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Investigation of milk melatonin and alpha casein levels in sheep

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Abstract

This study was conducted to determine melatonin and alpha (α) casein levels in sheep milk. The study material was 40 milk samples taken after milking from, healthy, 3-year-oldlactating sheep from 4 different farms (10 sheep from each farms). In the milk samples taken, melatonin levels were determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) and the density of α-casein levels by electrophoresis (SDS-PAGE).

It was determined that the lowest value of milk melatonin levels in the enterprises was 80.0 ng/L and the highest value was 92.4 ng/L. In addition, these values were determined as 82.7 ng/L and 84.3 ng/L in the other two enterprises. There was no significant difference between farms in terms of melatonin levels ($p>0.05$). When α-casein levels were examined, a significant difference was found between farms ($p<0.001$). α-casein level density was also lowest in the enterprise with the lowest melatonin level. It has also been determined that casein density is also high in farms where melatonin levels are high. As a result of the study, melatonin and α-casein levels were determined, also a parallelism was observed between the two parameters. Therefore, it has been concluded that farms can benefit from these parameters in the growth, development and disease resistance of the offspring.

Keywords: Melatonin; Milk; Alpha casein; Sheep; Production

1. Introduction

Due to the climatic conditions, land structure and the large and generally low quality of pastures, sheep breeding has an important place in the livestock branch [1]. It is an important source of income for the people in pastures and pastures that are not used agriculturally and have arid climate conditions [2]. Melatonin is a hormone secreted from the pineal gland in a light-free environment. It regulates conditions such as reproduction, sleep, immunity, body temperature, hormonal activity regulation and anti-inflammatory activity [3]. Reproduction is a very important process for all living things. The reproductive cycle successful is the light-dark cycle and the physiological conditions related to it. In these physiological conditions, the most important effect is exerted by the melatonin hormone, which regulates its production in a light-dark rhythm [4]. The most important factor affecting the synthesis and release of melatonin is light. In other words, the rhythm of light darkness and day and night controls the regulation of synthesis and release.

Casein, known as the main protein of milk, constitutes approximately 80% of the protein in milk. In particular, it has effects such as ensuring the growth, development, resistance to diseases and increasing muscle mass of the puppy. Milk casein level affects the growth, development, weight gain, etc. of lambs. Since conditions such as these cannot be known, the amount of α-casein in milk is actually important in determining the breeding animal and increasing productivity.

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Melatonin levels vary depending on seasonality and day length in sheep [5]. In parallel, milk components such as casein, which is effective in growth, development and muscle formation also differ. In this study, it was aimed to determine the melatonin and α-casein levels in the milk samples of Akkaraman sheep raised by public in Elazig province and to reveal the possible relationship between these two parameters.

2. Material and methods

2.1. Animals and experimental design

In the study, milk from sheep belonging to the TAGEM/23SAV2012-03 sub-project carried out within the scope of 'Public Small Cattle Breeding National Project' under the coordination of the General Directorate of Agricultural Research and Policies was used. This study was carried out with the approval decision of Animal Experiments Local Ethics Committee.

The material of the study consisted of 40 Akkaraman sheep. Within the scope of the study, 40 healthy, 3-year-old, lactating women, who had at least one birth, were subject to the same care and feeding, and had not received any antibiotic/drug treatment in the last 3 months. Milk samples were collected from sheep (4 different farms, 10 sheep from each farm). Farms were called as I, II, III and IV. A total of 40 milk samples were collected, 10 from each enterprise (n = 40 milk samples). Milk was obtained from morning (10:00-11:00) milking, when the owner of the farms was doing the only milking. Milk samples were taken, approximately 10 mL in total, by wiping the udder lobes and teat opening heads with wet cotton, and by hand milking from both lobes in ewes that were milked once after the first few milkings. The collected milk samples were brought to the laboratory in a cold chain and stored at -20 °C until application.

