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Original Article
Özgün Araştırma

Effects of night lighting with red light on melatonin and milk quality parameters in holstein cows

Rukiye Garip[1], Nuri Başpınar[2], Beyza Suvarıklı Alan[3*], Mustafa Garip[4], Pınar Coşkun[5], Emre Arslan[6]

[1,2,3] Department of Biochemistry, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye
[4,6] Department of Animal Science, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye
[5] Department of Biochemistry, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Türkiye

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Abstract: In this study, it was aimed to investigate the effects of red lighting on blood and milk melatonin (MLT) levels, and milk quality. The experiment was carried out on 6 lactating Holstein Dairy cows, which were being reared at the Hümeyra Özgen Research and Application Farm, affiliated to Selcuk University Faculty of Veterinary Medicine. After a control period of 15 days in which night lighting was not applied (control group), night lighting was provided by LED bulbs emitting red light with a wavelength of 652 nm during the following 15-day trial period (experimental group). On days 5th, 10th and 15th days of the control and experimental groups, milk and blood samples were taken at the 06:00 am and at 06:00 pm.Serum and milk melatonin concentrations in the morning of the control, on days 5th, 10th and 15th; were 8.64±1.4, 7.02±0.97, 8.71±2.3, and 5.01±0.92, 5.23±0.35 , 3.93±0.81pg/ml, respectively while control evening group were respectively 8.59±1.8,7.53±2.14,8.35±0.94, 6.91±1.73, 6.8±1.27. It was obtained as 3.67±0.97 pg/ml. Serum and milk melatonin levels in the trial morning group were 10.93±2.06, respectively, on the same days; 15.37±2.6; 11.25±1.71 and 2.97±0.64; 5.7±1.06; While it was measured as 3.33±0.73 pg/ml, it was 14.83±3.11 in the trial evening group; 14.5±3.57; 12.95±4.09 and 4.42±0.61; 2.51±0.56; It was obtained as 2.48±0.79 pg/ml. As a result, it was observed that serum and milk melatonin levels and milk parameters were not adversely affected in the use of red LED lamps (652 nm) at night for 12h, it was thought that there was no inconvenience in using them in indoor shelter lighting at night.

Keywords: Darkness, Holstein, Melatonin, Milk quality, Red light

Holştayn ineklerde kırmızı ışıkla gece aydınlatmasının melatonin ve süt kalite parametrelerine etkileri

Özet: Bu çalışmada kırmızı ışıkla aydınlatmanın, kan-süt melatonin düzeyleri ile süt kalitesi üzerine etkilerinin araştırılması amaçlandı. Deneme Selçuk Üniversitesi Veteriner Fakültesi'ne bağlı Hümeyra Özgen Araştırma ve Uygulama Çiftliğinde yetiştirilmekte olan laktasyondaki 6 Holştayn ırkı sağmal inek üzerinde gerçekleştirildi. Hayvanlarda 15 gün, gece aydınlatması uygulanmayan kontrol döneminden sonra (kontrol grubu) 15 gün boyunca gece aydınlatması (deneme grubu) 652 nm dalga boylu kırmızı ışık yayan LED ampüllerle sağlandı. Çalışma boyunca 5., 10., ve 15. günlerde, 06:00 ve 18:00'da kan ve süt örnekleri alındı. Kontrol sabah grubunda serum ve süt MLT düzeyleri, 5., 10., 15. günlerde, sırasıyla 8,64±1,4; 7,02±0,97; 8,71±2,3 ve 5,01±0,92; 5,23±0,35; 3,93±0,81 pg/ml olarak bulunurken, kontrol akşam grubunda, 8,59±1,8; 7,53±2,14; 8,35±0,94 ve 6,91±1,73; 6,8±1,27; 3,67±0,97 pg/ml olarak hesaplandı. Deneme sabah grubunda serum ve süt MLT düzeyleri, aynı günlerde, sırasıyla 10,93±2,06; 15,37±2,6; 11,25±1,71 ve 2,97±0,64; 5,7±1,06; 3,33±0,73 pg/ml olarak hesaplanırken, deneme akşam grubunda 14,83±3,11; 14,5±3,57; 12,95±4,09 ve 4,42±0,61; 2,51±0,56; 2,48±0,79 pg/ml olarak hesaplandı. Serum ve süt MLT düzeyleri, süt somatik hücre sayısı, süt kalite parametrelerinden iletkenlik dışında gruplar arasında veya grup içinde herhangi bir istatistiki fark belirlenmedi. Sonuç olarak, kırmızı LED ampuller ile gece aydınlatılmasının (652 nm) 12 saat boyunca kullanımında serum ve süt melatonin düzeyleri ile süt parametrelerinin olumsuz etkilenmediği görüldüğünden gece barınak içi aydınlatmalarda kullanılmasında bir sakınca olmadığı düşünüldü.

