of progesterone supplementation in estrus induction in Murrah buffaloes under field conditions in non-breeding season. Buffalo Bulletin, 40(1), 99-106.

Tippenhauer, C. M., Plenio, J. L., Madureira, A. M. L., Cerri, R. L. A., Heuwieser, W., & Borchardt, S. (2021). Factors associated with estrous expression and subsequent fertility in lactating dairy cows using automated activity monitoring. Journal of Dairy Science, 104(5), 6267-6282. https://doi.org/10.3168/jds.2020-19578

Uçar, M., Küçükkebapçı, M., Gündoğan M, Uğuz C, & Saban E (2004) Reproductive performance of Anatolian Water Buffaloes during postpartum period. Indian Vet J 81, 784–786.

Ünal, E. Ö., Işık, R., & Soysal, M. İ. (2020). Identification of novel single nucleotide polymorphisms in the growth hormone (GH) gene in Anatolian water buffalo (Bubalus bubalis) populations in Turkey. Revista Brasileira de Zootecnia, 49. https://doi.org/10.37496/rbz4920190260

Yilmaz, O., Yazıcı, E., Kahraman, A., Özenç, E., & Uçar, M. (2014). The relationship between ovarian follicle population and follicle size during different stages estrous cycle in Anatolian Water Buffaloes (Bubalus Bubalis). Revue de Médecine Vétérinairé (165), 111-115.

Yilmaz, O., Yazici, E., & Ucar, M. (2011). Measurements of some genital tract organ size in Anatolian Water buffalos. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17(6).

Yilmaz, O., Yazici, E., Cicek, H., Celik, H. A., Uyarlar, C., & Ucar, M. (2021). Postpartum uterine involution and ovarian follicular dynamics in crossbreed Anatolian water buffalo (Bubalus bubalis) during summer season. Turkish Journal of Veterinary & Animal Sciences, 45(2), 299-310. 10.3906/vet-2009-26

Yousuf, M. R., Martins, J. P. N., Husnain, A., Riaz, U., Riaz, H., Sattar, A., & Ahmad, N. (2015). Effect of oestradiol benzoate on oestrus intensity and pregnancy rate in CIDR treated anoestrus nulliparous and multiparous buffalo. Animal Reproduction Science, 159, 104-108. https://doi.org/10.1016/j.anireprosci.2015.06.003

Medit Vet J, 9(2): 333-336, 2024

Case Report

Sebaceous gland epithelioma with potential malignancy in a dog

Ömer Faruk Keleş[1], Yağmur Kuşçu[2], Caner Kayıkçı[2], Hacı Ahmet Çiçek[1]

[1]Department of Pathology, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye
[2]Department of Surgery, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Türkiye

Key Words:
dog
histopathology
Ki67 immunoreactivity
sebaceous epithelioma

Received : 31 January 2024
Revised : 24 June 2024
Accepted : 17 July 2024
Published : 31 August 2024
Article Code : 1429434

Correspondence:
ÖF. KELEŞ
(ofkeles@yyu.edu.tr)

ORCID
ÖF. KELEŞ : 0000-0002-7869-5311
Y. KUŞÇU : 0000-0002-7802-5481
C. KAYIKÇI : 0000-0001-7343-1045
HA. ÇİÇEK : 0009-0002-5154-7498

ABSTRACT

Sebaceous gland tumors consist of neoplastic proliferations of sebaceous gland cells located around hair follicles in the dermis. These tumors are subclassified as sebaceous epithelioma, sebaceous adenoma, sebaceous adenocarcinoma and nodular hyperplasia. In this case report, a nodular growth in the sacral region of an eight-year-old male Belgian Malinois dog was presented. Macroscopically, the nodular mass had a slightly soft consistency, grayish-white, and dark red-black appearance. The mass was measured in the dimension of 2x1x1 cm. Histopathologically, the tumor was observed to have a multilobular structure shaped by neoplastic cell islands. The tumor consisted mainly of eosinophilic cells with small cytoplasm resembling epithelial basaloid cells and to a lesser extent differentiated sebocytes. The parenchyma of the tumor consisted of irregular islets containing a small number of mature sebocytes. Their nuclei are oval with one to three small nucleoli. Immunohistochemically, tumor cells for Ki67 antibody showed strongly positive immunoreactivity. Based on the histopathological and immunohistochemical features, the tumor was diagnosed as sebaceous epithelioma having, a potential malignancy. Since no case of sebaceous epithelioma with malignant potential has been reported in our country, we aimed to present the case with histopathological and immunohistochemical features.