2.2. Determination of milk melatonin levels

Analysis of melatonin levels in milk samples was determined by the Enzyme Linked Immunosorbent Measurement (ELISA) method. Sheep melatonin ELISA kit (Sunred-201-07-0803) was used to determine melatonin level. Milk samples were centrifuged at 3000 rpm for 20 min. The resulting supernatant was studied as specified in the kit procedure. Absorbances were read spectrophotometrically at 450 nm in an ELISA reader (Bio-tek ELX800). Bio-tek device (ELX50) was used as an automatic washer for plate washing. Results are given as ng/L. The intra-assay value of the kit was: CV< 10%, CV< 10%, measurement range: 3-600 ng/L and sensitivity: 2.631 ng/L.

2.3. SDS-PAGE analysis of milk α-casein

Milk samples were diluted 1/10 (v/v) with 25 mM Tris HCl pH:7.4. The total protein amount of each sample was determined by nanodrop spectrophotometry (Thermo Fisher 2000c) and the samples were made ready for SDS-PAGE by boiling with 4X sample solution for 5 min, with a final volume of 30 µg/25µl. Milk samples were separated according to their molecular weight in 10% running gel at 130V current. In order to determine casein levels, a protein weight marker (Intronbio-24052) was added to the first well along with a 5 mg/ml casein standard (Sigma C7078) and the bands obtained at approximately 25 kDa were determined to be casein. The obtained bands were calculated as optical density (%) of casein fractions with the ImageJ analysis program.

2.4. Statistical analysis

A program IBM® SPSS 22 package program was used for statistical analysis. Differences between groups and intensity of the bands were subjected to one wasy analysis of variance (ANOVA), followed by a post hoc Tukey test was used in multiple comparisons of groups. Results are expressed as mean ± standard error (SE). Differences were considered significant at $p<0.05$.

3. Results

3.1. Milk melatonin levels

In farms, milk melatonin levels were determined by ELISA and presented in Figure 1. It is seen that the highest melatonin value in the farm I. It was determined that the melatonin value of farm I was 92.4 ng/L. The lowest value is 80.0 ng/L in farm IV. The melatonin value was determined 84.3 ng/L in farm II and 82.7 ng/L in farm III. There was no significant difference between farms in terms of milk melatonin levels ($p>0.05$). Additionally, the lowest and highest melatonin values of the farms are presented in Table 2.

