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Evaluation of Sheep Colostrums According to Time after Lambing by Brix Refractometer Method and Color Scoring

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ABSTRACT

The aim of the study was to determine the effects of breed and post-lambing time on sheep colostrum quality. In this study, colostrum samples taken at different times after lambing from 4 different sheep breeds (Tuj, Awassi, Morkaraman, Akkaraman) raised in Turkey were compared using Brix refractometer and color scoring. The sheep included in the study were placed in different paddocks according to their breeds. In the first week after lambing, a total of 7 colostrum samples were collected from each ewe, every 12 hours for the first 3 days and on the 7th day (mature milk sample). According to the data obtained from the study, it was determined that colostrum quality was affected by breed and milking duration ($p<0.05$). Brix values of colostrum in the first and second milking were found to be higher in the Akkaraman breed than in the Morkaraman breed ($p<0.05$). Additionally, it was determined that the color score decreased over time after lambing and this decrease was statistically significant ($p<0.05$). In conclusion, since the quality of sheep colostrum varies depending on breed and time, the quality of the colostrum to be fed for lamb health and performance must be determined in advance.

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INTRODUCTION

Animal protein sources are of great importance for human health (Aktan and Uçar, 2022). Adequate and balanced nutrition is very important for optimal health (Alkan *et al.*, 2019). People need to consume animal protein daily to maintain a healthy and balanced diet. A significant portion of animal proteins consumed by humans are provided by ruminant animals, and sheep have an important place among them (Ölmez *et al.*, 2023). Sheep attract more attention because of shorter gestation period, higher twinning rate, and lesser slaughter age as well as comprise of better roughage utilization capacity than that of cattle. The survival of each lamb born in sheep farming also affects animal welfare, farm economy and the country's economy. (Cannas *et al.*, 2019).

The first secretion produced by the mammary glands immediately after birth is called colostrum (Pattinson *et al.*, 1995). Colostrum is a thick substance rich in nutrients that is secreted during the first 72 h after birth (Todaro *et al.*, 2023). Colostrum is very different from normal milk in terms of color and composition (Yılmaz and Kaşıkçı, 2013). The most important of these differences is the very high concentration of immunoglobulins in the colostrum, which boost the immune system of lambs. In addition, the level of casein, fat, protein, and vitamins (A, B_{12}, D and E) is higher in colostrum, but it is poorer in terms of lactose. Proteins, growth factors, immunoglobulins (e.g. IgG, IgM and IgE), hormones, cytokines, lactoferrin, interleukins, nucleosides and nucleotides which are important for the health of lambs are the main bioactive components of colostrum. The ratio of these components in the colostrum is higher in the first few hours after lambing (Todaro *et al.*, 2023).

Colostrum contributes to the development of the gastrointestinal tract by providing passive immunity for newborns. It also affects the endocrine and metabolic systems and serves as an energy source to protect newborn animals from hypothermia. (Rauprich *et al.*, 2000). It also has a laxative effect, helping to remove the meconium from the intestines (Pattinson *et al.*, 1995). It contains a trypsin inhibitor that prevents immunoglobulins, which are important for passive immunity, from being digested in the intestine (Yılmaz and Kaşıkçı, 2013; Kaçar and Batmaz, 2023). Transferrin and lactoferrin contained in colostrum prevent the growth of some pathogenic bacteria by binding iron and thus prevent the development of diarrhea. (Selk, 1998). Colostrum performs these functions with the support of cellular (leukocytes, lymphocytes, macrophages, and natural killer cells) and humoral (immunoglobulins, lactoferrin, lysozymes and complements) mechanisms (Yılmaz and Kaşıkçı, 2013). Sheep colostrum also contains insulin (INS), insulin-like growth factor I (IGF-1), prolactin (PRL), growth hormone (GH), thyroxine (T4) and triiodothyronine (T3) (Canapana and Baunarucker, 1995; Kaçar and Batmaz, 2023).