Anahtar kelimeler: Holştayn, Karanlık, Kırmızı ışık, Melatonin, Süt kalitesi

Introduction

Proper lighting of animal farms is important for both animal welfare and safe, healthy working conditions for the farm staff (Belyaev and Gorbunova 1973; Köseman and Şeker 2019). Today, it is tried to provide advantages such as energy

Yazışma adresi / Correspondence: *Beyza Suvarıklı Alan, Department of Biochemistry, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye e-mail: beyza.alan@selcuk.edu.tr*

ORCID IDs of the authors: *[1]0000-0003-3586-9534 • [2]0000-0002-5481-1111 • [3]0000-0003-4698-9291 • [4]0000-0002-1429-2724 • [5]0000-0002-6991-3727 • [6]0000-0002-4609-8395*

saving and more efficiency by adjusting the illumination times in livestock farms. It was reported in studies that cows prefer a light environment over dark. Probably, the better lightning and visual contact between cows contribute for social hierarchy build-up and prevention of traumatism. This has LED researchers and agricultural equipment manufacturers to develop and apply technologies for the best possible lighting, thereby providing optimal growing conditions. (Penev et al. 2014). For this purpose, breeders use red light, known as 'invisible light' for cattle at night, for night illumination (Bunu 2019; Olsson 2020). LEDs reduce consumption of electricity for illumination in dairy barns and require less maintenance compared with several other types of light fixtures available for animal houses, which makes them increasingly popular. The use of LEDs also entails better control of light intensity as the diodes can be dimmed, as well as better control of the light spectrum as there are many different color types available. Artificial light supplements daylight, when daylight is available, and provides adequate levels of illuminance during the rest of the day to allow a daylight-like environment of 16h per 24h for lactating cows (ASABE 2014).

The retina contains 2 types of photoreceptors called cones and rods. Photoreceptors in the retina are stimulated by light. Like most mammals, are dichromats and have short-wavelength-sensitive (S-cones) and medium- to long-wavelength-sensitive cones (ML-cones) (Penev et al. 2014; Lindkvist et al. 2021). Melatonin (MLT) is a neuromodulatory hormone synthesized from the pineal gland. In both the pineal gland and ocular tissue, MLT synthesis and release parallels the circadian rhythm in relation to dark and light intensity. Retinal cells, which are hyperpolarized in the light, suppress MLT synthesis, while they depolarize in the dark and initiate synthesis in the pineal gland. The excitation threshold is light energy with a wavelength of about 482 nm (Ostrin 2019). The wavelength, intensity, and duration of light affect MLT biosynthesis. A light intensity of 5-10 lx in cattle is considered the threshold at which MLT synthesis is inhibited (Muthuramalingam et al. 2006; Dahl 2010). Short wavelength lights suppress MLT synthesis due to their high energies (Cajochen et al. 2005; Asher et al. 2015; Arnao and Ruiz 2018). Melatonin is amphilic, meaning it can diffuse freely across biological membranes (Simonneaux and Ribelayga 2003; Yu et al. 2016) into the circulatory system and from the bloodstream to milk (Vanecek 1998; Castro et al. 2011). Photoperiod application is important for milk production and health of dairy cattle (Dahl and Petitclerc 2003; Dahl et al. 2012; Crawford et al. 2015). Photoperiod feeding is also important in lactating dairy cows for increased milk yield (Peters and Tucker 1978; Dahl and Petitclerc 2003).

Melatonin released by dark stimuli induces relaxation and sleep in mammals. Therefore, there is some concern that the presence of light at night may interfere with rest in cows (Lawson and Kennedy 2001; Muthuramalingam et al. 2006). In a study by Lawson and Kennedy (2001) it was shown that MLT levels decreased in heifers under night-time lighting of 50 lx and higher intensity. On the other hand, it was noted that after 2h of 50 lx illumination, the suppression of MLT release disappeared. Muthuramalingam et al. (2006) suggest that it may be appropriate to use 10 lx and lower intensity light in areas where it is recommended to be dark. In a human study on MLT secretion with blue and red light, it was stated that blue light (479 nm) suppressed MLT release, while red light (627 nm) had no effect. In general, light intensity is an important parameter in measuring the suppression level of MLT, and as the brightness of the light stimulus increases, the suppression power increases (Papamichael et al. 2012).

In this study, since there were not enough satisfactory studies on the effects of night lighting with red light on blood-milk MLT levels and milk quality parameters in Holstein dairy cows, it was aimed to determine blood-milk MLT levels and milk quality parameters in Holstein dairy cows exposed to red light (with a wavelength of 652 nm and 50,267 lx) for 15 days.

Material and Methods

Animal and Feed Materials

Approval for the study was obtained from the Selcuk University Faculty of Veterinary Medicine Local Ethics Committee for Animal Experiments (dated 16.01.2020 and decision no:2020/02).

The study was carried out in a Research and Application Farm (Location: 38° 02′ 18″ K, 32° 30′ 15″ D) after a 5-day acclimation phase, for a total of 30 days (3 July - 2 August) with 15 days of dark and experimental groups. In the research, 6 Holstein cows were selected and the experiment was started. In the selection, animals aged between 3 and 9 years and with close milk yield levels were preferred (Table 1).

Table 1 Age and lactation days of the selected animals

Business Animal No	Lactation day	Age	Average Milk Yield kg/day
1	95	9	21,61±0,60
7	60	8	28,26±0,26
8	249	7	24,31±0,70
13	58	3	26,03±0,46
14	48	7	22,45±0,53
16	149	4	23,73±0,59
Average	**109,8**	**6,33**	**24,40 ±0,91**

During the experiment, the feeding program of the enterprise was applied. No changes were made to concentrate and roughage contents throughout the experiment. The roughage and concentrate feed ingredients and the feeding schedule are presented in (Tables 2, 3 and 4). Feeding was given ad libitum as a mixture of roughage and concentrated feed. The yield share as concentrated feed was given individually by the automatic voluntary milking system (VMS) in front of the animals during their milking.

Table 2. Mixture ratios of coarse and concentrated feed.

