

## RESEARCH ARTICLE

# The Effect of Using Zeolite (Clinoptilolite) as a Litter on Some Milk Yield and Welfare Parameters in Tent-Type Sheep Shelters

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## Abstract

This research was conducted to determine the effect of using zeolite used as a litter in tent-type sheep shelters on milk yield, milk quality, and animal welfare parameters. A total of 34 İvesi sheep in the control (n=17) and zeolite (n=17) groups were used as animal material in the study. Milk yield, milk quality, and blood parameters were monitored for 30 days period from the beginning of the study. In-shelter climatic conditions were recorded daily. The average milk yield in the control and zeolite groups were 1137.18 and 1168.94 g on the 2<sup>nd</sup> control (P>0.05); 536.83 and 790.88 g (P<0.05) on the 3<sup>rd</sup> control (P<0.05) respectively. In terms of somatic cell count, the zeolite group got a lower value on the 2<sup>nd</sup> and 3<sup>rd</sup> control of the study (P<0.05). According to the findings obtained from the research, other milk quality parameters were not affected by zeolite. Ammonia and humidity rates in the shelter are lower in the zeolite group (P<0.05). On the 3<sup>rd</sup> control of the study, significant differences were found between the groups in terms of BUN, urea, LDL, and TAC values (P<0.05). According to the our results, it may be stated that using zeolite as a litter contributes positively to the improvement of milk yield and animal welfare in the tent-type shelter environment.

**Keywords:** Milk quality, Milk yield, Sheep, Welfare, Zeolite

## Çadır Tipi Koyun Barınaklarında Altılık Olarak Zeolit (Klinoptilolit) Kullanılmasının Bazı Süt Verimi ve Refah Parametreleri Üzerine Etkisi

### Öz

Bu araştırma çadır tipi koyun barınaklarında altılık olarak kullanılan zeolit, süt verimi, süt kalitesi ve hayvan refahı parametreleri üzerine etkisini belirlemek amacıyla yapılmıştır. Araştırmada hayvan materyali olarak kontrol (n=17) ve zeolit (n=17) gruplarında toplam 34 baş İvesi koyun kullanılmıştır. Süt verimi, süt kalitesi ve kan parametreleri araştırma başlangıcından itibaren 30 günlük periyodlarla takip edilmiştir. Barınak içi iklimsel koşullar günlük olarak kayıt edilmiştir. Kontrol ve zeolit grubunda ortalama süt verimi sırasıyla 2. kontrolde 1137.18 ve 1168.94 g (P>0.05); 3. kontrolde sırasıyla 536.83 ve 790.88 g (P<0.05) olarak tespit edilmiştir. Somatik hücre sayısı bakımından zeolit grubu araştırmanın 2. ve 3. kontrolünde daha düşük değer almıştır (P<0.05). Araştırmadan elde edilen bulgulara göre diğer süt kalite parametreleri üzerinde zeolit etkisi olmamıştır. Barınak içi amonyak ve nem oranı zeolit grubunda daha düşük tespit edilmiştir (P<0.05). Biyokimyasal parametreler bakımından araştırmanın 3. kontrolünde gruplar arasında BUN, üre, LDL ve TAC değeri bakımından önemli farklar saptanmıştır (P<0.05). Elde edilen sonuçlara göre, koyun yetiştiriciliğinde çadır tipi barınaklarda altılık olarak zeolit kullanılmasının, süt verimi ve hayvan refahının iyileştirilmesine olumlu katkı sağladığı ifade edilebilir.

**Anahtar sözcükler:** Koyun, Refah, Süt kalitesi, Süt verimi, Zeolit

## INTRODUCTION

Sheep husbandry is practiced in different climatic and geographical conditions. Ewes are reared under different

environmental conditions depending on the purpose of breeding and farming business model <sup>[1]</sup>. The nomadic pastoralist system of sheep husbandry is practiced in regions with expanses of land, where different climates

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and pastures are suitable for housing and feeding animals in different seasons. In this type of sheep husbandry, sheep are transported from one region to another according to the climatic and pasture conditions. Livestock should be protected from extreme environmental factors in areas with large differences between temperatures during the day and those at night. Therefore, tent-type portable shelters are often used in nomadic sheep husbandry [2]. In addition, such shelters are generally preferred by farmers because of their low cost. However, adverse ground and ventilation conditions in shelters may affect animal health and welfare along with product quality [3].

