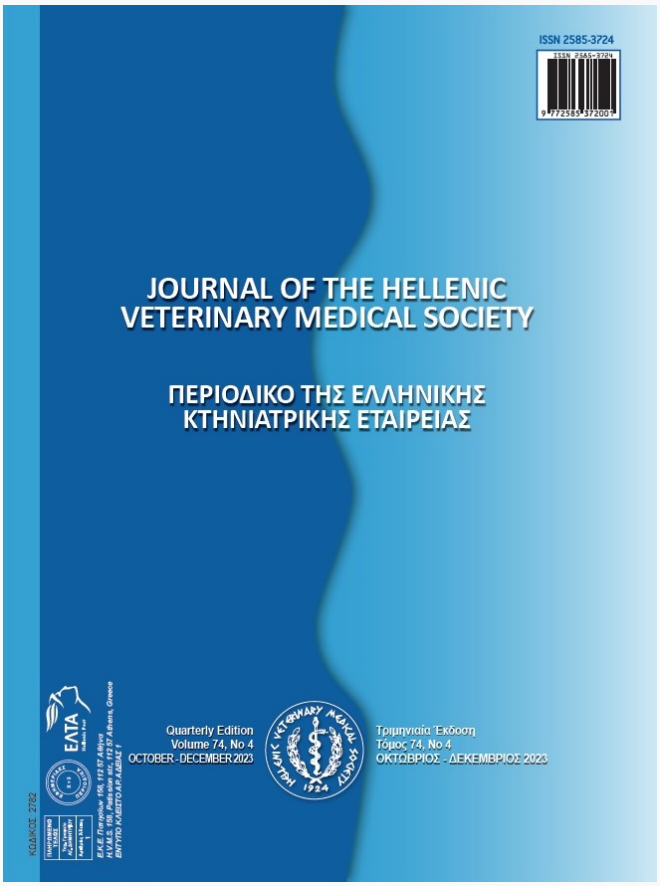
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Research article
Ερευνητικό άρθρο

Controlling the photoperiod to raise the melatonin content of sheep milk. Photoperiod control and milk melatonin content

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ABSTRACT: The aim of this study was to determine the effect of changing photoperiod time on the melatonin content of sheep milk. The animal material of the study consisted of 40 heads of Awassi sheep that gave birth in May 2021. The sheep were given roughage containing dry matter with 2.5% of their body weight and concentrated feed (2400-2600 kcal ME and 15-16% Crude Protein) as the 40% of their milk yield each day during the lactation period. The experimental ewes were randomly assigned into two groups with 20 animals in each. While the control group sheep were managed with normal day-time length during the lactation, the photoperiod restriction group were kept in the shed with 16 hours of darkness and 8 hours of daylight for 2 months. At the end of the two months, ewes of the both groups were managed in normal daylight until the end of lactation period. During the photoperiod restriction term, milk samples were taken at an interval of 14 days, while the milk composition was determined by a milk analyser and melatonin contents were determined by using LC MS/MS. In addition, lactation milk yield and lactation periods were estimated for all sheep. Statistical evaluation of the obtained data was done by using the SPSS package program. At the end of the study, the application of the extended dark period was found to be affected milk yield ($P<0.05$) as well as melatonin amount in the milk ($P<0.001$). Significantly higher melatonin amount was observed in the milk of sheep kept in a long-term dark environment compared to those exposed to normal photoperiod (11.06±7.24 pg/ml vs 6.12±4.55 pg/ml; $P<0.001$, respectively). As a result, it was determined that the prolonged dark period during a day increases the milk melatonin content. It can be also stated that the sheep's milk to be produced with this application could be a product that could take place in the functional food market.

***Keywords*:** Awassi, milk composition, antioxidant

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INTRODUCTION

It is known that seasonality in mating for small ruminants increases with the effect of the melatonin hormone, especially as we move away from the equatorial region. Because of this characteristic, the melatonin hormone has been used for many years in small ruminant husbandry for the purpose of oestrus synchronisation. However, the length of the mating season can also vary depending on the breeds of sheep and the regions where they are raised (Dellal and Cedden, 2002). This causes seasonal fluctuations in the price of sheep milk and may affect the profitability of the enterprises.

While there is a problem of insufficient or unbalanced nutrition in the world, the demand for functional foods is also increasing. It has been observed that the interest in foods that strengthen body resistance and the immune system has increased especially during the Covid-19 pandemic. In this context, one of the materials that attracted attention was melatonin which plays a role, especially in sleep regulation, immune system and increasing body resistance.