Feed İngredient	Amount in (kg)	Ration (%)
Straw	3,0	15
Clover	3,5	17,5
Corn Silage	6,0	30
Bran	0,5	2,5
Milk Feed	5,5	27,5
Cottonseed Meal	0,5	2,5
Beet Pulp	1,0	5
Vit Min Premix	0,005	0,02
Salt	0,005	0,02
Total	**20.01**	**%100**

Table 3. Roughage physical and chemical analysis results.

PHYSICAL ANALYSIS	
Name	**Tmr**
Amount	3 Kg
Where İt Came From	Farm
Arrived Date	09.09.2020
Packaging Shape	Plastic Bag
Appearance	Normal
Colour	Normal
Smell	Normal
Foreign Matter	No

CHEMICAL ANALYSIS		
	Natural	**Dry matter**
Dry Matter.%	96,44	96,44
Crude Ash.%	6,76	7,01
Crude oil %	1,53	1,58
Crude Cellulose.%	36,23	37,57
Crude Protein.%	6,39	6,63
ME, kcal/kg	1544	1601

Table 4. Physical and Chemical Analysis Results of Concentrate (Pellet) Feed.

PHYSICAL ANALYSIS	
Name	**Pellet Feed**
Appearance	Normal
Amount	3 Kg
Where İt Came From	Farm
Arrived Date	09.09.2020
Packaging Shape	Plastic Bag
Colour	Normal
Smell	Normal
Foreign Matter	No

CHEMICAL ANALYSIS		
	Natural	**Dry matter**
Dry Matter.%	91,15	91,15
Crude Ash.%	6,32	6,94
Crude oil %	1,48	1,62
Crude Cellulose.%	8,11	8,89
Crude Protein.%	16,33	17,92
ME, kcal/kg	2564	2813

Before starting the experiment, the animals were subjected to the acclimatization period 5-days before sampling by continuing the lighting program applied by the enterprise for the dark period. No changes in feed or worker were made until the end of the experiment. No application was carried out except for routine applications. No ration changes were applied during the study. On the days of blood and milk sampling, samples were taken from the roughage and concentrated feed ingredients in accordance with the procedures and content analyses were performed (Tables 2, 3 and 4).

Milk Sampling Periods

This study was conducted in two stages. Five days before the start of the experiment and the 5-days

between the dark and light periods were defined as the acclimatization period. In order to form the control group (Stage 1), milk samples from 6 cows were collected from the right fore udder on the 5th, 10th and 15th days of the study, at the end of the night (6.00 a.m.) and at the end of the day (06.00 p.m.). Before the light application, light sources were mounted in the facility and, the same animals were subjected to a 5-day acclimation period. For the purpose of the experimental group (Stage 2), 100 ml were collected from the right anterior nipple at the end of the night (06:00 am) and at the end of the day (06:00 pm) on days 5th, 10th and 15th days of the study. In addition, blood was taken from the coccygeal vein before the morning and evening milking of the animals during 1st and 2nd stages. The sera of the blood samples taken were separated.

Determination of Somatic Cell Counts (SCC):

In order to determine the SCC ethyl alcohol and acetonitrile were mixed in a bottle and kept in a water bath set at 60-70°C for a while, after adding methylene blue and mixing well, it was kept at 4°C overnight. It was then used by filtration after adding glacial acetic acid. After the milk samples brought to the laboratory, they were homogenized by keeping them in a shaking water bath set at 50-60°C for 30 minutes, 0.05 ml then milk sample was spread on an area of 100 mm^2 on a clean slide. After the prepared preparations were air-dried, they were covered with SCC paint solution to cover them completely and painted for 10 minutes. The stained preparations were washed, dried, and examined under the microscope with an immersion objective by dripping cedar oil.

The average number of cells in a microscope field was determined by counting the cells with clear nuclei in 100 microscope fields in the preparations with more cells and 20 in the preparations with few cells and SCC in 1 ml of milk was determined (Anonymous 1981).

Milk Content Analysis

Fat, dry matter, protein, lactose, density, freezing point and conductivity analyses in milk were performed by infrared analysis method (combined by ® Mayasan Biotech) using MILKANA® Express Plus device. Milk protein, fat, dry matter and lactose were measured using a Milko Scan FT device (Produced for Turkey with Serial No. 2008 Part No: 701028), based on Fourier transform infrared spectrum analysis.

Light Application

LED bulbs and carrier armature (1800 rotatable 50 Watt SMD Led Projector luminaire slim case) were used in the preparation of the light fixtures. SMD Powerled (6040 –F2525 A2) with a cooled 50 watt red light was mounted in bulb cases. Wavelength measurements of the light source were carried out with the aid of an electron microscope spectrophotometer (LED) Ocean Optics/Qe 65000, and the distance between the fiber tip and the led lamp was 10-15 cm. The wavelength of the light was calculated as approximately 652 nm (Figure 1).

The light fixtures, which were measured, were mounted in suitable places in the barn, where light insulation (the windows of which were covered with aluminum foil) was made, by the electric technicians working in the farm. After assembly and during the trial periods, light intensities were measured at the animal eye and the feeder levels. While the lighting program applied to the farm was continued to be applied to the selected animals during the control (dark, 1st stage) period of the experiment, blood and milk samples were collected on the 5th, 10th, and 15th days of the night, after the 5-day adaptation period. In the red light application, which is the main application of the experiment, after this period, the light mechanism was started, and the second stage of the experiment was started after the 5-day adaptation period. During this period, the animals were exposed to red light for 12h (between 06.00 pm and 06:00 am) after 06.00 pm, while they were exposed to daylight during the day. As in the control period, milk and blood samples were collected from the same animals on days5th, 10th and 15th days, twice, in the morning and evening, in accordance with the specified protocol. Light measurements were made and recorded during the periods when the samples of the dark and red light illumination phases were collected. The light intensity was measured with the help of the ILT 1700 radiometer at the eye level of the animals and the intensity of the light intensity was calculated as 4.67 x 10.76 fc (=~50,267 lx).

