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Melatonin Differences Between Day and Night Milk in Primiparous Holstein Friesian and Jersey Dairy Cattle

Saim BOZTEPE [1,*], İsmail KESKIN [1], Ahmet SEMACAN [2], Fikret AKYÜREK [3], İbrahim AYTEKIN[1], Özcan ŞAHIN [1]

[1] Selçuk University, Faculty of Agriculture, Department of Animal Science, Konya, Turkey
[2] Laranda Livestock Enterprise, Konya, Turkey
[3] Selcuk of University, Faculty of Medicine, Department of Biochemistry, Konya, Turkey

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ABSTRACT

This study was conducted to determine the levels of melatonin in the day and night milk of Holstein and Jersey cows. In the study, samples of daytime milk produced from 27 head of Holstein and 27 head of Jersey cows in the first lactation, which were raised in a private dairy cattle enterprise in the Kaşınhanı neighborhood of Meram district of Konya city Turkey, and night milk samples taken from the same cows that were blackened for one (1) week were used. Melatonin levels in milk samples taken from day and night milk were determined separately for Holstein and Jersey cows with the help of Bovine Melatonin (MLT) Elisa Kit.

In the study, it was determined that the ratio of melatonin in day and night milk in Holstein cows was 2.912 pg/ml and 11.314 pg/ml, respectively, and the ratio of melatonin in Jersey cows was 2.924 pg/ml and 6.954 pg/ml in the same order. The difference between the melatonin levels of the day and night milk of Holstein and Jersey cows was found to be statistically significant ($p<0.01$).

At the end of the study, it can be stated that night milk can be used for medical purposes and a new production source may arise for producers since there is a significant difference in melatonin between day and night milk.

1. Introduction

The presence of a higher amount of melatonin in milk produced in the dark than in day milk and its relationship with human health has recently attracted the attention of researchers and has led to a remarkable increase in the number of publications on the subject.

Özçelik et al. (2013) reported from studies conducted in humans that while the blood concentration of melatonin is around 0-20 pg/dl during the daytime, it rises to 50-200 pg/dl at night. An average of 30 mg of melatonin is synthesized overnight. There are many factors that affect melatonin synthesis. Light is one of these factors. This hormone, which is secreted during sleep, is secreted intensively, especially at night (between 23:00 and 05:00). For this intense secretion to occur, the environment must be dark. This mechanism is similar in other mammals (Jainudeen et al. 2000; Balch 2010).

Romanini et al. (2019) determined the highest concentration of melatonin in individual cow's milk as 41.94 pg/ml. They reported that there are seasonal changes and that the highest concentration of melatonin is reached at night in winter. There are also studies revealing the relationship between health and melatonin secretion in night milk (Valtonen et al. 2005). Related to melatonin helping to improve symptoms of jetlag (a problem more commonly experienced by people who travel a lot and flight crews in general, with interrupted sleep, early waking, excessive sleepiness, difficulty falling asleep, and reduced sleep quality being the most prominent effects of jetlag) are reports (Liu and Borjigin 2006; Srinivasan et al. 2008). At the same time, melatonin is a very powerful antioxidant, stronger than vitamin C, E or beta carotene, preventing harmful oxidation. In this way, it can reduce the risk of hypertension, heart attack and some types of cancer. In addition, it has been stated that it can be useful in the treatment of Alzheimer's and cancer, preventing the regulation of the immune system, memory loss, vascular occlusion and stroke. Also, there is no toxic level of melatonin consumption (Balch 2010). Romanini et al. (2019) reported that melatonin-

* Corresponding author email: *sboztepe@selcuk.edu.tr*

rich night milk produced and marketed has benefits for public health and business people.

Photoperiod, which is defined as the duration of daylight that the animal is exposed to for 24 hours, also significantly affects milk yield. Photoperiod is applied in two ways as long day photoperiod (16-18 hours of continuous daylight exposure and 6-8 hours of dark exposure) and short day photoperiod (8 hours of day-light and 16 hours of dark exposure). Changes in the exposure time in the photoperiod significantly affect the physiology of many species. Photoperiod has more gained importance in recent years to increase animal health and productivity level in dairy cattle herd management. Considering the climate changes, this management factor will be more important in the future.

This study was carried out to reveal the differences between the melatonin levels in the daytime (10 hours of light) and nightly (14 hours of darkness) synthesized milk of Holstein and Jersey dairy cows and in addition to determining whether the production possibilities of night milk rich in melatonin were and whether there is a difference between these.

