Revista de Chimie
https://revistadechimie.ro
https://doi.org/10.37358/Rev.Chim.1949

Vitamin A, E and D3 with Melatonin Concentration in Different Animals Milk According to Seasonal Changes and Periods

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Abstract: *Hormones and Vitamins are extremely important ingredients for healthy growth, development, and regularity of events in metabolism. In our study, it is aimed to compare melatonin hormone and vitamins A, E, D3 of goats, sheep and cows milk. Our sample consisted of 20 Simental Breed Cows, 20 Akkaraman Sheeps, and 20 Native Breeder Goats. Melatonin hormone was analyzed with Enzyme Plate Reader by using Rel Assay branded kit and to determine vitamin's levels high-performance liquid chromatography (HPLC). Goat milk melatonin level was at the highest level at night and summer. Regarding goats' milk, Retinol and tocopherol levels were higher in spring than in summer. Regarding sheep's milk, the melatonin level in the sheep's milk was most elevated at night and spring. There were also statistically significant differences between spring and summer milk of sheep in terms of Retinol, Tocopherol, and Vitamin D3. Retinol and tocopherol levels were higher in spring than in summer, but vitamin D3 was higher in summer than in spring. Regarding cow's milk, the amount of melatonin in milk was highest in winter and least in autumn. The amount of melatonin in spring was found to be higher than the amount of melatonin in summer. The amount of melatonin in spring was near to the amount of melatonin in winter, so there was not a statistical significance between them, and the amount of melatonin in summer was near to the amount of autumn, and no statistical significance was found between them.*

Keywords*: melatonin, vitamin, sheep, cow, goat*

1. Introduction

Hormones and vitamins are extremely important substances for healthy growth, development, and the regular functioning of metabolism. The recommended amount of milk consumption for adequate and balanced nutrition of healthy individuals varies according to age, gender, and physiological status (growth and development period, pregnancy, lactation, old age). Milk contains various levels of hormones with a limited nutritional or diagnostic value. Besides, many studies have been conducted to examine hormones physiological roles of human and bovine milk [1,2].

Melatonin (*N* -acetyl-5-metoksitriptamin) is a pineal hormone produced in dark periods and producing a transducer of photoperiodic information in many animal species, including cattle [3]. Melatonin is responsible for sleep control and circadian regulation seen at the highest concentration in the organism during the night [4]. With ageing, there is a significant decrease in the production of melatonin among the elderly, which may negatively affect sleep quality [5].

Serotonin serves as a precursor for the production of melatonin within the pineal gland [6]. Throughout the day, the pineal gland produces serotonin, and in the absence of light, serotonin is converted into melatonin. The production of melatonin within the pineal gland is stimulated by darkness and is inhibited by short-wavelength illumination [6]. Melatonin is a conversed molecule and is found in all organisms from prokaryotes to humans [7]. It is thought that another critical function of melatonin is that it is a potent free radical scavenger [7]. Melatonin levels can be influenced by many factors, such as heat, tide, and β-blocker drugs [8-10].

The number of somatic cells, which is one of the most critical factors determining milk quality, is related to oxidative metabolism and cellular immune functions in the mammary glands. Melatonin can have a positive effect on milk quality by protecting cellular structures from oxidative damage.

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Melatonin, which is easily soluble in water and lipids, quickly passes through the blood-brain barrier and cell membranes. Therefore, it is present in intracellular areas where free radicals are formed. In this way, the cell can protect its DNA from free radical attacks. In addition to its antioxidant characteristics and neutralizing free radicals, melatonin also stimulates the synthesis of numerous antioxidant defence enzymes that reduce the formation of free radicals through a nuclear receptor [11-13].

Vitamin A is a fat-soluble vitamin found in critical biological functions. It exists in three primary forms: retinol, retinal, and retinoic acid. The content of retinol in milk is affected by the β-carotene level in feeds [14, 15]. β-carotene in the diet is converted into retinal by the enzyme β-carotene-15-15'-dioxygenase in the intestinal epithelium and liver. Unlike cow milk, goat and sheep milk contains only retinol and usually does not contain a completely β-carotene converted from retinol [16]; thus this explains the reason for the observed differences in colour between dairy products of cattle and smaller ruminants [17].