Melatonin is a neurohormone synthesized by the pineal gland located deep in the middle of the brain of mammals including sheep, goats, cattle and humans. The most important feature of this hormone is that it is synthesized during the dark period of the day, depending on the length of the photoperiod. In other words, the length of the day affects the secretion of melatonin. The pineal gland works like a biological clock, increasing melatonin secretion during periods of increased night-time. As a consequence, plasma melatonin level also changes depending on the season (Dardente, 2007; Çınar et al., 2011). In many studies, it is stated that milk yield in sheep, goats and cows varies according to the length of photoperiod and therefore the melatonin concentration in the blood (Dahl et al., 2000; Misztal et al., 1997; Molik et al., 2006; Molik et al., 2013; Elhadi et al., 2022).

The change in the amount of melatonin hormone in milk according to the photoperiod is an important issue in terms of affecting the quality of milk. There are different studies stating that the melatonin hormone plays a role in regulating sleep, acts as an antioxidant and anticancer agent, and plays a role in the prevention of osteoporosis (Atasoy, 2019; Romanini et al., 2019).

In summary, melatonin is a hormone that plays an excessively critical role in the welfare of people. For this reason, studies to increase the melatonin content in milk may be important for functional purposes. The aim of this study, in this perspective, was to determine the effect of controlling the photoperiod time on the melatonin content of sheep milk.

MATERIAL AND METHODS

All experimental procedures were carried out according to the permission given by Hatay Mustafa Kemal University Local Ethics Committee with a batch number of 2021/02-18.

This study was carried out in Zengen village of Konya province Ereğli district (37°50ı N, 34°10ı E; Altitude 1080 m). In the study, 40 heads of Awassi sheep, which gave birth to singletons at their second lambing, were used. The animal material of the project was selected from among the sheep that gave birth at the beginning of May 2021, taking into account their age. The ewes were fed with alfalfa hay as well as concentrate feed containing 2394 kcal metabolizable energy and 17% crude protein in the dry matter during the experiment (Table 1).All sheep had access to potable water whenever they wanted. While the hay was offered as approximately 3% of the average body weight of the ewes, the daily concentrate feed was given as 40% of daily milk yield.

Table 1. Composition of the diet fed to the ewes

Ingredients	Percentage
Barley (890 g DM, 2937 Kcal ME and 110.4 g CP kg−1)	32
Maize (870 g DM, 2900 Kcal ME and 87.0 g CP kg−1)	25
Soybean cake (890 g DM, 2500 Kcal ME and 440.0 g CP kg−1)	10
Cotton seed cake(900 g DM, 2025 Kcal ME and 319.5 g CP kg−1)	18
Sunflower seed cake (890 g DM, 2300 Kcal ME and 300 g CP kg−1)	5
Yeast, vitamin and mineral mixture (obtained from a commercial source)	10
Calculated composition per kg fresh diet	
Metabolisable energy (Kcal)	2394
Crude protein (CP) (g)	173

The ewes were randomly assigned into two groups, each with 20 animals. While the control group (CG) were managed at normal day length during the lactation, the sheep in the extended dark group (EDG) were exposed the photoperiod restriction for 2 months beginning from June 30, 2021. This practice in the EDG was terminated on September 20, 2021. These ewes in the EDG were kept in darkness for 16 hours and in daylight for 8 hours every day.During this 2-month period, 5 sheep in the EDG group and 2 sheep in the CG group were excluded from the trial due to mastitis. After these two months, ewes of both groups were managed in normal daylight like as the control group until lactation was completed. During the photoperiod restriction, milk samples were taken from sheep at 14-day intervals starting from 30th of June, 2021. These samples were used to determine the melatonin contents and composition of the milk while also estimating lactation total milk yield per ewe.Individual milk yields and lactation periods of sheep were calculated according to the Holland method (Özcan, 1989).

Melatonin Analysis: Melatonin content was evaluated according to the method reported by Karunanithi et al. (2014). Melatonin standard was added to the milk samples brought to the laboratory in order to minimize errors caused also by extraction. Next, a purification step was applied to eliminate other elements that would interfere with the analysis in the LC-MS/MS. For this purpose, the samples were transferred to the solid phase extraction (SPE) cartridge. After all the samples passed through the cartridge, the cartridge was washed with solvent and the solution containing the solvent and the analyte was evaporated to remove the solvent. A solution was obtained by diluting the analyte with methanol, and the Thermo LC-MS/MS device, which is a combination of TSQ Quantum Access Max MS/MS system and Thermo UltiMate 3000 model UHPLC system(Thermo Fisher, USA), was used for the quantitative determination of this solution. The device consists of HPG-3400RS model gradient pump, TCC-3000RS model column furnace and WPS-3000RS model autosampler. Chromatographic separation was performed on a Kinetex C18 (100 mm×2.1 mm, 2.6 µm particle size) columns (Phenomenex, CA, USA).