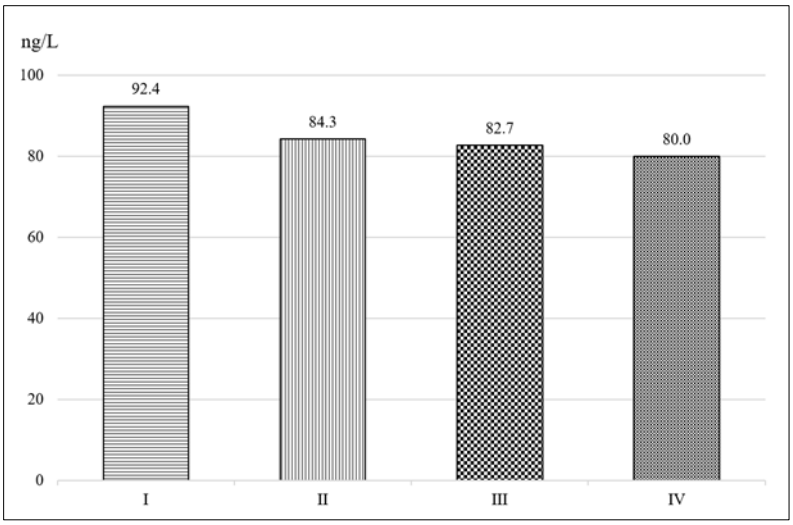


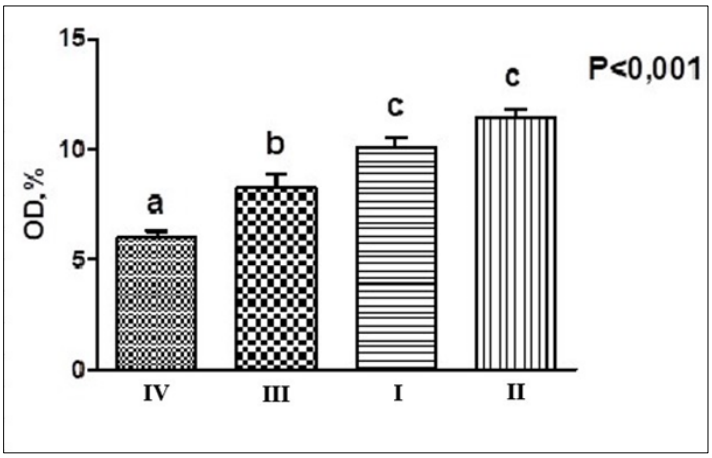
Figure 1 Milk melatonin levels in farms

Table 1 Minimum and maximum melatonin values of farms

Farms	Minimum (ng/L)	Maximum (ng/L)
I	76.3	108.7
II	71.2	93.0
III	62.9	98.1
IV	73.6	88.5

3.2. Determination of α-casein levels by SDS-PAGE

The density of casein levels in milk samples in farms are presented in Fig. 1. The highest casein density in the milk samples in farm II, while the lowest density determined in farm IV. It was determined that it was in operation. There were significant differences between farms in terms of casein levels in milk samples ($p<0.001$). The casein level in farm IV was lower than the other three farms ($p <0.001$). Casein levels in farm II increased compared to the other farms. This increase was significant in farms III and IV ($p<0.001$), while it did not express any significance in farm I.



a,b,c: Different letters on the columns indicate statistical difference; OD: Optical density (density)

Figure 2 Casein levels in farms

4. Discussion

It is known that milk is an important food for living. Milk, it is the 'most perfect food' in terms of nutrition because it contains protein, fat, carbohydrates, minerals, vitamins and amino acids. Seasonal variation in milk composition has been associated with several factors, among which environmental conditions such as photoperiod may play an important role [6]. It has also been found that melatonin concentrations increase and prolactin secretion decreases in short-day animals exposed to reduced photoperiod [7]. Molik et al. [8] showed that the milk yield of sheep varied according to day length. It has been observed that sheep lactating on a short-day regime produce 50% less milk than sheep milked on a long-day regime.

In species such as sheep that show seasonal breeding, the chemical composition of milk is significantly affected by environmental factors, climatic conditions and season (photoperiod length) [7, 9]. It has been determined that the fat content of the milk of ewes lambing in autumn is higher than the milk of sheep lambing in winter [10, 11]. Sheep milk contains more magnesium, calcium, manganese, phosphorus and zinc than cow milk [12, 13]. Therefore, calcium and phosphorus, which are necessary for the growth and bone development of newborns, are abundant in sheep milk. Calcium plays an important role in milk digestion. It is stated that calcium bioavailability in sheep milk is associated with high casein levels [14]. One of the most important nutritional benefits of milk is its high protein content. Approximately 80% of the protein part consists of casein. It has been stated that the amount of casein in milk is highest in sheep milk, followed by buffalo, goat and cow milk, respectively [15].

In this study, it was determined that the lowest milk α-casein level was in the farm IV. In addition, the highest value is in the farm II. Generally, it was determined that there was a significant difference between the farms in terms of milk casein levels ($p<0.001$). The casein levels are ranked from low to high, the order is farm IV, III, I and II. Therefore, casein is of great importance in feeding puppies with milk in terms of calcium bioavailability and digestibility of milk. These differences in farms are important in terms of growth and bone development of milk-fed puppies, especially calcium bioavailability has a strong relationship with high casein levels. It will be inevitable for the puppies to be fed with milk from farms with high milk casein levels to make a difference compared to other puppies in terms of bone, growth and development.