When vitamin D3 (cholecalciferol) is exposed to ultraviolet radiation on the skin, it is formed in most animal tissues by breaking one of the chain bonds of 7-dehydrocholesterol. Cholecalciferol is a white and crystalline compound soluble in fat and organic solvents and is stable against heat and oxidation in light alkali or acid solutions [18]. The existence of an anti-sterility vitamin (a phrase used to describe the effects of vitamin E, prevention, and treatment of infertility) was suggested by Sure [19]. This factor was named "Vitamin E" by Sure [19]. Vitamin E works with selenium and ascorbic acid in the glutathione peroxidase enzyme to stop the chain reactions of polyunsaturated fatty acid peroxidation [20].

In our study, it is aimed to compare melatonin hormone and vitamins A, E, D3 in goat's, sheep's and cow's milk in terms of the period of day and seasons.

2. Materials and methods

2.1. Sample

The sample of the study consists of 20 Simmental breed Cows, 20 Akkaraman breed Sheep, and 20 Domestic Hair Goats. The cow group was actively milked in the four seasons. Goats and sheep were milked in the spring and summer seasons. Milk samples were taken separately as morning and evening milk on a day determined for each season. The milk samples of the cows forming the subject group were taken from a farm located on the Van Erciş road. Milk samples belonging to the other group consisting of goat and sheep were taken from the farm owned by Van Yüzüncü Yıl University. To animals were given green grass in summer, spring, and autumn. In the winter, dry grass and prepared feed were given to feed them.

2.2. Methods

Milk samples were taken four times in the morning and evening for cows. Goats and sheep are seasonally polyestrous animals, and breeding activities are associated with short-day and long-day cycles. As the days begin to shorten, the duration of receiving light decreases, and melatonin secretion from the pineal gland increases. Sheep’s milk samples were taken in the morning and evening in two seasons. Likewise, goat’s milk samples were taken in the morning and evening. According to seasonal changes and daily milking periods, samples were taken at 06:00 am for the morning milk and 5:00 pm for the evening milk, and melatonin hormone and vitamin levels were determined for each animal type by keeping them in the cold and without waiting much.

2.3. Reading processes for melatonin levels

2.3.1. Tools and materials used in the determination of melatonin

Eliza Plate Reader (Mega Medicine Rel Assay brand Gaziantep, Turkey)
Eliza washer (Mega Medicine Rel Assay brand Gaziantep, Turkey)
Sheep Melatonin (MT) Elisa Kit (Mega Medicine Rel Assay brand Gaziantep, Turkey)
Bovine Melatonin (MT) Elisa Kit (Mega Medicine Rel Assay brand Gaziantep, Turkey)

Goat Melatonin (MT) Elisa Kit (Mega Medicine Rel Assay brand Gaziantep, Turkey)

50 µL milk sample was taken, and the measurement was performed at 450 nm with Enzyme Plate Reader by using Rel Assay branded diagnostics kit. The graph output was obtained based on the optical densities received by working on the samples whose concentrations are known, and the concentrations of the samples with unknown concentration values were obtained from the graph. It was carried out by a private company (Mega Tıp, using Rel Assay branded diagnostics kit, Gaziantep, Turkey) by following the procedures found in the diagnostics kit [21].

2.4. Determination of vitamin A, E, D3

High-performance liquid chromatography (HPLC) (Thermo Scientific Pinligan Surveyor), HPLC column (C-18, 250x4.6 mm, Supelco) were used.

2.5. Extractions of the milk samples

2.5.1. Chemicals

Retinol standard (vitamin A) (Sigma)
α-tocopherol standard (vitamin E) (Sigma)
Cholecalciferol standard (vitamin D3) (Fluka)
Ethanol (Merck)
Methanol (HPLC Grade, Merck)
n-Hexane (Merck)

A sample of 200 µL milk was put in plastic tubes for Vitamin A, D3 and E analyses. 200 µL ethanol was added to it and mixed via the vortex for 1 min. On top of it, 800 µL n-hexane was added and then vortexed for 1 min, after which it was centrifuged at 2000 RPM for 10 min. 600 µL was taken from the emerging hexane phase and dried under the nitrogen gas. The remnant was solved in 500 µL methanol and injected into the HPLC column [22, 23].