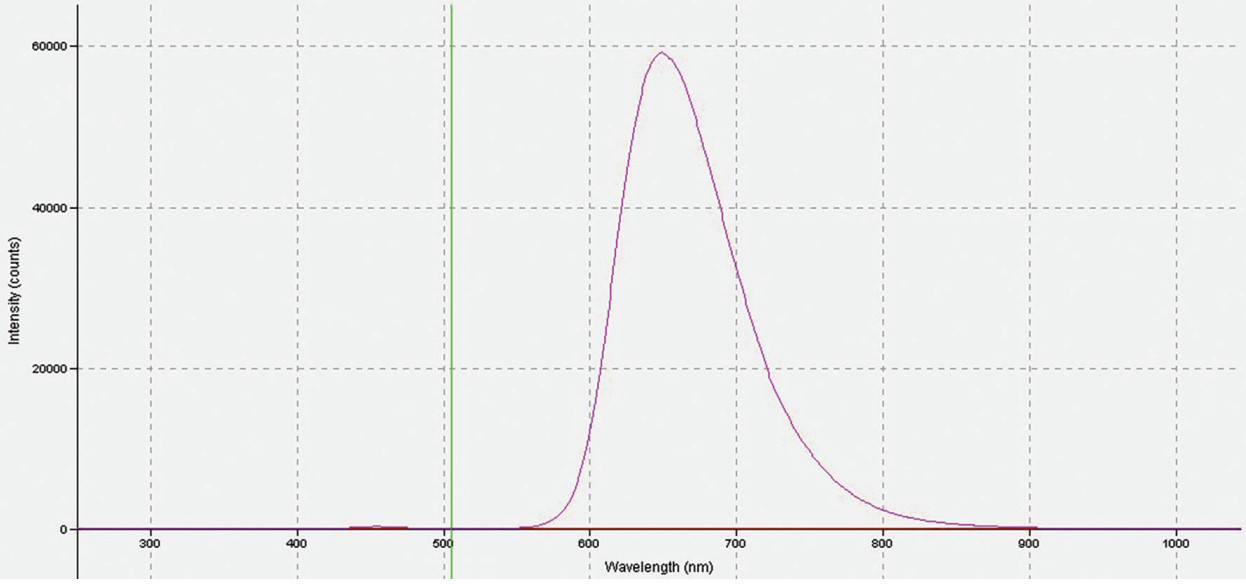

Figure 1. Wavelength of the red LED bulb light used in the experiment.

Temperature and Humidity Measurements

The light quantity and spectral compositions were measured using a calibrated handheld fiber-optic spectrometer (IL 1700 Research Radiometer). In addition, measurements of humidity, (Trotec Heat humidity meter BC 06) and air flow rate (Air Velocity Meter Trotec BA 06) were carried out. In addition, values such as altitude, humidity, day length and moon position of the location of the enterprise were obtained from the Regional Directorate of Meteorology. The average air temperature for July, the period of the research, was 25.51 °C (max 36.16-min 11,5 °C), and the monthly precipitation was 0.6 mm. For the beginning of August, the average air temperature was 23.79 °C (max 36.43-min 8.31 °C), monthly rainfall was recorded as 12.8 mm. The animals were housed in the barn (22±2°C and 55±5% humidity) with an automatic fan with temperature and humidity control inside the barn.

Analysis of Milk and Serum Melatonin Levels

Collected milk samples were brought to the biochemistry laboratory in the cold chain and stored at -20°C until use. Analysis of milk MLT levels was performed with the human direct saliva MLT ELISA kit (RE54041, IBL International, Germany) modified for cow's milk by Kollmann et al. (2008) and Milagres et al. (2014). Milk samples were analyzed according to the kit procedure (Milagres et al. 2014). Before sampling, milk samples were diluted 4 times. Serum was applied directly to the kit procedure. The levels are given as pg/ml.

Statistical Analysis

IBM SPSS 25 package program was used for statistical analysis of the data. The Kolmogorov-Smirnova test was used to determine whether the data showed a normal distribution. Since the data did not conform to normal distribution, after Kruskal Wallis test for nonparametric analysis of variance, Mann-Whitney U test was used for pairwise comparisons and the Dunnett test was used for multiple comparisons to determine the differences in the groups. Spearman's test was used to evaluate the relationship between blood and milk MLT concentration (night and day milk average), SCC and milk nutrient content levels (dry matter, fat, protein, density, conductivity, lactose) in each group and the relationship between milking time and blood milk in each group.

Results

The serum and milk MLT levels and SCC findings of the data obtained in the study are given in Table 5, and data on milk content parameters are given in Table 6.