In particular, IgG found in the colostrum plays a key role in preventing passive transfer failure that may occur in newborns. In the early hours of life after birth, the intestines of newborn mammals allow large molecular structures of IgG to pass into the circulatory system. However, this feature disappears after few hours of birth (Tsioulpas *et al.*, 2007; Stelwagen *et al.*, 2009; Aytaç and Yazıcı; 2020). Since colostrum IgG concentration and intestinal IgG permeability reduce quickly in the first 24 hours after birth, it is vital for newborn animals to consume adequate quantity and quality of colostrum without delay (Moore *et al.*, 2005). Due to the structure of the placenta in ruminant animals, the transfer of immunoglobulin from mother to offspring is not carried out during pregnancy. Neonatal lambs and kids are born hypogammaglobulinemic and require colostrum containing immunoglobulin for immunological protection after birth (Dwyer *et al.*, 2016). It is the most sensitive and dynamic period of life for all newborns after the transition from the intrauterine environment to the extra uterine environment (Tokan and Geçkil, 2019; Altay, 2021). In order to ensure passive immunity, it is of great importance that newborn lambs consume sufficient amount and high-quality colostrum in the first hours

after birth. (Martins and Oliveira., 2020).

The quality of colostrum is very important for the profitableness of livestock enterprises as it affects the viability of newborns (Belkasmi *et al.*, 2022). Colostrum quality is influenced by many factors such as the animal's age, breed, pre-pregnancy nutrition level, length of drying period, dystocia, size and behavioral factors (Şireli, 2017). Accurate measurement of colostrum IgG level is essential to provide colostrum containing sufficient levels of immunoglobulin to newborn in animal farms for the continuation of a healthy generation (Baltrukova *et al.*, 2019). Additionally, for high-quality colostrum, it is recommended that the total number of bacteria and coliforms in the colostrum must be <100,000 cfu/ml and <10,000 cfu/ml respectively (Lago *et al.*, 2018). Colostrum IgG level can be determined by various methods. These methods; radial immunodiffusion (RID), hydrometer, refractometer, first milking weight, colostrum color, high performance liquid chromatography (HPLC), electrophoresis (Gapper *et al.*, 2007; Rivero *et al.*, 2012).

The purpose of this study was to compare color and Brix values with optical refractometer in colostrum samples collected at different times after birth from four different sheep breeds such as Awassi, Morkaraman, Akkaraman and Tuj, and to evaluate the effectiveness of Brix refractometers in sheep colostrum.

MATERIAL AND METHOD

Animal Materials and Experimental Groups

In this study, 28 sheeps obtained from a production farm (Awassi, Morkaraman and Tuj) affiliated with Ataturk University Food and Livestock Application and Research Center and a sheep farm (Akkaraman) in Erzurum, Türkiye, were used as experimental animals. The study was conducted in Erzurum province in northeastern Turkey during winter. Straw was used as litter material in the paddocks. Sheeps were selected by considering that they were healthy as well as their live weights, and body condition scores were also similar. The animals used in the study were included in the experiment considering that they were 2 or older. In addition, it was also ensured that all the animals used in the study were multiparous and gave birth to single offspring at each birth. Since the colostrum characteristics of the breeds were examined in the study, sheep of different breeds were placed in different paddocks. The study was conducted in 4 groups according to their breed, and with an equal number of sheep (7 per group) in each group. In the experimental trial, animals were fed with same diet (Table 1).

The animals in the experiment were given water, meadow grass and alfalfa grass ad libitum and were also fed 500 g of sheep milk feed. During the study, the feeding program implemented by the farm was applied to the animals, and no different feeding program was applied. Crude protein, crude ash and ether extract analyzes of the feeds used in the study were performed according to AOAC's Weende Analysis System (AOAC, 2005). NDF and ADF analysis of feeds was performed according to Van Soest and Robertson (1985) and Goering and Van Soest (1970). The current study was conducted with the guidelines and permission of Ataturk University Animal Experiments Local Ethics Committee, Erzurum, Turkey (E-36643897-000-2300020138).

Table 1. Nutrient composition (%)

Nutrient composition	%
Dry matter (%)	88
Crude protein (%)	14.4
Ether extract (%)	60
Acid detergent fiber (ADF) (%)	25.4
Neutral detergent fiber (NDF) (%)	38.9

Collection of colostrum and milk samples

All the colostrum samples were collected by the same person. After lambing the colostrum samples were collected into falcon tubes every 12 h for the first 3 days and on the 7th day (mature milk), a total of 7 samples were collected from each animal (Table 2). During colostrum samples collection, the colostrum (50 ml) that came after milking the udder several times was collected into sterile sample containers. The collected colostrum samples were brought to the laboratory in a cold chain and stored at -20^{o}C until further analysis (Abdel-Salam *et al.*, 2019). A backup copy (50 ml) of each colostrum sample was stored in another deep freezer (at -20^{o}C) until the analyzes were completed.