2.6. Liquid chromatography

Vitamin A, D3, and E standards were used, and the setup was made ready for the analyses. Afterwards, 20 µL was taken from the extracts prepared and injected into the liquid chromatography column. Determination of vitamins A, D3, and E was performed at 325, 265, and 290 nm wavelengths using the DAD (diode-array detector). As the mobile phase, methanol-water (98:2) was used at the flow rate of 1.5 mL/min. C18 column (4.6 mm x 25 cm) was utilized for the separation of the vitamins, and the calculations were made according to the peak area and concentrations of the vitamins A, D3, and E [24, 25].

2.7. Statistical analysis

The data were analyzed with IBM SPSS V25. The normal distribution compliance was examined through the Shapiro Wilk. Paired samples t-test was used for the comparison of the values according to the animal species. The significance level was considered as $p<0.05$.

3. Results and discussions

To compare melatonin, retinol, tocopherol, and vitamin D3, paired sample t-tests were conducted. The results are shown in Table 1 below and Figure 1, 2. As it is seen in the table, goat's milk melatonin level was at the highest level at night and summer. The mean differences between day and night level [$t(19) = 10.135$, $p < 0.001$] and spring and summer level [$t(19) = -9.249$, $p < 0.001$] were found to be statistically significant. The level of melatonin in goat milk was higher at night and in summer than during the day and spring. There were also statistically significant differences between spring and summer milk in terms of Retinol [$t(5) = 5.196$, $p < 0.005$] and Tocopherol [$t(5) = 8.051$, $p < 0.001$]. Retinol and tocopherol levels were higher in spring than in summer. However, there was not a statistically significant difference between spring and summer milk in terms of Vitamin D3 ($p > 0.05$).

Table 1. Melatonin, retinol, tocopherol and vitamin D_3 level of goats milk from different meals and seasons

		M	n	Sd	*t*	*P*-value
Pair 1	Melatonin Night Level	28.54	20	3.59	10.135	0.000
	Melatonin Day Level	21.01	20	2.67		
Pair 2	Melatonin Spring Level	22.22	20	3.13	-9.249	0.000
	Melatonin Summer Level	27.33	20	2.78		
Pair 3	Retinol Spring Level	0.15	6	0.02	5.196	0.003
	Retinol Summer Level	0.09	6	0.05		
Pair 4	Tocopherol Spring Level	2.38	6	0.14	8.051	0.000
	Tocopherol Summer Level	2.08	6	0.20		
Pair 5	Vitamin D_3 Spring Level	0.011	6	0.00	1.168	0.296
	Vitamin D3 Summer Level	0.010	6	0.00		

Paired samples t-tests were conducted to compare night, day, spring, and summer sheep's milk in terms of Melatonin, Retinol, Tocopherol, and Vitamin D3. As it is seen in Table 2, Figure 3 and 4 the melatonin level in the sheep's milk was highest at night and spring. The mean differences between day and night level [$t(19) = 9.935$, $p < 0.001$] and spring and summer level [$t(19) = 2.778$, $p < 0.05$) were found to be statistically significant. The level of melatonin in sheep's milk was higher at night and spring than during the day and summer. There were also statistically significant differences between spring and summer milk of sheep in terms of Retinol [$t(5) = 2.794$, $p < 0.05$], tocopherol [$t(5) = 7.621$, $p < 0.005$] and vitamin D3 [$t(5) = 7.621$, $p < 0.005$]. Retinol and tocopherol levels were higher in spring than in summer, but vitamin D3 was higher in summer than in spring.