2. Materials and Methods

Material

The research was carried out in January and the photoperiod is 10 hours of light and 14 hours of darkness in Konya, where the research was conducted during this period. The average daily milk yield and days in milk of the cows in the first lactation, which constitutes the research material, were determined as 30.63±0.72 kg-75.91±4.21 day and 17.16±1.44 kg-73.25±4.01 day for Holsteins and Jerseys, respectively. Milk samples to be analyzed were obtained by milking the milk produced by 27 Holstein and 27 Jersey cows lighted 150 lux at eye level in night time in the first lactation in the afternoon (at 15:00-17:00 milking time) during the day. Then, Holstein and Jersey cows were kept in the barn, which was completely dark at night, for a week, and the nightly synthesized milk was obtained by milking before sunrise (at 3:00-5:00 milking time). Samples of both day and night milk for both breeds were taken into 50 ml tubes homogeneously to represent the total milk with the help of the milk sampling apparatus that can be mounted on the milking system.

Method

The milk samples taken were brought to the laboratory in the cold chain (+ 4 °C). Milk samples were homogenized at +4 °C, taken into 8 ml polypropylene tubes and transferred to the laboratory in a cold chain and dark environment.

After all samples were obtained, the analysis phase was started. Centrifugation was performed twice at 4500 rpm for 15 minutes at +4 °C in the laboratory. After each centrifugation, the fat layer accumulated in the upper part was separated, and the milk samples, which were completely separated from the fat, were kept at -80 degrees until the working day. On the working day, the samples were thawed first at -20 °C, then at +4 °C and finally at room temperature. After dissolution, homogenization was achieved by vortexing. Homogenized samples were analyzed using IBL brand Melatonin direct Saliva ELISA (Non-Extraction) and in accordance with the kit procedure, Rayto RT-2600 Microplate Washer (India) and BMG LABTECH (German) Enzyme-Linked Immuno Sorbent Assay in Selcuk University Faculty of Medicine Biochemistry Laboratory. (ELISA) Melatonin levels were determined in pg/ml via ELISA reader. The quantitation limits of the melatonin kit used are between 0.5 - 50 pg/ml and the samples were not diluted.

Statistical analysis

Analysis of the differences between melatonin levels in day and night milk in Holstein and Jersey cows was performed according to the repeated measurements experiment design (Two Factors Experiments with Repeated Measurements On One Factor Levels) in order to prevent dependence since the samples were taken from the same animals before and after darkening (Gürbüz et al. 2003). Statistical analyzes were made with the help of the RStudio statistical package program.

3. Results and Discussion

At the end of the study, it was determined that the level of melatonin in Holstein cows was 2.912 pg/ml in milk synthesized during the day and 11.314 pg/ml in milk synthesized at night.

In the samples examined in Holstein cows, it was observed that the lowest melatonin value in day milk was 1.168, the highest melatonin value was 5.887, and the melatonin ratio in night milk was between 3.520 and 21.510 (Table 1). In Jersey cows, on the other hand, melatonin level was determined as 2.924 pg/ml in milk synthesized during the day and as 6.954 pg/ml in night milk. The melatonin ratios in day milk of Jersey cows ranged from 1.085-4.577, and in night milk from 3.422 to 15.722 (Table 1). The melatonin level in night milk of Holstein cows was approximately 3.9 times the milk synthesized during the day and this difference was statistically significant ($p<0.01$). In Jersey cows, the melatonin level in night milk was approximately 2.4 times the milk synthesized during the day, and this difference was statistically significant ($p<0.01$). While the difference between the melatonin levels of milk synthesized during the day in Holstein and Jersey cows was statistically insignificant ($p>0.05$), the difference in milk synthesized at night was statistically significant ($p<0.01$).

Table 1
Melatonin levels and standard errors in day and night milk

Breed	Samples	n	$\bar{X} \pm S_{\bar{X}}$	Min-Max
Holstein Friesian (HF)	Daytime milk (pg/ml)	27	2.912±0.266	1.168-5.887
	Nighttime milk (pg/ml)	27	11.314±1.100	3.520-21.510
Jersey (J)	Daytime milk (pg/ml)	27	2.924±0.216	1.085-4.577
	Nighttime milk (pg/ml)	27	6.954±0.567	3.422-15.722