Table 2. Days and times to collect colostrum samples

Breed	1th hour (T0)	12th hour (T1)	24th hour (T2)	36th hour (T3)	48th hour (T4)	60th hour (T5)	Day 7 (mature milk sample-T6)
Tuj	x	x	x	x	x	x	x
Awassi	x	x	x	x	x	x	x
Morkaraman	x	x	x	x	x	x	x
Akkaraman	x	x	x	x	x	x	x

Brix refractometer analysis

Total protein concentration in the colostrum was determined at room temperature using a commercially available optical Brix refractometer (ATC Colostrum Refractometer S1310). Calibration and sample readings of the refractometer used during the study were performed in accordance with the manufacturer's instructions. (Santiago *et al.*, 2020). The brix percentage of the refractometer used varied between 0-30. All the colostrum samples were allowed to reach room temperature before refractometer analysis. The samples that reached room temperature were mixed homogeneously with a vortex and then measured with a refractometer and the results were recorded. The Brix refractometer analysis of all the colostrum samples was performed by a single person.

Color Analysis

The color scoring was performed as described by Prom *et al.*, (2022) using a scale of 1 to 4; 1 point was almost white (the color of milk) and 4 points were the color of orange juice.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 20 (Statistical Package for the Social Sciences; SPSS Inc., Chicago/IL, USA). Pearson probability value (P value) was calculated using the One-Way ANOVA test to compare the data obtained from the study. A P value of less than 0.05 was considered statistically significant. The data obtained as a result of color scoring were subjected to

Kruskal-Wallis and Friedman tests.

RESULTS

In this study, the brix values and color scores of colostrum samples taken from different breeds of sheep at different times were examined. The brix values of colostrum are presented in Table 3, and the results obtained from color scoring are presented in Table 4.

Table 3. Change in colostrum Brix values (%) according to time after lambing

	T0	T1	T2	T3	T4	T5	T6
Tuj	24.8±1.02^{ab}	21.2±1.09^{ab}	16.9±1.05	15.9±0.46	13.9±0.61	14.4±0.52	13.8±0.29^{a}
Awassi	26.8±1.12^{ab}	22.7±1.78^{ab}	18.5±1.34	16.9±1.19	14.4±0.67	12.5±0.56	11.8±0.55^{b}
Morkaraman	23.0±2.01^{a}	17.9±1.0^{a}	15.8±0.57	15.2±0.65	14.7±0.62	13.6±0.62	12.5±0.43^{ab}
Akkaraman	28.3±0.9^{b}	24.3±1.02^{b}	19.0±1.27	18.0±0.63	16.1±0.41	15.4±0.34	12.3±0.34^{ab}

a-b: Means with different superscripts in the same column indicate significant differences ($p<0.05$).

Table 4. Change in colostrum color scores according to time after lambing

	T0	T1	T2	T3	T4	T5	T6
Tuj	3 (2-3)	2 (2-3)*	2 (1-2)*	1 (1-2)*	1 (1-1)^{a*}	1 (1-2)*	1 (1-1)^{a*}
Awassi	3 (3-4)	3 (2-3)*	3 (1-3)*	2 (1-4)*	2 (1-3)^{ab*}	2 (1-3)*	1 (1-2)^{ab*}
Morkaraman	3 (2-3)	3 (1-3)*	2 (1-3)*	2 (1-3)*	2 (1-2)^{b*}	2 (1-2)*	1 (1-1)^{a*}
Akkaraman	3 (2-4)	3 (2-4)*	2 (1-4)*	2 (1-2)*	2 (1-2)^{ab*}	2 (1-2)*	2 (1-2)^{b*}

a-b: Means with different superscripts in the same column indicate significant differences ($p<0.05$). * The same line shows the difference in color scoring according to time within the group regardless of breed ($p<0.05$). The values in parentheses indicate the smallest and largest values obtained in the measurements made on the relevant samples.