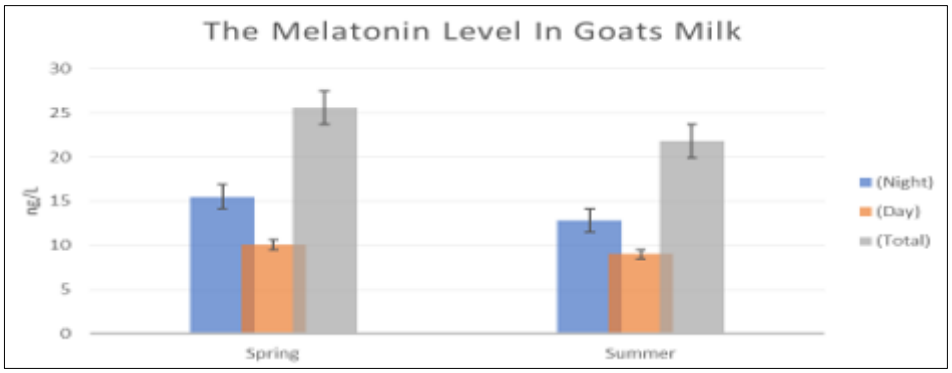

Figure 1. Temporal change of average melatonin hormone in Goats

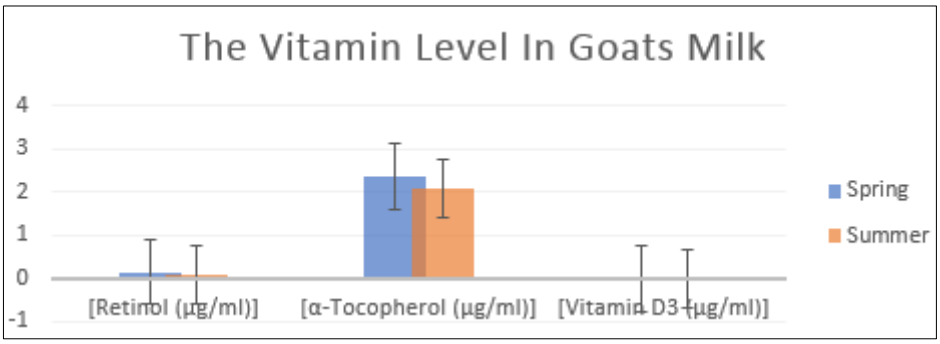

Figure 2. Temporal change of average Vitamin level in Goats Milk

Table 2. Melatonin, retinol, tocopherol and vitamin D3 level of sheep's milk from different during day and night and seasons

		M	n	Sd	*t*	*P*-value
Pair 1	Melatonin Night Level	14.16	20	2.58	9.935	0.000
	Melatonin Day Level	9.54	20	1.75		
Pair 2	Melatonin Spring Level	12.79	20	2.14	2.778	0.012
	Melatonin Summer Level	10.91	20	2.74		
Pair 3	Retinol Spring Level	0.17	6	0.02	2.794	0.038
	Retinol Summer Level	0.15	6	0.01		
Pair 4	Tocopherol Spring Level	2.02	6	0.18	7.621	0.001
	Tocopherol Summer Level	1.79	6	0.12		
Pair 5	Vitamin D_3 Spring Level	0.010	6	0.00	-7.181	0.001
	Vitamin D3 Summer Level	0.016	6	0.00		

Table 2, Figure 5, 6 paired-samples t-tests were conducted to compare night, day, spring, and summer, autumn, and winter cow's milk in terms of Melatonin, Retinol, Tocopherol, and Vitamin D3. Regarding the melatonin level, Night milk contained more melatonin than day milk. The amount of melatonin in milk was highest in winter and at least in autumn. The amount of melatonin in spring was found to be higher than the amount of melatonin in summer. The amount of melatonin in spring was near to the amount of melatonin in winter, so there was not a statistical significance between them, and the amount of melatonin in summer was near to the amount of melatonin in autumn, and no statistical significance was found between them ($p > 0.05$). Regarding the retinol level in cow's milk, it can be said no significant difference was observed as the seasons changed. However, in the autumn, the retinol level was significantly different from the amount of retinol in winter milk [$t(5) = -4.029$, $p < 0.05$]. When it comes to the tocopherol level, the amount of tocopherol in milk was highest in winter and least in autumn. The amount of tocopherol in spring was found to be higher than the amount of tocopherol in summer. There were statistically significant differences between pairs, as can be seen in Table 3. Vitamin D3 in summer was significantly higher than Vitamin D3 in spring, autumn and winter. However, there was not a significant difference between spring, autumn, and winter in terms of Vitamin D3 ($p > 0.05$).