INTRODUCTION

Sebaceous gland tumors are neoplastic proliferations of sebaceous gland cells around hair follicles in the dermis of the skin. These tumors are classified as sebaceous epithelioma, sebaceous adenoma, sebaceous adenocarcinoma, and nodular hyperplasia according to the degree of differentiation of the proliferating sebaceous gland cells (Hendrick 2016). These are dog's third most common skin tumors, accounting for 21-35% of all cutaneous epithelial tumors (Vail and Withrow, 2007). Sebaceous epitheliomas are primarly seen in dogs and account for approximately one-third of all sebaceous gland tumors and 2% to 3% of all skin tumors (Goldschmidt and Goldschmidt, 2017). Epitheliomas have typical macroscopic appearances ranging from a few millimeters to a few centimeters in diameter, with well-defined borders, hard consistency, ulcerated, nodular, and fungiform structures (Skelly and Franklin, 2002). Sebaceous epitheliomas are frequently seen on the head, ears, and dorsum in dogs between 8 and 13 years of age (Goldschmidt and Goldschmidt, 2017). It has been reported that sebaceous epithelioma is a common sebaceous tumor comprising 35.7% of all sebaceous tumors in dogs (Gross et al., 2005). A case of sebaceous epithelioma resulting in death due to metastases was reported by Bettini et al. (2009). Ki67, CK18, p63, CK14 and Bcl-2 antibodies were used to evaluate the potential malignancy of sebaceous epitheliomas in dogs (Yoon and Park, 2016). However, there are no reports on histopathological and immunohistochemical characterization of sebaceous epithelioma which has malignant potential and results in death in our country. In this study, histopathological and immunohistochemical features of sebaceous epithelioma were investigated using anti-Ki67 antibody.

MATERIALS and METHODS

An 8-year-old male Belgian Malinois dog was brought to the surgery clinic with the presentation of a mass. According to the history, the swelling had developed approximately 15 days prior at the level of sacral vertebrae. The patient was reported to have no loss of appetite but had experienced weight loss. During the clinical examination, the mass was firm, elastic in consistency, and ulcerative. For induction, 0.1 mg/butorphanol, 0.025 mg/kg medetomidine and, 5 mg/kg ketamine were administered intramuscularly. Anesthesia maintenance was achieved by continuous propofol infusion of 0.5 mg/kg/minute rate. The tumor, along with the surrounding macroscopically unaffected tissues, was excised entirely. The mass was sent to the pathology laboratory. The tumor mass was 2x1x1 cm in size and weighed 7.4 g. After routine tissue processing, the tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections of about 4 µm were stained with hematoxylin and eosin (H&E). Immunohistochemical analysis was performed using antibodies for Ki67 (MIB-1, Dako). Ki 67 immunoreactivity was determined according to the avidin-biotin complex procedure. Evaluation of the proliferation index by Ki67 immunolabelling was performed according to the method of Yoon and Park (2016).

FINDINGS

Macroscopically, the nodular mass appeared grayish-white

To cite this article:
Keleş ÖF, Kuşçu Y, Kayıkçı C, Çiçek HA. Sebaceous gland epithelioma with potential malignancy in a dog. Medit Vet J. 2024; 9(2): 333-336.

and dark red-black, with a slightly soft consistency. The cut surface of the mass was multilobular in appearance separated by thin fibrous tissue, and hemorrhagic, and had a similar color to its outer surface (Figure 1 A, B).

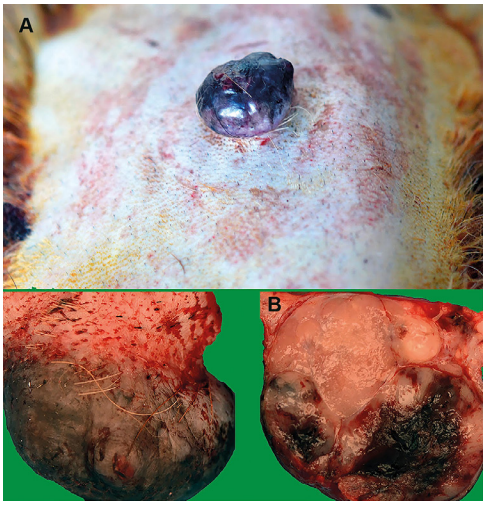

Figure 1. A) A tumoral nodular mass observed in the sacral region showing a grayish-white and dark red-black appearance. B) The cut surface was a multilobular structure separated by thin fibrous tissue, hemorrhagic, and had a similar color to its outer surface.