Milk Quality Analysis: In milk samples, fat, fat-free dry matter (FDM), lactose, protein, salt and density were detected by Milkotester Master Classic (LM2 P1, Bulgaria).

Statistical analysis: Statistical evaluations of the obtained data were made using the SPSS package program(SPSS inc., USA).The mathematical model for milk yield characteristics is as follows:

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

Y_{ij}, recorded value of the j^{th} animal in the i^{th} group

μ, mean of population

α_i , effect of the group, i = CG, EDG

e_{ij}, error term

The mathematical model for melatonin content of the milk is as follows:

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

Y_{ijk}, recorded value of the k^{th} animal in the i^{th} group and j^{th}sample collection day

μ, mean of population

α_i , effect of the group, i = CG, EDG

β_j , effect of the sample collection days, j = 1, 2, 3, 4, 5, 6, 7

e_{ij}, error term

Comparisons between group averages were made by using the Duncan multiple range test in the same software.

RESULTS

The daylight time the CG group was 14 hours and 45 minutes on 30th of June 2021 when the photoperiod restriction started on the EDG group. After this date, the nights continued to get longer and daylight time was determined as 12 hours and 25 minutes on 22th September 2021 when the treatment was terminated. In other words, daylight for the CG group decreased naturally as 2 hours and 20 minutes during the treatment period.

The average lactation duration, lactation and treatment period milk yields as well as melatonin amounts in the milk for the control (CG) and extended dark (EDG) groups during the study are given in Table 2.

As seen in this table, the group exposed to long-term darkness in order to increase the melatonin content in milk ($p<0.001$) gave less milk than the control group both during the treatment period and during all lactation ($p<0.05$). However, the lactation durations

of both groups were found to be similar (p>0.05).

As seen in Table 3, prolonged dark application caused increases in melatonin content in milk on all control days. The date of 30 June, 2021, when the first milk samples were taken, is one day before the start of the extended dark application. The last milk sample was also taken on September 22, 2021, one day after the end of this practice. As seen in the figure, while there was no significant change in milk melatonin contents between the groups at the beginning and end of the application, the practice caused significant increases in milk melatonin content in the other control days.

The fat-free dry matter, protein, fat, lactose and

Table 2. Some milk yield characteristics in the groups ($\bar{x}$ ± s.e.)

Groups	TPMY (kg)	LMY (kg)	LD (days)	MA (pg/ml)
CG	34.79±3.47	132.02±3.48	189.76±5.26	6.12±4.55
EDG	25.03±2.57	113.68±4.75	178.52±5.83	11.06±7.24
Significance	P<0.05	P<0.05	P>0.05	P<0.001

CG, control group; EDG, extended dark group; TPMY, treatment period milk yield; LMY, lactation milk yield; LD, lactation duration; MA, melatonin amount in milk

Table 3. Changing of melatonin contents (pg/ml) in milk produced by the ewes in the groups

days	$\bar{x}$ ± s.e.	min	max
Extended dark group			
30th of June, 2021	5.21±0.75[a] (19)	1.28	13.93
14th of July, 2021	18.72±1.05[d] (16)	10.71	24.35
28th of July, 2021	11.63±1.39[b] (15)	4.70	21.31
11th of August, 2021	10.81±1.13[b] (15)	5.24	18.40
25th of August, 2021	10.53±0.88[b] (15)	5.28	18.33
8th of September, 2021	15.60±1.24[c] (15)	2.91	23.70
22nd of September, 2021	6.21±0.66[a] (15)	1.40	10.47
P	<0.001		
Control group			
30th of June, 2021	4.05±0.14[a] (20)	1.84	6.34
14th of July, 2021	4.25±0.20[a] (19)	2.04	7.46
28th of July, 2021	6.59±0.79[b] (19)	1.30	10.64
11th of August, 2021	6.92±0.48[b] (19)	2.92	10.55
25th of August, 2021	6.75±1.09[b] (19)	0.13	17.72
8th of September, 2021	6.50±0.59[b] (18)	2.37	9.77
22nd of September, 2021	7.07±0.57[b] (18)	2.28	12.52
P	<0.01		