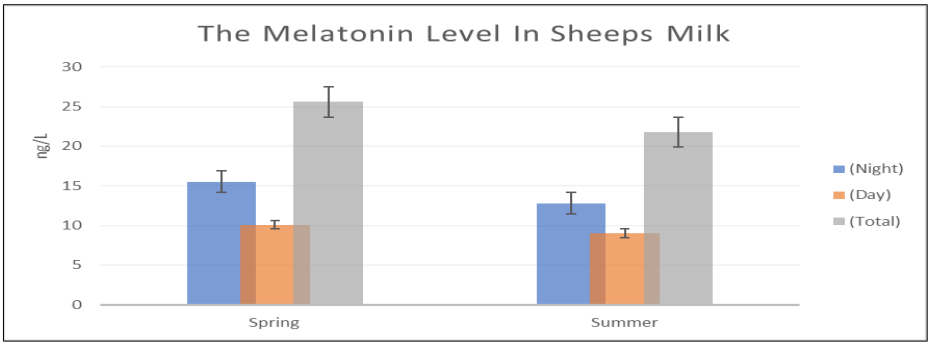

Figure 3. Temporal change of average melatonin hormone in Sheep's Milk

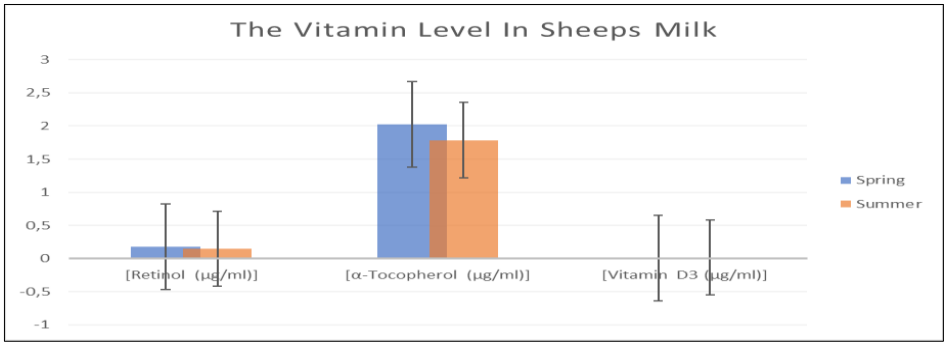

Figure 4. Temporal change of average Vitamin level in Sheep's Milk

Table 3. Melatonin, retinol, tocopherol and vitamin D3 level of cows milk from different meals and seasons