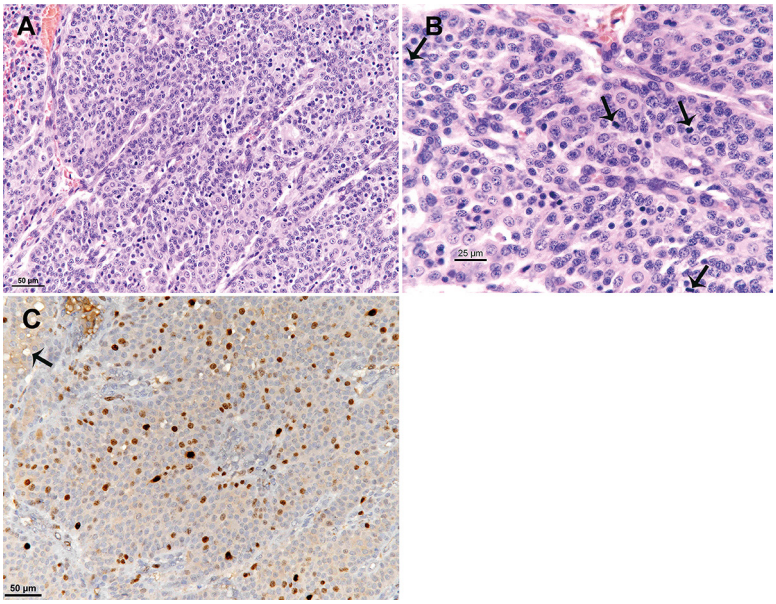

Figure 2. A) Tumor cells separated by a thin fibrovascular stroma consisted mainly of cells resembling epithelial basaloid cells with eosinophilic scant cytoplasm, round or oval nuclei, and one or three small nucleoli. HE, Scale bar = 50µm. B) Numerous mitotic figures (arrows) were observed in the parenchyma of the tumor. HE, Scale bar = 25µm. C) Immunohistochemical staining for Ki67 shows prominent immune expressions in the nuclei of many cells. The neoplastic cells have intracytoplasmic lipid vacuoles (arrow), Streptavidin Biotin Peroxidase method. Scale bar = 50µm.

Histopathological examination of the tumor mass revealed a multilobular structure consisting of tumor cell islets separated by an extensive fibrovascular connective tissue. Congestion, hemorrhage, and extensive areas of necrosis were observed in the tumor parenchyma. Tumor cells separated by an irregularly thin fibrovascular stroma reflected the characteristics of a sebaceous epithelioma pattern (Figure 2 A). The parenchyma of the tumor consisted mainly of cells resembling epithelial basaloid cells with eosinophilic scant cytoplasm, round or oval nuclei, one or three small nucleoli, and to a lesser extent of

differentiated sebocytes containing intracytoplasmic lipid vacuoles. Numerous mitotic figures, some of which were atypical in appearance, were observed in the parenchyma of the tumor (Figure 2 B). Mitoses were 4 to 6 per high power field (×400 magnification). However, mitoses are found only in the tumor cells resembling epithelial basaloid cells, not found in differentiated sebocytes. It was also observed that the foci of ductal differentiation were seen as small ducts lined by squamous epithelium and containing small amounts of keratin in some areas of the tumor parenchyma. Also, the local invasion of tumor cells was detected in peripheral lymphatics and blood vessels. To investigate the malignant potential of this case, immunohistochemistry for Ki67 was performed on sections of the tumor. Immune expressions were observed prominently in the nuclei of many cells for Ki-67. The proliferative activity index of tumor cells stained with Ki67 antibody was 35.7% (Figure 2 C). Based on these findings, the case presented here was diagnosed as sebaceous epithelioma with malignant potential.