The production performance of farm animals is affected by various factors. To obtain high-yield performance, animals with high genetic potential should be used and optimal care, feeding, and housing conditions should be provided [4]. Scientific studies and field applications have demonstrated that improving housing conditions increase the yield in animal husbandry [5].

Zeolites find application in many sectors and are widely used in geology, chemistry, physics, agriculture, animal husbandry, and medicine for scientific and commercial purposes. The most important feature of zeolite minerals is that they keep liquid and gas molecules away from the environment by trapping them in the spaces within their structure. They are used in eliminating the unpleasant odor of fertilizers in agriculture and animal husbandry [6] and in pH regulation of acidic volcanic soils [7]. Clinoptilolite is the most abundant natural zeolite in the world and has optimal properties. It is widely used as a feed additive in organic livestock breeding due to its nonfibrous mineral structure; a composition that is free of harmful elements; and high quality [8]. Although there is a limited number of studies on the effect of zeolites on ruminant barn odor, ruminant litter quality, and environmental humidity, it has been reported that zeolites improve litter quality and ensure dry feces in poultry, reduce the ammonia ratio, and ensure clean air in the poultry house [6,9].

In this study, the effects of application of zeolite on the ground on animal welfare, milk yield, milk quality, and serum biochemical parameters were investigated in tent-type shelters, which are widely used in rural areas with subtropical climates of Turkey.

## MATERIAL AND METHODS

### Ethical Statement

This study was approved by the Harran University Animal Experiments Local Ethics Committee (Approval no: 2018/08-01-07), Şanlıurfa, Turkey

### Animals and Feedings

The animals used as research material were selected

from a herd (approximately 500 heads) whose oestrus was synchronized. Thirty-four İvesi sheep on the  $80 \pm 10^{\text{th}}$  days (mean  $\pm$  SD) of milk production of first lactation and multiparous were selected for this research. Their body weights were an average  $54.16 \pm 6.65$  kg. The ewes were fed in the pen during the lambing period. During the pasture period, the animals were not taken out of the pen until the hoarfrost rises from the pasture in the morning, and they were kept in the tent-shelter at night. The sheep were kept in pasture for 14 h and 10 h in tent. They were fed with concentrate feed (18% CP, 2600 kcal/kg ME) and wheat hay in the evening after returning from pasture.

### Experimental Design

The ewes divided into two similar groups according to the age, average daily milk yield and milk composition at the beginning of the experiment. Within the scope of the research, a tent-type shelter used in the nomadic pastoralist system was designed. The tent was divided into two parts, and 36 m<sup>2</sup> of floor space was prepared for each group. The tent is placed in such a way that both groups are exposed to the same amount of daylight at the same time. Each group is provided with ventilation with a 2 m<sup>2</sup> window area.

The granular form of clinoptilolite was obtained from a commercial enterprise, and it was applied to the litter homogeneously over the entire natural soil ground (2.7 kg/m<sup>2</sup> on average each time) in the shelters of only one of the two experimental groups every 15 days (days 0, 15, 30 and 45). Throughout the experiment, a total of 389 kg clinoptilolite was used for the zeolite group [6,7].

### Ammonia, Temperature and Humidity Measurements

Since the beginning of the study period, the ammonia level in the tent was measured by using a gas measuring device (Industrial Scientific) once a day in the morning before taking the sheep to the pasture. Humidity ratios and temperature were measured daily with a TESTO-branded humidity and temperature measuring device. Measurement data were recorded after the readings stabilized.