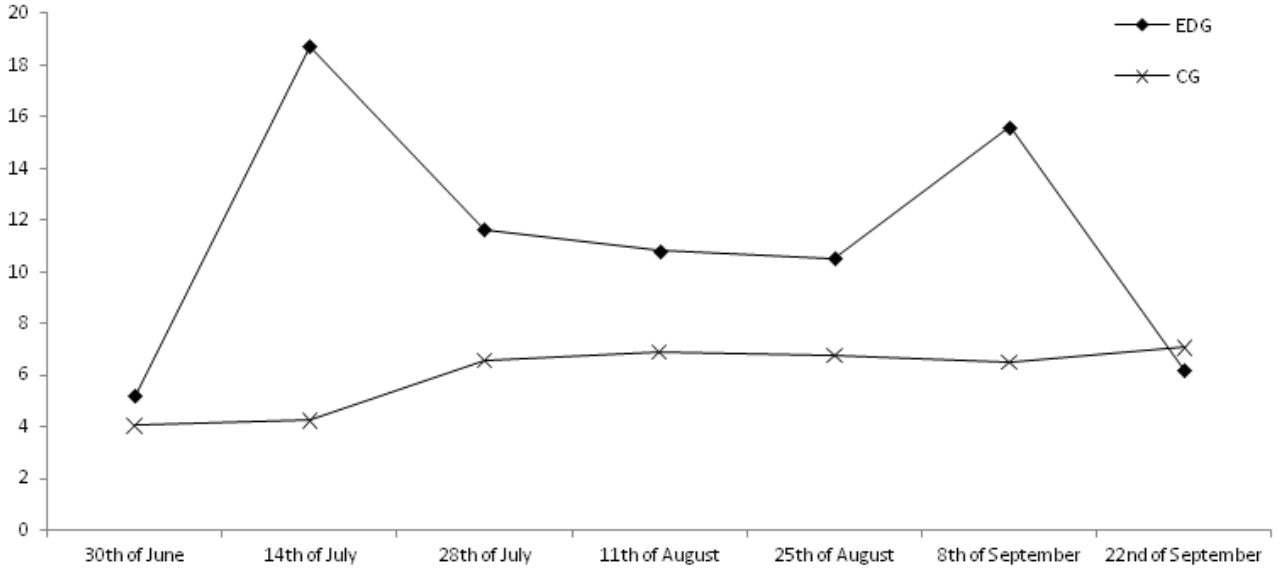


Figure. Changing of milk melatonin content (pg) in the groups during restricted light period

Table 4. Average milk compositions of ewes in the groups ($\bar{x} \pm$ s.e.)

Groups	FDM%	Fat%	Protein%	Lactose%	Salt%	Density
CG	10.91±0.80	7.48±0.40	4.01±0.03	6.02±0.04	0.84±0.01	1.04±0.28
EDG	10.79±1,38	7.02±0.19	3.88±0.05	5.93±0.07	0.89±0.07	1.04±0.44
Significance	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

CG, control group; EDG, extended dark group; FDM, fat-free dry matter

salt contents and density values determined from the milk of the ewes in the experimental groups are given in Table 4. As seen this table, prolonged dark application did not have any effect on the investigated milk components.

DISCUSSION

The lactation length and lactation milk yield (Table 2) of ewes showed similarities as well as differences with the reports of different studies on Awassi sheep. The differences between the milk yields of the groups were found to be lower than the values reported for the same breed by Kaygısız and Dağ (2017).On the other hand, they were similar to the values reported by Nudda et al (2002). Because, as in other farm animals, milk yield characteristics of Awassi sheep are formed under the effects of environmental conditions together with the genetic structure of the animals. In particular, Kaygısız and Dağ (2017) carried out their study with the nucleus selectionflock reared in Ceylanpınar Agricultural Enterprise, and it can be expected that the milk yield is relatively higher in this flock. Reiad et al. (2010) reported the lactation period as 154.7 days and the lactation milk yield as 243 litres in their study with animals selected under intensive conditions in Syria. On the other hand, Üstüner and Ogan (2013) stated the milk yield as 197 kg in lactation lasting 184 days in Awassi sheep studied in Eskişehir, Turkiye.