Table 5. Serum and milk MLT levels (pg/ml) and milk SCC levels (Mean ±SE)

		MORNİNG			EVENİNG		
n=6		**CONTROL**	**TRİAL**	*P*	**CONTROL**	**TRİAL**	*P*
Serum MLT	5. gün	8,64±1,4	10,93±2,06	*0,699*	8,59±1,8	14,83±3,11	*0,310*
Serum MLT	10. gün	7,02±0,97	15,37±2,6	*0,090*	7,53±2,14	14,50±3,57	*0,310*
Serum MLT	15. gün	8,71±2,3	11,25±1,71	*0,310*	8,35±0,94	12,95±4,09	*0,589*
	p	*0,697*	*0,322*		*0,947*	*0,891*	
Milk MLT	5. gün	5,01±0,92	2,97±0,64	*0,180*	6,91±1,73	4,42±0,61	*0,093*
Milk MLT	10. gün	5,23±0,35	5,7±1,06	*0,240*	6,80±1,27	2,51±0,56	*0,004*
Milk MLT	15. gün	3,93±0,81	3,33±0,73	*0,394*	3,67±0,97	2,48±0,79	*0,394*
	p	*0,359*	*0,139*		*0,113*	*0,118*	
*SCC**	5. gün	37,32±5,63	91,23±37,82	*0,180*	111,96±82,21	140,99±34,40	*0,180*
*SCC**	10. gün	62,20±23,82	99,52±35,19	*0,485*	62,2±14,00	248,81±146,78	*0,589*
*SCC**	15. gün	91,23±28,49	161,72±92,82	*0,818*	140,99±101,51	157,58±117,87	*0,818*
	p	*0,147*	*0,845*		*0,452*	*0,584*	

(*pıeces x 1000).

Table 6. Milk parameters (Mean ±SE) in all experimental groups.

		MORNING			EVENING		
		CONTROL	**TRIAL**	*P*	**CONTROL**	**TRIAL**	*P*
% DEN	5. gün	27,22±1,92	29,58±0,38	*0,520*	24,22±2,85	27,38±1,37	*0,589*
% DEN	10. gün	28,63±1,32	30,17±0,55	*0,589*	23,78±2,98	24,28±1,74	*0,937*
% DEN	15. gün	25,66±4,51	30,32±0,16	*0,818*	27,85±1,10	28,62±0,90	*0,589*
	p	*0,645*	*0,345*		*0,554*	*0,075*	
% FAT	5. gün	3,30±1,62	1,38±0,32	*0,630*	2,28±0,50	3,13±1,20	*0,937*
% FAT	10. gün	1,55±0,53	0,93±0,23	*0,485*	5,42±1,48	5,27±1,43	*0,937*
% FAT	15. gün	1,10±0,51	0,55±0,18	*0,937*	3,01±1,11	1,86±0,69	*0,310*
	p	*0,431*	*0,076*		*0,359*	*0,108*	
% FP	5. gün	53,53±1,02	54,53±0,35	*0,699*	46,97±4,39	53,58±0,53	*0,310*
% FP	10. gün	53,05±1,53	54,82±1,01	*0,485*	52,00±2,45	51,95±0,65	*0,485*
% FP	15. gün	54,62±0,70	54,38±0,36	*0,818*	54,02±0,79	53,67±0,61	*0,818*
	p	*0,875*	*0,470*		*0,402*	*0,144*	
% LAC	5. gün	4,35±0,11	4,47±0,03	*0,699*	3,81±0,37	4,36±0,06	*0,485*
% LAC	10. gün	4,36±0,13	4,50±0,07	*0,485*	4,10±0,30	4,16±0,09	*0,485*
% LAC	15. gün	4,52±0,07	4,48±0,02	*0,699*	4,41±0,06	4,39±0,06	*0,937*
	p	*0,778*	*0,443*		*0,548*	*0,125*	
% PROT	5. gün	2,96±0,05	3,01±0,02	0,*748*	2,60±0,24	2,97±0,02	*0,394*
% PROT	10. gün	2,94±0,08	3,02±0,05	*0,485*	2,85±0,18	2,88±0,03	*0,240*
% PROT	15. gün	3,03±0,05	3,00±0,02	*0,818*	3,0±0,04	2,96±0,03	*0,589*
	p	*0,719*	*0,450*		*0,321*	*0,115*	

		MORNING			EVENING		
		CONTROL	**TRIAL**	*P*	**CONTROL**	**TRIAL**	*P*
% SNF	5. gün	7,90±0,16	8,07±0,05	*0,631*	6,91±0,66	7,91±0,10	*0,394*
% SNF	10. gün	7,87±0,22	8,11±0,13	*0,485*	7,50±0,52	7,6±0,13	*0,485*
% SNF	15. gün	8,17±0,14	8,06±0,05	*0,699*	7,99±0,11	7,93±0,09	*0,589*
	p	*0,781*	*0, 475*		*0,436*	*0,119*	
Z mS/cm	5. gün	4,53±0,19	5,13±0,10	*0,015*	4,79±0,15b	5,19±0,17	*0,240*
Z mS/cm	10. gün	4,99±0,21	5,17±0,06	*0,589*	5,04±0,18a	5,18±0,10	*0,485*
Z mS/cm	15. gün	4,78±0,13	5,18±0,15	*0,093*	5,27±0,05a	5,25±0,11	*0,699*
	p	*0,082*	*0,890*		*0,030**	*0,932*	

* The difference at the P<0.05 level is significant. DEN: Density 1.0260 g/cm3 – 1.0330 g/cm3 (±0.0005g/cm3) FP: Freezing Point % (-1°C) – 0°C (±0.015°C) , SNF: Oil-free dry substance 6%–12% (±0.2%) FAT: oil 0.5%–9% (±0.1%). Z: Conductivity:2 mS/cm – 20 mS/cm (±1%) (18°C).

Correlation tables between the relevant parameters are presented in Table 7 for the 5th day, Table 8 for the 10th day and Table 9 for the 15th day in the experimental and control groups.

Table 7. Correlations between control and trial day 5 morning and evening milk MLT and milk quality parameters.