### Milking Data Collection

The births on the sheep farm started in February. Ewes were selected on the days when the births increased and care was taken to keep the date of lactation onset close to each other. Milking controls were started when the firstborn lamb was 15 days old. On control days, the lambs were separated from their mothers at 06:00 pm on the previous day and left until after milking the next test day. The morning and evening milk yields in the control milking were summed and the milk yields on the control day were calculated. Control milkings were done in 15 days from the beginning of lactation to the peak of lactation. From the data obtained on the control days, the daily

milk yields of each sheep on days 15, 30, 45, 60, 75 were determined. Milk yield was calculated according to the Trapez II method [10]. According to the data obtained, two homogeneous groups were formed. After the beginning of the research, milking controls were made in 30 days periods. Average daily milk yields were determined with control milking performed at the beginning (80<sup>th</sup> day of lactation-1<sup>st</sup> Control), 110<sup>th</sup> day of lactation (2<sup>nd</sup> Control), and 140<sup>th</sup> day of lactation (3<sup>rd</sup> Control) of the study. The ewes were milked using a machine twice a day, at 06:00 am and 06:00 pm. The amount of milk was determined by using a balance with a sensitivity of 1 g. The vacuum pressure, pulsation rate, and pulsation ratio of the milking machines were set to 40 kPa, 120, and 60:40, respectively [11]. Routine practices regarding milking hygiene were performed before and after machinery milking.

### Sampling and Laboratory Analyses

Blood samples were collected from the jugular vein on control days to determine the biochemical parameters examined within the scope of the study. Samples were centrifuged at 3500 rpm for 10 min, and then the serum was separated into microcentrifuge tubes and delivered under cold chain conditions to the analysis laboratory (Yasamlab, Adana/Turkey). For analyses of the status of oxidative stress and antioxidant levels, blood samples collected in ethylenediaminetetraacetic acid-coated tubes on control days were centrifuged at 3000 rpm for 10 min, and plasma was stored at -80°C until the time of analysis. Total oxidative stress (TOS) and total antioxidant capacity (TAC) were determined according to the commercial kit (Rel assay, Turkey) protocol, and subsequently, the oxidative stress index (OSI) was calculated [TOS/(TACx10)] according to the protocol specified in the kit.

One hundred and two milk samples collected during morning milking on the control days were used to determine the milk quality parameters. Dry matter, fat, lactose, and protein ratios and somatic cell count (SCC) in milk were determined with a combined milk analyzer (Bentley); color properties with a portable color meter (Lovibond); and pH value with a portable pH meter (Mettler Toledo) [12,13].

### Statistical Analysis

Descriptive statistical values related to the parameters examined in the research groups were given as the mean  $\pm$  standard error of mean (SEM). The groups were by the normal distribution according to the Shapiro-Wilk test. After observing the normal distribution in the data, the comparison of the control and zeolite groups was analyzed by using independent sample T-Test. SPSS (SPSS Version 18.0®, Chicago, IL, USA) program was used for all statistical analyses. The differences between the groups in terms of the parameters examined were considered significant at the  $P < 0.05$  level.

## RESULTS

Meteorological data of the study period are presented in *Table 1*. As expected, the highest and lowest temperatures were measured in July and May, respectively.

*Table 1. Research period climatic conditions*

Month	Temperature (°C)			Rainfall (mm) Mean
	Mean	Maximum	Minimum	
May	22.1	28.6	15.2	26.7
June	28.0	34.6	20.4	4.4
July	31.9	38.7	24.2	2.0

Milk yields and milk quality parameters obtained in the study are presented in *Table 2*. The groups had similar milk yields at the beginning of the study. The difference became significant on the 3<sup>rd</sup> control; milk yields of the control and zeolite groups were 0.54 kg and 0.79 kg, respectively ( $P < 0.05$ ). The difference between the groups in terms of SCC became apparent on the 2<sup>nd</sup> control of the study and continued to increase on the 3<sup>rd</sup> control ( $P < 0.05$ ). There were no significant differences between the groups in terms of fat, protein, lactose, dry matter ratio, freezing point, pH and color ( $L^*$ ,  $a^*$ ,  $b^*$ ) properties ( $P > 0.05$ ).

The climatic parameters in the shelter determined during the study are presented in *Table 3*. The differences between the groups in terms of ammonia levels and humidity ratios were found to be significant ( $P < 0.05$ ).