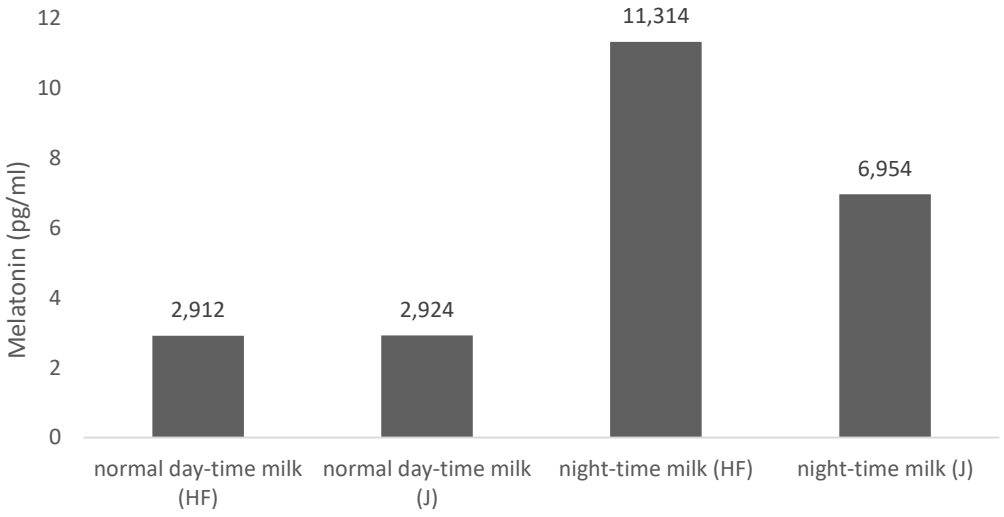

Figure 1
Melatonin levels in day and night milk in Holstein Friesian (HF) and Jersey (J) cows

The differences between melatonin levels in night and day milk of Holstein and Jersey cows can also be seen in Figure 1. According to Holzmann et al. (2019), the ration supplemented with vitamins (C) did not affect the concentration of melatonin in total milk. However, in the night milk, the melatonin level was 6.57 pg/ml in the cows fed the unsupplemented ration, while it was 11.06 pg/ml in the fortified ration group. Milk expressed at night showed melatonin concentrations 1.43 to 2.38 times higher than total milk per day. According to Romanini et al. (2019), in their study investigating melatonin levels and sources in cow's milk, reported differences in levels of melatonin in night milk and total milk according to winter and summer months. In the low yield group, the level of melatonin was found to be around 22 pg/ml in night milk in winter and around 10 pg/ml in summer, while it was around 8 pg/ml in winter and 6 pg/ml in summer in total milk. In the high yield group, it was found as 16 pg/ml in night milk in winter and 11 pg/ml in summer, while it was determined as 9 pg/ml in winter and 4 pg/ml in total milk. The values found in the current study were similar to those reported by Romanini et al. (2019). While Asher et al. (2015) found lower levels of melatonin levels in milk under dark and limited lighting conditions at night, researchers reported values between 15-20 pg/ml in the group with limited lighting, while melatonin level was around 30 pg/ml in night milk. Sahin et al. (2021), it was determined that the melatonin level in day milk of first lactation cows of Holstein breed was 103.70±6.61 pg/ml and 163.13±8.96 pg/ml in night milk. The difference between melatonin levels of day and night milk was statistically significant ($P<0.01$). The probable reason for this difference is that the kits used to determine the melatonin level are different (In some kits, the reading range for melatonin level varies between 0 and 1000 ((MYBIOSOURCE brand Bovine Melatonin (MLT) ELISA Kit (Competitive ELISA)), in some kits this reading is between 0.5 and 50. (IBL, Melatonin direct Saliva ELISA (Non-Extraction) varies) It may also be that the season and yield level of milk samples are different (high or low yield) (Asher et al. 2015).

4. Conclusion

Melatonin has a vital role for humans and is necessary for a healthy life. Sources rich in melatonin, especially milk, should be evaluated for a healthy and happy life. In Finland and Germany, melatonin-rich milk produced at night from cattle under the name of "night milk" has begun to be produced commercially (Valtonen et al. 2005; Mullins 2010). Valtonen et al. (2005) reported that even the lowest melatonin dose of 0.1 mg was 10 times higher than the total melatonin secreted at

night. In other words, it is not possible to meet this amount by consuming milk rich in melatonin. Accordingly, "night milk" produced from cows should be consumed at least half a liter based on the reports of Valtonen et al. (2005) in adults, especially children and the elderly, as a supplement to the melatonin secretion of patients. Regarding this issue, Valtonen et al. (2005) reported that melatonin secretion decreases with age and that they use night milk as a material for the elimination of sleep disorders in elderly people, and that they obtain positive results when the patients are given about 0.61 liters/day. Bae et al. (2016) compared the effects of consuming regular milk containing 100 pg melatonin and 47.5 mg tryptophan amino acids and a glass of overnight milk containing 1000 pg melatonin and 58.24 mg tryptophan amino acids. As a result, it has been reported that a glass of night milk containing 1000 pg melatonin provides an increase in sleep comfort and a decrease in insomnia throughout the day.

In some countries, milk rich in melatonin is marketed as “night time milk” separately from normal milk. In Turkey, it is necessary to make legal regulations in this regard and to produce and market this milk rich in melatonin. In this way, milk producers will be able to provide an additional income.

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