CONTROL			**TRIAL**							
Morning **Evening**	*Serum MLT*	*Milk MLT*	*FAT*	*SNF*	*DEN*	*PROT*	*FP*	*LAC*	*Z*	*SCC*
Serum MLT	1	0,60	0,89*	-0,31	-0,49	-0,20	-0,31	-0,31	0,00	0,44
Serum MLT	1	-0,64	0,89*	-0,43	-0,84*	-0,32	-0,43	-0,60	-0,26	-0,81*
Milk MLT	0,41	1	0,43	-0,83*	-0,94**	-0,78	-0,83*	-0,83*	0,35	0,09
Milk MLT	0,03	1	-0,52	0,06	0,46	0,01	0,06	0,23	0,70	0,87*
FAT	0,37	-0,14	1	-0,20	-0,37	-0,14	-0,20	-0,20	-0,43	0,79
FAT	0,94**	0,26	1	-0,77	-0,99**	-0,70	-0,77	-0,83*	-0,26	-0,78
SNF	0,03	0,12	-0,54	1	0,94**	0,99**	1,00**	1,00**	-0,32	0,09
SNF	-0,14	-0,49	-0,43	1	0,84*	0,99**	1,00**	0,94**	0,14	0,32
DEN	-0,14	0,29	-0,89*	0,83*	1	0,93**	0,94**	0,94**	-0,23	-0,09
DEN	-0,31	-0,54	-0,60	0,94**	1	0,78	0,84*	0,90*	0,23	0,72
PROT	0,03	0,25	-0,29	0,91*	0,68	1	0,99**	0,99**	-0,22	0,04
PROT	-0,14	-0,49	-0,43	1,00**	0,94**	1	0,99**	0,90*	0,09	0,28
FP	0,06	0,09	-0,49	0,99**	0,81*	0,93**	1	1,00**	-0,32	0,09
FP	-0,14	-0,49	-0,43	1,00**	0,94**	1,00**	1	0,94**	0,14	0,32
LAC	0,03	0,12	-0,54	1,00**	0,83*	0,91*	0,99**	1	-0,32	0,09
LAC	-0,14	-0,49	-0,43	1,00**	0,94**	1,00**	1,00**	1	0,31	0,41
Z	0,03	-0,26	-0,37	0,37	0,49	0,26	0,49	0,37	1	-0,85*
Z	-0,03	0,54	-0,09	0,43	0,37	0,43	0,43	0,43	1	0,35
SCC	-0,88*	-0,30	-0,29	0,29	0,29	0,40	0,30	0,29	0,10	1
SCC	0,51	-0,78	0,34	0,07	0,07	0,07	0,07	0,07	-0,68	1

*P<0.05 correlations mean significant, **P<0.01 level correlations mean important. (The correlation coefficients in bold (r) belong to the Evening group, and the italics belong to the Experimental group).

Table 8. Correlations between control and trial day 10 morning and evening milk MLT and milk quality parameters.

CONTROL			**TRIAL**							
Morning **Evening**	*Serum MLT*	*Milk MLT*	*FAT*	*SNF*	*DEN*	*PROT*	*FP*	*LAC*	*Z*	*SCC*
Serum MLT	1	0,60	0,89*	-0,31	-0,49	-0,20	-0,31	-0,31	0,00	0,44
Serum MLT	1	0,99**	-0,12	0,41	0,12	0,32	0,41	0,41	0,35	0,36
Milk MLT	-0,15	1	0,43	-0,83*	-0,94**	-0,78	-0,83*	-0,83*	0,35	0,09
Milk MLT	0,09	1	-0,14	0,43	0,14	0,26	0,43	0,43	0,31	0,44
FAT	0,26	-0,09	1	-0,20	-0,37	-0,14	-0,20	-0,20	-0,43	0,79
FAT	0,66	0,60	1	-0,94**	-1,00**	-0,77	-0,94**	-0,94**	-0,14	-0,03
SNF	-0,18	0,56	-0,46	1	0,94**	0,99**	1,00**	1,00**	-0,32	0,09
SNF	-0,54	-0,03	-0,77	1	0,94**	0,83*	1,00**	1,00**	0,31	0,03
DEN	-0,22	0,50	-0,72	0,94**	1	0,93**	0,94**	0,94**	-0,23	-0,09
DEN	-0,37	-0,26	-0,83*	0,94**	1	0,77	0,94**	0,94**	0,14	0,03
PROT	-0,17	0,57	-0,40	0,99**	0,92*	1	0,99**	0,99**	-0,22	0,04
PROT	-0,54	-0,03	-0,77	1,00**	0,94**	1	0,83*	0,83*	0,26	-0,37
FP	-0,17	0,47	-0,39	0,99**	0,90*	0,99**	1	1,00**	-0,32	0,09
FP	-0,54	-0,03	-0,77	1,00**	0,94**	1,00**	1	1,00**	0,31	0,03
LAC	-0,18	0,56	-0,50	0,99**	0,96**	0,99**	0,98**	1	-0,32	0,09
LAC	-0,54	-0,03	-0,77	1,00**	0,94**	1,00**	1,00**	1	0,31	0,03
Z	-0,71	0,13	0,10	-0,13	-0,08	-0,14	-0,15	-0,13	1	-0,85*
Z	-0,43	-0,14	-0,77	0,89*	0,94**	0,89*	0,89*	0,89*	1	-0,30
SCC	-0,11	-0,40	-0,36	-0,59	-0,28	-0,64	-0,64	-0,55	0,33	1
SCC	0,39	0,33	0,82*	-0,88*	-0,88*	-0,88*	-0,88*	-0,88*	-0,70	1

* P<0.05 correlations mean significant, **P<0.01 level correlations mean important.