Blood biochemical parameters of the ewes used in the study are shown in *Table 4*. Significant differences were detected between the groups in terms of blood urea nitrogen (BUN), urea, low density lipoprotein (LDL), and TAC levels on the 3<sup>rd</sup> control ( $P < 0.05$ ). There were no significant differences in other biochemical parameters ( $P > 0.05$ ).

## DISCUSSION

In the present study, the effects of using zeolite-litter supplementation in shelters in nomadic sheep husbandry, which is widely practiced in the Middle East, were experimentally established. The care and feeding conditions of the animals were also established in accordance with this system. The study was initiated after the lambs were weaned. In this way, we ensured that the lambs received a sufficient amount of milk from their mothers and that the environmental conditions suitable for nomadic sheep husbandry were provided. In addition, the study was initiated after the peak period of lactation, and thus the chances of the increase in yield being attributed to lactation physiology were eliminated [14].

To obtain an optimum yield from sheep, the optimum ambient temperature of the shelters should be set within

**Table 2.** Milk yield and milk quality traits

Traits	Groups	1 <sup>st</sup> Control	2 <sup>nd</sup> Control	3 <sup>rd</sup> Control
Daily Milk (g)	Control	1321.59±69.25	1137.18±100.18	536.35±83.26 <sup>b</sup>
	Zeolite	1338.29±72.29	1168.94±91.83	790.88±89.23 <sup>a</sup>
Fat (%)	Control	6.33±0.09	6.80±0.31	7.54±0.22
	Zeolite	6.35±0.12	6.80±0.27	7.06±0.28
Protein (%)	Control	4.91±0.12	5.61±0.10	5.58±0.16
	Zeolite	5.05±0.11	5.66±0.13	5.38±0.18
Lactose (%)	Control	5.27±0.05	4.60±0.09	4.17±0.14
	Zeolite	5.13±0.05	4.66±0.15	4.38±0.13
Dry Matter (%)	Control	17.89±0.19	18.18±0.40	18.26±0.33
	Zeolite	17.93±0.19	18.40±0.38	17.93±0.42
Freezing Point (°C)	Control	0.58±0.00	0.57±0.01	0.57±0.00
	Zeolite	0.58±0.00	0.57±0.00	0.58±0.00
SCC (x10 <sup>3</sup> cell/mL)	Control	133.13±40.68	154.38±39.51 <sup>a</sup>	227.88±61.53 <sup>a</sup>
	Zeolite	110.81±33.38	63.12±12.60 <sup>b</sup>	51.69±11.33 <sup>b</sup>
pH	Control	6.54±0.02	6.02±0.07	5.92±0.04
	Zeolite	6.57±0.02	5.94±0.06	5.82±0.05
L*	Control	70.85±0.71	73.55±0.37	73.00±0.41
	Zeolite	71.39±0.48	74.33±0.24	72.91±0.46
a*	Control	-6.66±0.14	-5.76±0.11	-5.69±0.11
	Zeolite	-6.57±0.10	-5.79±0.15	-5.68±0.09
b*	Control	6.46±0.25	7.05±0.26	7.71±0.33
	Zeolite	7.02±0.17	6.89±0.22	7.64±0.26

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P<0.05; X±SEM: Mean±Standart error of mean

**Table 3.** The climatic parameters in the shelter

Groups	NH <sub>3</sub> (ppm)	Temperature (°C)	Humidity (%)
Control	21.03±0.79 <sup>a</sup>	20.88±0.28	68.87±0.76 <sup>a</sup>
Zeolite	15.55±0.56 <sup>b</sup>	20.99±0.41	65.88±1.07 <sup>b</sup>

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P<0.05; X±SEM: Mean ± Standart error of mean

the range of 6°C-14°C for sheep, 12°C-14°C for lambs, and 14°C-16°C for butchery muttons [15]. In contrast, the thickness and quality of fleece on sheep can adapt to lower temperatures depending on air flow. Considering the average temperature in Şanlıurfa during the study period, the environmental temperature was found to be higher in our study compared to that reported [15] in the literature (Table 1). This was predicted by the investigators before commencing the study. Therefore, to reduce the effect of this stress factor, the study was commenced after the ewes in the farm were sheared.