DISCUSSION

Sebaceous glands are microscopic glands commonly found in all hairy skin areas and secrete an oily substance that lubricates the skin and hair. They also constitute the majority of hormonal metabolism in the skin (Chen and Zouboulis, 2009). The etiology of sebaceous gland tumors is not known for certain (Jakab, 2003). However, it has been emphasized that hormonal dysfunctions may play an essential role in their development (Rungsipipat et al., 2003). These tumors sometimes show locally aggressive behavior and metastasize to regional lymph nodes and other organs (Bettini et al., 2009). Although sebaceous gland tumors have been reported in all domestic animals, they are mostly reported in cats and dogs (Amaravathi et al., 2017; Goldschmidt and Goldschmidt, 2017). According to their histological characteristics and development, tumors are classified as nodular hyperplasia, epithelioma, glandular adenoma, ductal adenoma, and adenocarcinoma (Goldschmidt and Goldschmidt, 2017). Although adenomas have been widely reported in dogs, carcinomas (Amaravathi et al., 2017) have rarely been reported. Sebaceous carcinomas arise primarily on the head (39%), neck (11%) and thorax in dogs, and are similar on grossly to sebaceous adenoma and epithelioma. In carcinomas, a multilobular, solid, grey-whitish nodular mass and local invasion is the most common finding (Graham et al., 2004; Goldschmidt and Goldschmidt, 2017). Sebaceous epitheliomas typically appear as well-demarcated, firm, nodular, fungiform, or plaque-like masses in varying sizes, frequently with ulceration (Gross et al., 2005), and occur most often on the head, ears, and dorsum in dogs between 8 and 13 years of age (Goldschmidt and Goldschmidt, 2017). In the case presented here, the macroscopic appearance of the tumor mass was similar to those of sebaceous epithelioma described by Gross et al. (2005) and Goldschmidt and Goldschmidt (2017).

It has been reported that in sebaceous adenocarcinomas, histologically, the neoplastic cells have intracytoplasmic lipid vacuoles, but the degree of lipidisation varies from cell to cell and without significant amounts of well-differentiated small basaloid cells. The nuclei of tumor cells are reported to be large, chromatic, with prominent nucleoli and moderately pleomorphic. It was also reported that the number of mitotic figures was variable but atypical mitoses were also present. Mitotic figures will be found involving differentiated sebocytes as well as reserve cells, whereas in sebaceous epitheliomas mitoses are found only in the reserve cells (Goldschmidt and Goldschmidt, 2017). It has been reported that the histological pattern of sebaceous epitheliomas is very characteristic; the parenchyma of the tumor is mainly composed of a predominance of small basophilic reserve cells with fewer sebocytes. The reserve cells may show considerable mitotic activity. Also, there are foci of ductal differentiation lined with squamous epithelium and containing small amounts of keratin. The differentiation from sebaceous adenocarcinoma is based on the fact that the tumor cells have less cellular and nuclear pleomorphism and mitoses are not found in differentiated sebocytes (Bettini et al., 2009; Goldschmidt, 2017). In the case presented here, the parenchyma of the tumor was similar to those of sebaceous epitheliomas described by Bettini et al. (2009) and Goldschmidt and Goldschmidt (2017). In addition, the tumor was considered to have a potential malignancy due to the presence of numerous mitoses, some of which were atypical, in the reserve cells and local infiltration of peripheral lymphatics and blood vessels.

It has been reported that sebaceous epitheliomas are of low-grade malignancy and local infiltration frequently occurs. Therefore, recurrence occurs in incompletely excised neoplasms, but widespread metastases are rarely observed, and tumor cells spread to the mandibular lymph nodes and head region via lymphatics in metastasis cases (Gross et al., 2005; Bettini et al., 2009; Goldschmidt and Goldschmidt, 2017). The application of anti-CK14, CK18, p63, Ki67 and Bcl-2 antibodies in sebaceous epithelioma cases has been reported to be useful in the diagnosis and prognosis of these tumors (Yoon and Park. 2016). To differentiate between sebaceous carcinomas and benign sebaceous tumors in humans, proliferating cell nuclear antigen and Ki67 markers are used (Cabral et al., 2006). Bettini et al. (2009) recorded the proliferative activity index of Ki67 as 51.6% to 68% in a dog with metastatic (multiple lung and central nervous system metastases) sebaceous epithelioma. Yoon and Park (2016) recorded the proliferative activity index of Ki67 as 13.1% in a dog. In our case, the proliferative activity index of tumor cells stained with Ki67 was 35.7% and the tumor cells were histologically found to be locally invaded into the lymphatic and blood vessels. These findings indicate that the tumor might have a high metastatic potential. But in this case, the metastatic tumor foci were not observed in the other regions of the skin. Also, there was no information about the presence of metastases in the internal organs during the operation. However, although the tumor mass was completely incised with a successful surgical excision, the anamnesis taken six months later revealed that the general condition of the dog continuously deteriorated and the dog died.