		M	n	Sd	*t*	*P*-value
Pair 1	Melatonin Night Level	45.79	20	5.62	11.700	0.000
	Melatonin Day Level	36.30	20	6.73		
Pair 2	Melatonin Spring Level	44.97	20	10.12	2.324	0.031
	Melatonin Summer Level	38.42	20	10.83		
Pair 3	Melatonin Spring Level	44.97	20	10.12	5.357	0.000
	Melatonin Autumn Level	32.51	20	5.92		
Pair 4	Melatonin Spring Level	44.97	20	10.12	-0.909	0.375
	Melatonin Winter Level	48.28	20	12.05		
Pair 5	Melatonin Summer Level	38.42	20	10.83	1.908	0.072
	Melatonin Autumn Level	32.51	20	5.92		
Pair 6	Melatonin Summer Level	38.42	20	10.83	-3.227	0.004
	Melatonin Winter Level	48.28	20	12.05		
Pair 7	Melatonin Autumn Level	32.51	20	5.92	-6.279	0.000
	Melatonin Winter Level	48.28	20	12.05		
Pair 8	Retinol Spring Level	0.16	6	0.02	-1.192	0.287
	Retinol Summer Level	0.17	6	0.02		
Pair 9	Retinol Spring Level	0.16	6	0.02	1.168	0.296
	Retinol Autumn Level	0.14	6	0.02		
Pair 10	Retinol Spring Level	0.16	6	0.02	-1.451	0.206
	Retinol Winter Level	0.17	6	0.01		
Pair 11	Retinol Summer Level	0.17	6	0.02	2.087	0.091
	Retinol Autumn Level	0.14	6	0.02		
Pair 12	Retinol Summer Level	0.17	6	0.02	0.131	0.901
	Retinol Winter Level	0.17	6	0.01		
Pair 13	Retinol Autumn Level	0.14	6	0.02	-4.029	0.010
	Retinol Winter Level	0.17	6	0.01		
Pair 14	Tocopherol Spring Level	1.42	6	0.27	-3.215	0.024
	Tocopherol Summer Level	1.90	6	0.11		
Pair 15	Tocopherol Spring Level	1.42	6	0.27	4.379	0.007
	Tocopherol Autumn Level	0.98	6	0.06		
Pair 16	Tocopherol Spring Level	1.42	6	0.27	-3.823	0.012
	Tocopherol Winter Level	2.06	6	0.18		
Pair 17	Tocopherol Summer Level	1.90	6	0.11	15.606	0.000
	Tocopherol Autumn Level	0.98	6	0.06		
Pair 18	Tocopherol Summer Level	1.90	6	0.11	-2.669	0.044
	Tocopherol Winter Level	2.06	6	0.18		
Pair 19	Tocopherol Autumn Level	0.98	6	0.06	-12.549	0.000
	Tocopherol Winter Level	2.06	6	0.18		
Pair 20	Vitamin D_3 Spring Level	0.007	6	0.001	-13.594	0.000
	Vitamin D_3 Summer Level	0.017	6	0.001		
Pair 21	Vitamin D_3 Spring Level	0.007	6	0.001	1.052	0.341
	Vitamin D_3 Autumn Level	0.006	6	0.002		
Pair 22	Vitamin D_3 Spring Level	0.007	6	0.001	-1.000	0.363
	Vitamin D_3 Winter Level	0.007	6	0.002		
Pair 23	Vitamin D_3 Summer Level	0.017	6	0.001	11.390	0.000
	Vitamin D_3 Autumn Level	0.006	6	0.002		
Pair 24	Vitamin D_3 Summer Level	0.017	6	0.001	8.442	0.000
	Vitamin D_3 Winter Level	0.007	6	0.002		
Pair 25	Vitamin D_3 Autumn Level	0.006	6	0.002	-3.503	0.017
	Vitamin D_3 Winter Level	0.007	6	0.002		