Table 9. Correlations between control and trial day 15 morning and evening milk MLT and milk quality parameters.

CONTROL			**TRIAL**							
Morning **Evening**	*Serum MLT*	*Milk MLT*	*FAT*	*SNF*	*DEN*	*PROT*	*FP*	*LAC*	*Z*	*SCC*
Serum MLT	1	-0,20	-0,49	-0,71	-0,32	-0,71	-0,71	-0,67	0,14	0,00
Serum MLT	1	0,06	0,26	-0,31	-0,37	-0,14	-0,14	-0,37	0,03	0,12
Milk MLT	0,26	1	0,20	0,20	0,20	0,20	0,20	0,09	-0,54	0,41
Milk MLT	0,93**	1	-0,41	0,64	0,52	0,84*	0,84*	0,52	0,38	-0,72
FAT	-0,66	-0,14	1	0,61	0,00	0,61	0,61	0,59	-0,49	0,01
FAT	-0,70	-0,83*	1	-0,60	-0,77	-0,49	-0,49	-0,77	-0,89*	0,62
SNF	-0,14	0,37	0,14	1	0,75	1,00**	1,00**	0,99**	-0,09	-0,55
SNF	0,23	0,43	-0,03	1	0,94**	0,94**	0,94**	0,94**	0,43	-0,93**
DEN	-0,64	-0,06	0,06	0,58	1	0,75	0,75	0,76	0,29	-0,72
DEN	0,52	0,77	-0,60	0,77	1	0,83*	0,83*	1,00**	0,66	-0,93**
PROT	-0,12	0,46	0,12	0,99**	0,59	1	1,00**	0,99**	-0,09	-0,55
PROT	0,29	0,37	0,14	0,94**	0,60	1	1,00**	0,83*	0,37	-0,93**

CONTROL						**TRIAL**				
Morning **Evening**	*Serum MLT*	*Milk MLT*	*FAT*	*SNF*	*DEN*	*PROT*	*FP*	*LAC*	*Z*	*SCC*
FP	-0,26	0,49	0,09	0,94**	0,64	0,93**	1	0,99**	-0,09	-0,55
FP	0,12	0,37	-0,09	0,94**	0,83*	0,83*	1	0,83*	0,37	-0,93**
LAC	-0,14	0,37	0,14	1,00**	0,58	0,97**	0,94**	1	0,06	-0,60
LAC	0,12	0,37	-0,09	0,94**	0,83*	0,83*	1,00**	1	0,66	-0,93**
Z	-0,49	0,26	0,31	-0,20	-0,06	-0,23	0,09	-0,20	1	-0,32
Z	0,58	0,31	0,09	0,09	-0,14	0,37	-0,14	-0,14	1	-0,62
SCC	-0,06	-0,46	-0,55	-0,55	0,24	-0,53	-0,46	-0,55	-0,12	1
SCC	-0,03	-0,27	-0,03	-0,94**	-0,70	-0,82*	-0,88*	-0,88*	0,15	1

*P<0.05 correlations mean significant, **P<0.01 level correlations mean important.

On the 5th day of the study, serum MLT and FAT were positively high in the evening group (r=+0.89, p<0.05), serum MLT and DEN (r=-0.84, p<0.05) and SCC (r=-0.81, p<0.05) a negative high correlation was detected. There was no significant relationship between milk MLT and milk parameters in the control group (P>0.05). Significant between SNF (r=-0.83, p<0.05) and LAC (r=-0.83, p<0.05), DEN (r=-0.94, p<0.05) in the trial morning group with milk MLT (<0.01), very significant negative associations were observed between a very significant positive correlation (r=+0.94) was found between serum MLT and FAT in the trial evening group. It was observed that there was a high positive (r=+0.87, p<0.05) correlation between milk MLT and SCC in the trial evening group. When the correlations in terms of SCC were examined, no significant relationship was detected in the control evening group, while there was a highly negative (r=-0.88, p<0.05) relationship with serum MLT in the morning group. In the experimental groups, there was a high negative correlation (r=-0.85, p<0.05) with only conductivity (Z) in the morning group (Table 7).

A significantly high correlation was detected between serum MLT and milk MLT in the evening in the control group on the 10th day of the study (r=0.99, P<0.05). In terms of milk quality characteristics, a high positive (r=0.89, p<0.05) correlation was determined between morning serum MLT and FAT in the morning and evening groups on the 10th day of the trial. No significant relationships could be determined with serum MLT in terms of other characteristics. Significant correlations were found between milk MLT and SNF, DEN, FP, and LAC in the morning control group (p<0.05). There was no significant correlation between the control evening milk MLT and any of the features (p>0.05). In the control evening, positive (r=0.82) relationships between FAT, which is between SCC and milk components, and negative (r=-0.88) relationships between SNF, DEN, PROT, FP and LAC were determined. It was calculated that there was only a negative significant correlation (r=-0.85) between SCC and Zara in the trial morning group. On the 10th day of the study, very high positive correlations were observed between the control SNF and DEN parameters and PROT, FP, LAC, Z, provided that they were similar in the morning and evening groups. In the same groups, very high positive correlations were observed between PROT and FP, LAC and Z (Table 8).