CONCLUSION

As a result, in such cases, even if the animal is diagnosed with sebaceous epithelioma, a surgical intervention must be performed very carefully and the postoperative process must be managed very well due to the high metastatic potential of

these tumors.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: ÖFK, CK.

Data collection and analysis: ÖFK, YK, CK.

Drafting of the manuscript: ÖFK, HAÇ.

Critical review: ÖFK, CK, HAÇ.

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

Acknowledgements

Not applicable.

REFERENCES

Amaravathi, M., Murthy, R., Naik, S., Nasreen, A., Srilatha, C., Sujatha, K., & Saibaba, M. (2017). Sebaceous gland adenocarcinoma in a dog. Journal of Livestock Science, 8, 18-20. ISSN No. (online) 2277-6214.

Bettini, G., Morini, M., Mandrioli, L., Capitani, O., & Gandini, G. (2009). CNS and lung metastasis of sebaceous epithelioma in a dog. Veterinary Dermatology, 20(4), 289-294. https://doi.org/10.1111/j.1365-3164.2009.00762.x

Cabral E.S., Auerbach A., Killian J.K., Barrett T.L., & Cassarino D.S. (2006). Distinction of benign sebaceous proliferations from sebaceous carcinomas by immuno-histochemistry. The American Journal of Dermatopathology, 28, 465–471. DOI: 10.1097/01.dad.0000245200.65600.a4

Chen, W.C. & Zouboulis, C.C. (2009). Hormones and the pilosebaceous unit. Dermato-endocrinology, 1(2), 81-86. https://doi.org/10.4161/derm.1.2.8354

Goldschmidt M.H., Goldschmidt K.H. (2017). Epithelial and Melanocytic Tumors of the Skin. In: D. J. Meuten (Ed.). Tumors in Domestic Animals (pp. 88-141). Iowa State Press. https://doi.org/10.1002/9781119181200.ch4

Graham, J.E., Kent, M.S., & Théon, A. (2004). Current therapies in exotic animal oncology. Veterinary Clinics: Exotic Animal Practice, 7(3), 757-781. https://doi.org/10.1016/j.cvex.2004.04.004

Gross T.L., Ihrke P.J., Walder E.J., & Affolter V.K. (2005). Sebaceous tumors. In: Skin Diseases of the Dog and Cat. Clinical and Histopathologic Diagnosis. (2th ed., pp. 641-644). Blackwell Publishing. Doi: 10.1002/9780470752487

Hendrick, M.J. (2016). Mesenchymal tumors of the skin and soft tissues. Tumors in domestic animals (pp. 142-175). https://doi.org/10.1002/9781119181200.ch5

Jakab, C. (2003). Histopathological analysis of tumours of the sebaceous gland in spaniels. Kisallat Praxis, 4, 36-38. https://eurekamag.com/research/003/795/003795973.php

Rungsipipat, A., Sunyasootcharee, B., Ousawaphlangchai, L., Sailasuta, A., Thanawongnuwech, R., & Teankum, K. (2003). Neoplasms of dogs in Bangkok. The Thai Journal of Veterinary Medicine, 33(1), 59-66. thaijo.org/index.php/tjvm/article/view/37802

Skelly, B.J., & Franklin, R.J. (2002). Recognition and diagnosis of lysosomal storage diseases in the cat and dog. Journal of veterinary internal medicine, 16(2), 133-141. https://doi.org/10.1111/j.1939-1676.2002.tb02344.x

Vail, D.M., & Withrow, S.J. (2007). Tumors of the skin and subcutaneous tissues. Withrow, SJ; MacEewen, EG Small animal clinical oncology, 4, 375-401. https://doi.org/10.1016/B978-072160558-6.50021-6

Yoon, J. S., & Park, J. (2016). Immunohistochemical characterization of sebaceous epithelioma in two dogs. Iranian Journal of Veterinary Research, 17, 134-136. PMCID: PMC5090144