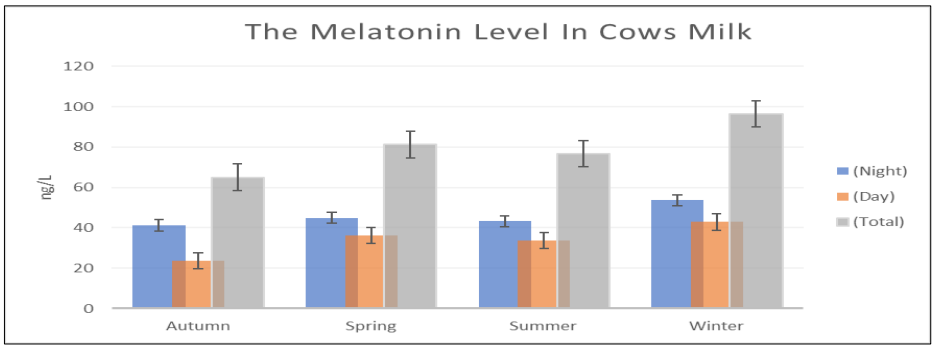

Figure 5. Temporal change of mean melatonin hormone in cows

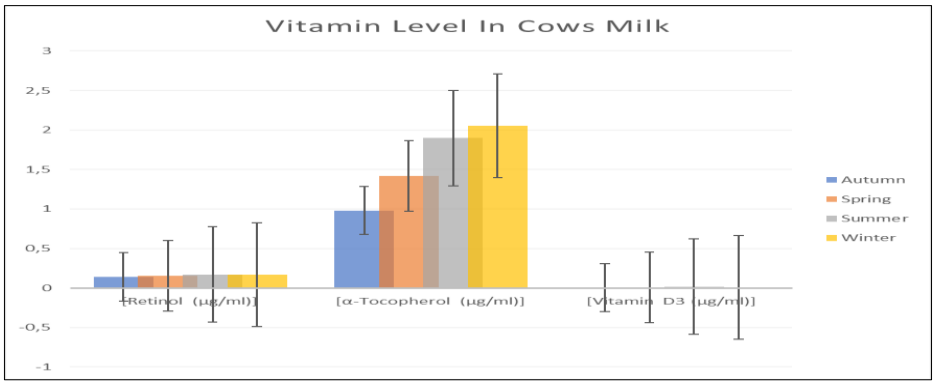

Figure 6. Temporal change of average Vitamin level in Cows Milk

Our study results revealed that for all animals' milk (goats, sheeps, and cows) melatonin level was at the highest level at night and summer. The mean differences between day and night levels and spring and summer levels were found to be statistically significant. The level of melatonin in goat milk was higher at night and in summer than during the day and spring. The melatonin level in milk reaches the maximum at midnight and drops to a minimum at noon. It is stated that, by comparison, the milk obtained at night (around 12:00 at midnight) contains a higher level of melatonin than the milk obtained during the day [26]. In the study conducted by Schaper et al. [27], concentration determination was executed with Elisa every two months to determine the milk melatonin concentrations of 37 Holstein-Friesian cows seasonally. Mean melatonin concentrations reach 5.4 pg/mL in February, 11.8 pg/mL in August, statistically the highest levels in August and June, the lowest concentrations in February. Valtonen et al. [28], Increasing the dark phase to 17 h of retention of cows, reported that it increased the amount of melatonin in night milk. Haigh [29], found that cows exposed to a low light intensity of 50 lux increased the amount of melatonin at night relative to higher concentrations.

It is stated in the study conducted by Schaper et al. [26], by comparison, the milk obtained at night (around midnight) contain a higher level of melatonin than the milk obtained during the day [27]. Asher et al. [5] and Schaper et al. [27] also found that melatonin hormone amount to be higher in the night milk compared to day milk. Our study findings also support the results of the specified studies. Therefore, it can be concluded that night milk and summer milk consist of more melatonin than day milk and winter milk. Sheep exhibit anoestrus in the summer months and continue oestrus when days start becoming shorter [30]. While melatonin is known to mediate the changing photoperiodic effect in seasonal animals, studies have been conducted on the effects of melatonin administration in sheep [31]. It imitates the long and short profiles of melatonin in these animals and boosts the effects of the photoperiod on reproduction. Melatonin administration in sheep raising seasons has become beneficial to ease the economic burden of the reproduction season [27].

In our study regarding goat's and sheep's milk, it was found that there was a statistically significant difference between spring and summer milk in terms of Retinol. Retinol level was higher in spring than

in summer. However, regarding cow's milk, winter milk was contained more retinol than autumn milk. Among other seasons no differences were found. Results Vissers et al. [32] are incompatible with the findings that the entire vitamin A content of milk is lower in winter and higher in summer. Similarly, it is stated that the content of vitamin A in all milk decreases in winter, and South Africa increases in summer. The change in vitamin A concentration from different regions may be related to the difference in cow/buffalo ratio and nutritional status. The change in the vitamin A content of milk indicates the difference in the breed. Comparison of the species showed that Holstein breeds had the highest vitamin A activity and Guernsey contained the lowest vitamin A content.