DISCUSSION

In this study, the changes in colostrum quality of different breed sheep over time were evaluated according to Brix values and color scoring, it was found that colostrum quality was affected by breed and milking time. Also, the brix values of colostrums at T0 and T1 milking were higher in the Akkaraman breed than in the Morkaraman breed. In addition, the comparison of the brix values of the samples collected that had turned into milk after one week of birth, showed that the samples taken from the Tuj breed had a higher brix value than those from the Awassi breed. As a result of the analysis of the data obtained, it was determined that there was a significant difference in color between the first colostrum samples taken after lambing (T0) and the samples taken on the 7th day (T6). In the comparisons made at the breed level, it was observed that the lowest quality was obtained in the Tuj breed sheep in the colostrum samples (T4) at the 5th milking (score 1) and that there was no significant difference between the other breeds. In the 7th sample (T6) taken at the end of the experiment, it was found that the colostrum with the highest score was from the Akkaraman breed, and the colostrum with the lowest score was from the Tuj and Morkaraman breeds.

In the literature review, it was observed that studies on determining the colostrum quality

focused mostly on dairy cows. In addition, the number of studies on the evaluation of colostrum quality in different sheep breeds using brix refractometer is limited (Castro *et al.*, 2018; Santiago *et al.*, 2020; Kessler *et al.*, 2021; Agenbag *et al.*, 2023). Therefore, this study was conducted to compare the colostrum qualities of four different domestic sheep breeds using Brix refractometers.

The high brix value in colostrum is directly related to the colostrum total protein content (IgG) (Kessler *et al.*, 2021). In a study conducted by Agenbag *et al.*, (2023) on South Australian Merino sheep, it was determined that the brix values were lower than those obtained from this study. In this study, brix % ranged from 21.6 to 44.7% at 0 hours postpartum, 15.1 to 45.3% at 4 hours, and 12.0 to 40.4% at 24 hours. However, the results obtained from this study were similar to those obtained by Kessler *et al.*, (2021). Kessler *et al.*, (2021) reported that in their study of increasing samples from their study comparing 10 different sheep and 10 different goat colostrums (Kessler *et al.*, 2019), the brix % ranged between 15.4-40.0. Kessler *et al.*, (2019) also stated that colostrum quality varies depending on breed. In a study conducted by Santiago *et al.*, (2020) on Santa Inés sheep, both colostrum quality and total serum protein levels of lambs were measured using a Brix refractometer. In this study, colostrum samples were collected at 6, 12, 24 and 48 hours after lambing and brix values were recorded. The results obtained were reported to be over 20% of the brix value in the first 24 hours. It was found that at the 48th hour after lambing, the brix value decreased to 15%. In addition, the ability to detect total serum protein levels with brix refractometers is another indication that brix refractometers are useful.

No studies have been conducted yet on the visual color scoring of colostrum samples in sheep. However, in a study conducted on Valle del Belice sheep (Todaro *et al.*, 2023), colostrum color analysis was performed with the help of "Chroma Meter". In this study, we tried to reveal the correlations between colostrum composition and color. In a study conducted to determine the effectiveness of β-carotene supplementation on colostrum in cattle during pregnancy, a color scoring system similar to that in our study was developed (Prom *et al.*, 2022). According to the study results, it was reported that as the color score increased, the brix value also increased. In our study, it was observed that the color score decreased as the brix value decreased over time. It was determined that the color score decreased with time after lambing and that this decrease was statistically significant. Additionally, it was observed that the colostrum samples taken from the Tuj breed were of lower quality than the T4 samples. In T6 samples, it was determined that the highest color score was in the Akkaraman breed.

CONCLUSION

In this study, it was determined that breed and the time elapsed after lambing were effective in improving the colostrum quality in sheep. It was thought that the difference between the obtained brix values and rank scores may be due to the colostrum IgG content. Brix refractometers used in the study is a useful, cheap and easy method that can be easily used by sheep breeders. Owing to the use of this method in sheep farming, lamb deaths due to consumption of poor quality colostrum can be prevented. Studies on colostrum quality in the literature have focused on dairy cows, and studies on sheep have been limited. Studies on the subject in sheep will make valuable contributions to the knowledge gap in the literature.

Ethical Approval

The current study was conducted with the guidelines and permission of Ataturk University Animal Experiments Local Ethics Committee, Erzurum, Turkey (E-36643897-000-2300020138).

Conflict of Interest

There is no Conflict of Interest. All authors contributed to the study conception and design. Material

preparation, data collection and analysis were performed by Soner UYSAL, Ayşe UYSAL, and Cihan ÖZ, Mükremin ÖLMEZ. The first draft of the manuscript was written by Ayşe UYSAL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Additional information's

The summary of this study was presented at the 2023 Sheep Goat Health and Management Congress.

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