In the study, the effects of prolonged dark application during the day on the amount of melatonin in milk were focused. Meanwhile, some other milk properties were also investigated. As can be seen from Table 2, extended dark application per day during the June-September period caused a decrease in milk yield in the current study. In the study conducted by Mikolayunas et al. (2008), it was stated that long-term photoperiod application during the lactation period increased the total lactation milk yield. Similarly, in the study conducted by Molik et al. (2013), it was stated that dark application reduces lactation milk yield. In another study by Molik et al (2006), it was stated that ewes lambed in June produced 50% less milk than ewes lambed in January, and decreased day length conditions reduced milk yield. In addition, Jimenez

et al. (2020) also stated that the milk yield in sheep giving birth in autumn, which is the period when days get shorter, is lower than those giving birth in spring.

As seen in Table 2, the CG group had also a higher mean in terms of lactation duration, but the difference between the two groups was calculated to be insignificant (P>0.05). This may be due to the relatively high standard error value calculated for the animals in each group, which represents the variation between the ewes (Bek and Efe, 1983).

In the study, the mean amounts of melatonin in the samples of both the extended dark and control groups were detected as 11.06±7.24 pg/ml and 6.12±4.55 pg/ml, respectively (P<0.001). In other words, the extended dark period per day caused an increase in the amount of melatonin in milk. As seen in Table 3 and Figure, 16 hours of dark exposure during the day affected the milk melatonin content of sheep on all control days, causing this content to be higher. It is known that light has the key role in secretion of melatonin in sheep milk which is higher than cows' milk just before the sunrise in the morning (Singh et al., 2011). In another study conducted by Şahin et al (2021), it was stated that the content of melatonin in night milk is higher than that in day milk and that night milk can be used for medical purposes.

It has also been reported by different researchers that the decreased photoperiod increases the amount of melatonin in milk in different breeds of sheep (Molik, et al., 2006; Şahin et al., 2021). Likewise, it was reported in the study conducted with cows that the melatonin content of night milk is higher than the milk produced during the day, and the melatonin content of milk produced in the winter is higher than the milk produced in the summer season (Romanini et al., 2019).

On the other hand, extended dark application per day did not have any effect on milk composition (Table 4). As can be seen in Table 4, the average fat- free dry matter, fat, protein, lactose and salt contents as well as density values of the milk in both groups were found to be similar to each other (p>0.05).These kinds of parameters can be affected by the management and feeding characteristics of animals and by the milk yield itself. For this reason, there may be differences among herds or even among years observed in the same herd. In the current study, extended dark treatment per day affected the amount of melatonin in milk, but no effects were observed for milk composition. Similarly, in a study by Cosso et al (2021) with sheep, melatonin administration did not affect the milk yield and composition. In their study, the ewes received 400 g of commercial concentrated food per head with addition to pasture at the daily milking period. The researchers randomly distributed the ewes into two equal groups of 50 ewes each (M and C). On March 1, group M received one subcutaneous implant (18 mg) of melatonin while group C was not treated. The researchers recorded individual milk yield every 15 d from 1 March to 30 April and analysed the milk samples for milk composition. On the other hand, in their study by Molik et al. (2011), it is stated that the application of shorterday-time significantly affects milk components. In this study, the researchers randomly assigned the ewes to three groups. Group I ewes raised under natural day length; Group II ewes raised under natural day length and were implanted with melatonin; Group III ewes exposed to an artificially short photoperiod (16D: 8L). During the lactation period milk samples were collected every 28 days to determine chemical composition and fatty acid content. The ewes were received 1.5 kg pelleted diet per day (7.5 MJ net energy and 220 g crude protein) as addition to pasture and hay from the 5th month of pregnancy to the end of experiment. All animals had free access to water and a mineral lick. The researcher informed that the administration of exogenous melatonin and the simulation of a short-day photoperiod during the summer period had significant effects on the milk levels of solids, protein, fat and lactose, and on the fatty acid content of sheep milk. The difference between our current study and this study may be due to breed, region or management differences.

CONCLUSION

Although there are many individual studies on the quantification of melatonin in milk or the importance of the melatonin hormone for both animals and humans, there are hardly any studies regarding the increase in the content of this hormone in milk naturally. With this point of view, it can be said as a conclusion that the prolonged exposure of Awassi sheep to the dark caused a decrease in milk yield and an increase in milk melatonin content without affecting milk composition based on the observations of the present study. The results obtained can be evaluated by producers who want to take place in the functional food market by producing milk with higher melatonin content.

CONFLICT OF INTEREST

The authors report no declarations of interest.

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