In the present study, the difference detected between the groups in lactation milk yield on the 3<sup>rd</sup> control was found to be significant (Table 2). No study has been found in the literature in which zeolite (clinoptilolite) was used as litter material in sheep or goat shelters. It may be observed that the milk yield of the zeolite group on the 3<sup>rd</sup> control

(corresponding to the last days of lactation in the study) was higher than that of the control group. It is believed that this finding will be favorable in warranting further studies in terms of increasing the milk yield of sheep throughout the entire lactation period.

Previous studies on clinoptilolite in the past generally evaluated this substance as a feed additive [16,17]. Among these studies, the only study evaluating lactation milk yield was conducted on dairy goats by Katsoulos et al. [17]. In that study, they reported that adding zeolite to feed did not result in a difference in lactation milk yield between experimental and control groups. Although the yield obtained in their study was similar to the average lactation milk yield (0-60 days) observed in the present study, a difference of approximately 100 g between the two groups was striking. High milk yield requires plenty of fresh air with suitable quality because insufficient

**Table 4. Blood biochemical parameters in sheep**

Traits	Groups	1 <sup>st</sup> Control	2 <sup>nd</sup> Control	3 <sup>rd</sup> Control
Blood Urea Nitrogen (BUN) (mg/dL)	Control	18.85±1.47	20.32±0.58	14.81±0.96 <sup>a</sup>
	Zeolite	16.32±1.06	18.30±1.14	10.66±0.53 <sup>b</sup>
Urea (mg/dL)	Control	40.29±3.14	43.50±1.25	31.71±2.04 <sup>a</sup>
	Zeolite	34.93±2.27	39.13±2.46	22.82±1.13 <sup>b</sup>
Creatinine (mg/dL)	Control	0.56±0.04	0.54±0.02	0.74±0.03
	Zeolite	0.52±0.02	0.50±0.02	0.66±0.02
Creatine Kinase (IU/L)	Control	309.65±46.25	198.30±16.18	120.53±6.20
	Zeolite	273.29±15.90	163.88±7.51	130.18±8.70
Triglyceride (mg/dL)	Control	15.06±1.19	17.30±2.31	17.24±1.53
	Zeolite	14.64±1.17	19.88±2.73	15.18±1.21
Cholesterol (mg/dL)	Control	66.65±3.51	61.70±3.02	62.65±2.68
	Zeolite	66.86±2.98	61.00±3.02	59.35±1.68
High Density Lipoprotein (mg/dL)	Control	43.29±2.10	41.10±1.68	34.06±1.70
	Zeolite	46.50±2.17	40.13±1.54	35.29±0.84
Low Density Lipoprotein (mg/dL)	Control	20.53±2.31	17.00±1.91	25.24±1.38 <sup>a</sup>
	Zeolite	17.43±1.27	16.88±2.07	21.00±1.29 <sup>b</sup>
Aspartate Transaminase (U/L)	Control	115.71±2.10	120.10±4.63	108.29±3.50
	Zeolite	112.57±4.00	115.13±5.27	111.00±4.65
Alanine Amino Transferase (U/L)	Control	22.24±1.57	25.00±1.94	21.82±1.23
	Zeolite	22.64±1.99	23.00±1.88	23.47±1.61
Gamma Glutamyl Transferase (U/L)	Control	70.59±2.97	51.90±3.55	57.12±2.15
	Zeolite	66.79±3.99	63.00±3.17	56.24±2.98
Total Bilirubin (mg/dL)	Control	0.13±0.01	0.15±0.02	0.12±0.01
	Zeolite	0.15±0.01	0.13±0.01	0.11±0.01
Total Protein (g/dL)	Control	7.51±0.09	7.48±0.20	7.19±0.15
	Zeolite	7.58±0.14	7.64±0.11	6.98±0.12
Albumine (g/dL)	Control	3.13±0.07	3.36±0.07	3.10±0.06
	Zeolite	3.18±0.07	3.54±0.13	3.03±0.07
C-Reactive Protein (mg/dL)	Control	0.0031±0.00	0.0150±0.00	0.0147±0.00
	Zeolite	0.0049±0.00	0.0125±0.00	0.0124±0.00
Lactate Dehydrogenase (U/L)	Control	585.29±16.95	570.70±17.12	516.88±20.43
	Zeolite	610.50±17.61	578.63±30.96	532.76±17.55
Total Antioxidant Capacity	Control	1.00±0.03	1.09±0.01	1.15±0.02 <sup>a</sup>
	Zeolite	0.97±0.03	1.05±0.03	1.06±0.02 <sup>b</sup>
Total Oxidative Status	Control	8.98±0.49	11.19±0.77	7.65±0.67
	Zeolite	9.10±0.50	12.42±1.05	6.55±0.41
Oxidative Stress Index	Control	0.91±0.05	1.03±0.07	0.67±0.06
	Zeolite	0.95±0.05	1.19±0.10	0.62±0.04