Regarding goats and sheep, there were statistically significant differences between spring and summer milk in terms of Tocopherol. Tocopherol levels were higher in spring than in summer. However, regarding cows, the amount of tocopherol in milk was highest in winter and least in autumn. The amount of tocopherol in spring was found to be higher than the amount of tocopherol in summer. The amount of tocopherol was also in summer than in the amount in spring. However, in the study conducted by Givens et al. [33]. Vitamin E concentration in milk has been reported to be high in summer and low in winter. Feeding is the most important that creates significant changes in vitamin E concentration in raw milk. The type of feed changes with seasonal change and eventually affects the vitamin content of milk. It was reported by Focant et al. [34] the study was conducted according to natural events. In cows, the highest mean value of melatonin was obtained in the winter evening. Among the three species, the highest melatonin value was discovered in the cow species. In our study, we did not keep any variable stable or intervene in any variable. The fact that only the winter value was higher than the other seasons was attributed to long winter evenings. In winter, evening samples were collected one hour earlier. This increased the duration of staying in the dark for the cows. Therefore, the winter value was thought to be higher. The melatonin value in the evening samples was found higher than the day samples. Compared to the cows, there was a difference in terms of the place where sheep and goats took it. Only two seasons were taken into account for two animal species. The sheep and goats which were fed during the day were being kept at a place half-covered, but open on the sides and surrounded by short walls for seasonal convenience. The chemistry of vitamin E is rather complex since there are eight compounds (four tocopherols and four tocotrienols) that exhibit vitamin E activity. In milk, α-tocopherol accounts for nearly all of vitamin E. In bovine milk, α-tocopherol is the main compound with vitamin E activity [35], while very small amounts of β-tocopherol and γ-tocopherol are present. Sheep milk fat is a good dietary source of vitamin E. Vitamin E is found in 3 forms (α-, β-, and γ -tocopherols), with α-tocopherol being the most abundant form [36]. Goat milk is poor in vitamin E [15], while sheep milk generally has higher contents of it than cow and goat milk [16]. However, it is also known that milk composition can be affected by internal (breed) and external factors (season, nutrition, stage of lactation, and milking system) [37, 38]. Yasmin et al. [39], looked at vitamin E concentration in buffalo and cow milk in Punjab, Pakistan. In the study, they observed seasonal changes in vitamin content in milk. In June, they found the maximum amount of vitamin E (0.226 mg / 100 g), and in November, they found a minimum (0.041 mg/100 g). The change in the vitamin E concentration of milk from all regions demonstrates the increase in the vitamin E level of milk from all regions from April to August, and then observed a permanent decrease in vitamin E from October to February. In this case, the level of tocopherol was highest in cows in winter and lowest in autumn. In Sheep and Goats, it was found highest in spring and lowest in autumn. However, vitamin E content was increased in the winter months than summer months in some localities [37]. This work is parallel to our work.

4. Conclusions

The studies given in the literature complies with our studies more or less; however, they differ in terms of the experimental study results. The reason for the differences in our study may result from the variation in the vitamin A, E, D content of the milk, different species of animals, the difference between the cow, goat and sheep proportions, the amounts, and types of the nutrients in the feed. Our study is thought to help analyze the seasonal changes in the milk composition concerning vitamins for the

available and latest quality products. In the studies conducted by [40-42], the fact that the samples were generally taken from the animals which lived in pastures at high altitudes and fed with ready feeds and the ready food stored limitedly in winter did not prove any seasonal differences in vitamin administrations or any statistical difference [40-42]. The content of fat-soluble vitamins A, D, and E in milk is strongly influenced by the food ration composition of the dairy cow [43].

More commonly known as the sleep hormone, melatonin also has antioxidant, anti-inflammatory, antiapoptotic and many other important properties [44]. Photoperiod is strictly linked to the secretion of melatonin. In cattle, melatonin secretion increases at night and decreases during the day with low concentrations of melatonin during the long-day photoperiod and an increase during the short-day photoperiod. It should be noted that melatonin secretion in cows, as determined by spectral analysis, did not differ among seasons, though the duration of melatonin secretion was longer during winter [45,46]. Milk rich in melatonin can be preferred in old age and in providing sleep patterns. In our work, we took into consideration the night, day and seasonal factors. It can be recommended in milk with high Vitamin A, E, D values, both in the growth phase, in sickness and old age. In addition, levels of hormones and vitamins varied according to species, seasons and milking time. It is hoped that this study will contribute to future studies and literature.

Acknowledgement: We would like to thank Van Yüzüncü Yıl University, Graduate School of Natural and Applied Sciences Projects Department for supporting the study with the project number of FYL-2017-5785.

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Manuscript received: 4.05.2021