In another study where casein levels were determined in different animal milks [16], the amount of α-1 casein fraction was determined as 1.51 μg/L in sheep milk, 1.29 μg/L in goat milk, 1.40 μg/L in cow milk and 1.33 μg/L in buffalo milk. It was statistically determined that the α-1-casein fraction amount of casein was highest in sheep milk and lowest in goat milk. In a study conducted in cows, Barry and Donnelly [17] reported that casein concentration increased in early lactation and then decreased during the remainder of lactation. Similar results were found by Lucey and Fox [18], reported that late lactation milks may have significantly lower casein concentrations than mid-lactation milks. In casein protein, the fact that α-caseins contribute to a large portion of total casein and are maximum in mid-lactation in control milk is also supported by the findings of Ostersen et al. [19], found that the proportion of α-casein in total casein decreases at the end of lactation. The varying ratios of casein fractions according to lactation stages may be due to the increase in plasmin activity in the late lactation stage, because more plasmin enters the mammary glands in late lactation [20]. The decrease in plasmin activity observed in normal milk in the mid-lactation phase is probably associated with a decrease in pH that inhibits enzyme expression, therefore it has been stated that the maximum casein concentration in milk is observed in the mid-lactation phase [21].

Ispir et al. [22], measured melatonin and total protein levels in raw, pasteurized and boiled on goat milk. Melatonin levels were found to be 4.20 pg/ml in boiled and 3.19 pg/ml in raw milk samples. Therefore, melatonin level was found to be higher in boiled milk. It was also determined that the total protein level was higher in boiled milk. The study conducted is also appropriate in this aspect. It has been shown in many studies that melatonin release is associated with the reproductive system [23, 24]. Melatonin levels may vary depending on animal species, age, dose and time of administration. Additionally, melatonin levels vary in animals showing seasonal estrus.

In this study, milk melatonin levels were determined and the highest melatonin value belonged to farm I (92.4 ng/L) and the lowest value belonged to farm IV (80.0 ng/L). There was no difference ($p>0.05$) between farms in terms of milk melatonin levels. II. Therefore, although these values did not differ between farms, they were parallel to milk casein levels. Milk melatonin values were found to be high in I and II farms, also high milk casein levels in two farms. Ispir et al. [22] conducted on goat milk, the total protein level was also high in the group with high melatonin level, which is consistent with the study.

In some studies of investigating melatonin levels [25, 26], it was determined that the melatonin value in cow and goat milk was 5-25 pg/ml. In addition, it has been determined that the melatonin level in cow milk milked at night is ten

times higher than in daytime milk [27]. In a study where milk melatonin level was measured by solid phase extraction and mass spectrometry [28], the average melatonin level in breast milk and cow's milk was measured as 16.52 pg/ml and 14.57 pg/mL, respectively. In another study examining the melatonin level in milk [29], the average melatonin level was measured as 6.98 pg/ml in cow milk, 5.62 pg/ml in UHT milk and 4.71 pg/ml in bulk tank milk. The average melatonin concentration was found to be 14.87 pg/ml in night milk and 6.98 pg/ml in daily milk. In addition, it was determined that the melatonin concentration of milk in winter was higher than in summer. In another study [13], the average melatonin level in sheep was determined to be 13.7 ng/L in the spring morning, 9.7 ng/L in the spring evening, 13.1 ng/L in the summer morning and 9.3 ng/L in the summer evening. The highest melatonin value was determined in the spring morning (evening milk). Melatonin values in this study are similar to those of the researchers.

As a result of the study, it was determined that the highest value of sheep milk melatonin level was 92.4 ng/L and the lowest value was 80.0 ng/L. It was determined that there was no significant difference in terms of melatonin level between farms. Additionally, it was determined that there was a significant difference between the farms in terms of milk α-casein levels.

5. Conclusion

Also, the casein level was also high in farms with high melatonin levels, and the casein level was also low in milk with low melatonin levels, and a parallelism was observed between these two parameters. For this reason, we believe that farms should benefit from these parameters in cases such as growth, development, immunity and resistance to diseases in the offspring. Therefore, it gives the impression that it will contribute to the literature and raise awareness of breeders on this issue.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

This study was carried out with the approval decision of Animal Experiments Local Ethics Committee.This study was carried out with the approval decision of Fırat University Animal Experiments Local Ethics Committee (Number:2022/08).

Clarification

This article is a summarized from master's thesis of Sahin's.

Availability of Data and Materials

The data results obtained in this study are available from the corresponding author upon request.

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