<sup>a,b</sup> Values within a row with different superscripts differ significantly at P<0.05; X ± SEM: Mean ± Standart error of mean

oxygen slows down the metabolism, which affects milk production. Insufficient ventilation air exchange inside the barn causes increased air humidity as well as the concentration of harmful gases produced as a result of the decomposition of animal waste. High air humidity can have a negative impact on animal welfare and lead to the growth of pathogenic bacteria [18,19]. The interior surface

area of clinoptilolite is 300 m<sup>2</sup>/g, which accounts for its high absorptive capacity [20]. Zeolites are incorporated into the litter to reduce ammonia and moisture levels, thanks to their ability to adsorb ions and water [20]. Due to these properties, zeolite can improve environmental conditions, production performance, and hygiene of farm animals.

Varying results have been reported with respect to SCC in the milk of healthy sheep [21,22]. In the present study, significant differences were found between the two groups in terms of SCC (Table 2). Similar results have been reported in studies where clinoptilolite was added to the feed in dairy goats [17], and it has been reported that zeolite enhanced milk hygiene. Furthermore, there are similar studies showing that zeolite reduces SCC in cattle [23]. When the results obtained were compared with other studies conducted in sheep regardless of the zeolite litter, they were determined to be similar to the values reported by Yağcı and Kaymaz [24] in normal milk samples and lower than the values reported by Leitner et al. [21]. In dairy ewes, mastitis has a significant effect on reducing milk yield and quality, resulting in greater economic losses than those reported for dairy cattle [25]. SCC in milk is positively correlated with the pathogens causing mastitis and severity of infection [26]. Clinoptilolites are minerals that have a variety of characteristics, including water absorption, ion adsorption, and cation exchange capacity [20]. Litter chemical modifiers are substances that improve the physical, chemical, and microbiological integrity of the litter [20]. It has been reported that natural clinoptilolite is able to selectively adsorb bacteria and bind certain toxins [27]. Loch et al. [28] confirmed that zeolite added to the litter directly affects the survival of microorganisms by reducing pH and water activity. Because of these properties, zeolite may reduce the somatic cell count in dairy animals by reducing the contamination from the litter in the mammary tissue. Increased SCC poses a risk for subclinical mastitis in flocks especially during the last days of lactation [13,21,24]. In our study, although the increase in SCC continued in the control group, it decreased in the zeolite group as lactation progressed, which was evaluated as a factor that eliminated the risk of subclinical mastitis. The difference in milk yield detected in the zeolite group also supports this finding. In the present study, there was no difference between the groups in terms of the parameters determining milk composition and quality (Table 2). These data were consistent with the results of other studies in which different feed additives were used [29-31].

Climatic conditions in the shelter greatly affect the stress levels of animals in warm areas [32]. Poor housing conditions are regarded as an important reason for the decrease in livestock production and animal welfare [3]. Excessive heat and relative humidity in the shelters as a result of sheep breeding can be harmful to animal health. Relative humidity in the shelter should not exceed 80% unless required [15]. High relative humidity decreases the quality as well as quantity of fleece and affects the health of sheep [33]. The values found in the study for the control and zeolite groups (Table 3) were consistent with the findings of studies by Alkan [34] and Mutaf and Sönmez [35]. Intensive animal farming, which is currently practiced, has a negative impact on the environment due to the spread of various gases and unpleasant odors. Ammonia build-up

in the shelter environment irritates the respiratory mucosa, leading to cough and decreased lung capacity [36]. In the present study, the ammonia level in the shelter decreased significantly, especially in the zeolite group (Table 3). A study conducted on cattle reported that adding zeolite to the litter in sloping shelters with straw litter could be a promising way to reduce carbon dioxide, nitrous oxide, and ammonia emissions [37]. The present study concluded that a decrease in the level of ammonia in the shelter environment due to zeolites will contribute to the practice of sheep husbandry.

Biochemical analyses are often used to assess the physiological condition of animals [38]. In our study, quantitative changes in biochemical parameters were monitored to ascertain whether the sheep were in normal physiological condition. Urea and BUN levels generally indicate kidney health. The ammonia released during protein digestion is converted into urea and excreted by the kidneys. BUN and urea levels increase in renal failure, hypovolemia, dehydration, bleeding, shock, and high protein diet intake, whereas they decrease in liver diseases, fasting, and low protein intake [39]. Urea, BUN, and creatinine levels were consistent with the reference values reported by Nisbet et al. [40], Şimşek et al. [41], Toprak et al. [42], and Comba et al. [43]. Although serum levels of muscle enzymes including those of creatine phosphokinase (CPK or CK), aldolase, and lactic dehydrogenase (LDH) are used to diagnose muscle disorders, CK level is the most commonly used indicator in practice. CK values obtained in the study were consistent with those reported by Comba et al. [43]. Measurement of parameters such as triglyceride, cholesterol, high-density lipoprotein, and LDL levels is referred to as lipid profile tests [44,45]. These tests are critical in cardiovascular diseases. Triglyceride values found in the study were consistent with those reported by Udum et al. [44], whereas they were lower than the values reported by Kurt et al. [45]. Fatty liver is a problem frequently associated with intensive animal farming. This may be one of the reasons for decreased blood cholesterol levels. In the literature, it has also been reported that zeolite added to feed adsorbs bile acid salts on the surface of the digestive tract, leading to a decrease in serum cholesterol level [46]. The present study did not find any differences in cholesterol levels between the groups. This may be due to the fact that zeolite was not given to animals as feed, but was only used as litter material. Serum cholesterol values found in this study were similar to those reported by Nisbet et al. [40], Kurt et al. [45], and Şimşek et al. [41]. Serum or plasma concentrations of different oxidants and antioxidants can be measured separately by direct or indirect methods. However, there are advantages and disadvantages of measuring each parameter separately. These measurements do not provide a general cumulative measurement of oxidative and antioxidant status. Individual measurements require time consuming, costly, and complex techniques. Therefore, we used measurements of TAC, TOS, and OSI in this

study, which are more economical<sup>[47]</sup>. TAC and TOS values obtained in the present study were similar to those reported by Mis et al.<sup>[48]</sup>, whereas there was a difference in terms of OSI values. This may be due to the difference in the methods used to calculate OSI values or having used a different breed of sheep in the present study. Blood parameters differed between animal species, as well as by the race, age, and sex of animals, regions where they were bred, and their diets<sup>[40]</sup>.

In the present study, it was observed that the use of zeolite litter in tent-type sheep shelters decreased the ammonia level in the shelter environment and improved milk yield and SCC. It was determined that mixing zeolite with litter material did not have a negative effect on milk quality parameters, blood biochemistry, and oxidative markers of sheep. According to the results of the present study, it is recommended to use zeolite litter in the shelter environment to increase milk yield and improve animal welfare.

#### AVAILABILITY OF DATA AND MATERIALS

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

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#### CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

#### AUTHOR CONTRIBUTIONS

MK and AD planned, designed the research, analyzed all data and drafted manuscript, GG, BDD, AY and MÜB provided and help in the research. All authors discussed the results and contributed to the final manuscript.

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