On the 15th day of the study, a very high positive correlation was detected between serum MLT and milk MLT levels in the trial evening group (r=+0.93 p<0.01). There was a significant negative correlation between milk MLT and FAT in the trial evening group (r=-0.83, p<0.05). On the 15th day in the control group, significant negative correlations (p<0.05) were observed between SCC and SNF and between SCC and PROT, FP and LAC. It was revealed that there were very high negative relationships(r=-0.93 p<0.01) between SCC and SNF, DEN, PROT, LAC, and FP in the red light trial groups (Table 9).

Discussion

Currently, it is thought that different light applications in livestock farms may be beneficial in terms of animal productivity. By adjusting the light and dark times, it is tried to provide advantages such as energy saving and more efficiency. The eyes of all mammals contain light-sensitive rods that allow vision in the dark and cones that allow us to see shapes and colors in daylight. Cow eyes are large and contain a high amount of rods, which may facilitate vision

at low light intensities (Phillips et al. 2000). Today, it is not known exactly how well cattle can see in the dark, and there is no complete information about the color discrimination in cattle. All primates are trichromatic and have three types of cones, while the mullein has only two types of cones and is dichromatic. For this reason, it is thought that cattle cannot distinguish between green and red (Sjaastad et al. 2003). At night, breeders use red light, known as "invisible light"' for cattle. This suggests that red light is useful for night-time lighting. Thus, the farmers think that they will not disturb the animals when they control them at night (Olsson 2020). In our study, no significant changes were observed in the milk and serum MLT levels and SCC in the control and light trial groups, which is consistent with the finding that red light is beneficial in night lighting.

Light is the most important factor affecting the synthesis and release of the MLT hormone, which is an indole compound and synthesized from the amino acid tryptophan. The secretion of melatonin by pineal gland cells depends on the sensitivity of pinealocytes to light. As a result of this sensitivity, the inhibition caused by light disappears in the dark and MLT secretion by melanocytes increases. Whether the environment is light or dark, in other words, day and night, is the main factor in MLT synthesis and release (Özgüner et al. 1995). Melatonin secretion reaches its highest level, especially at around 23.00-05.00 at night. The level of MLT in the cell and in the blood increases 3-10 times compared to that in the daytime. While the blood concentration of melatonin in humans is approximately 0-20 pg/dl during the daytime, it rises to 50-200 pg/dl at night (Claustrat et al. 2005). Also, MLT biosynthesis is sensitive to the wavelength of light. Synthesis is suppressed by short light wavelengths (Cajochen et al. 2005; Asher and Corsi 2015). In general, light intensity is an important parameter for measuring the suppression level of MLT, and as the brightness of the light stimulus increases, the suppression power also increases (Özçelik et al. 2013). In a study on MLT secretion with blue and red light in humans, it was stated that blue light (479 nm) suppressed MLT secretion, while red light (627 nm) had no effect. In another study conducted in humans, it was reported that MLT secretion was suppressed by exposure to short-wavelength light (446-477 nm) (Brainard et al. 2001). It has been shown that light in the short-wavelength spectrum (465-485 nm) suppresses MLT production in mammals (Lockley et al. 2003; Mainster, 2006; Brainard et al. 2008). On the other hand, it is known that the effects of light on MLT synthesis and release are not similar among different species. For example, it has been reported that light application at a wavelength of 640 nm in hamsters inhibits MLT secretion from the pineal gland, MLT suppression occurs at 460 nm in humans, and serum MLT levels decrease slightly at 630 and 700 nm (Hanifin et al. 2006). In this study, it was determined that 50 lx red light at wavelength 652 nm at the animal eye level, applied for 15-days, did not significantly change serum MLT levels in milk cows compared to dark application. This is important in terms of showing that MLT synthesis does not decrease in farms that will be illuminated at night with red light.

The presence and levels (5-25 pg/ml) of the MLT hormone, which is photoperiodically synthesized from the pineal gland, in human, cow, and goat milk have been demonstrated (Eriksson et al. 1998; Valtonen et al. 2003; Castro et al. 2011). Milk MLT levels in cows have been reported at different levels by different researchers: Milk MLT levels in Ayrshire cows are 7±2 pg/ml, (Erikkson et al. 1998), in dry period Holstein-Friesian cows 5.4 pg/ml in February, 11 in August 8 pg/ml (Schaper et al. 2015) and 2.9±0.6 pg/ml in dairy cows in June (Castro et al. 2011). Milk MLT levels in the study (in the range of 3.93-5.23 pg/ml) were slightly lower than the levels reported by Erikkson et al. (1998) and Schaper et al. (2015) and slightly higher than the levels reported by Castro et al. (2011) was determined as. Serum MLT levels obtained in the morning and evening hours in the control group were 7.02 pg/ml in the control group, close to Erikkson et al. (1998) and slightly higher 8.71 pg/ml in the evening group. In the red light experimental groups, it was in the range of 7.53-8.59 pg/ml.

The concentration of MLT in milk follows a diurnal course in parallel with the serum concentration. The highest concentration is at night and the lowest at noon. For this reason, milk expressed at night (between 2 and 4 at night) contains higher MLT than milk expressed during the day (Milagres et al. 2014). In the study, no difference was observed in terms of MLT levels between blood and milk samples taken around 06.00 a.m. and blood and milk samples taken around 06.00 p.m. This may be related to the short half-life of the MLT hormone. The half-life of melatonin in the blood is approximately 40 minutes (Çevik and Yurdaydın 1998). It has been reported that the release of MLT, which has a special circadian rhythm, starts to increase at 09.00-10.00 p.m. hours in the evening, reaches a maximum level at 02.00-04.00 a.m. hours, starts to decrease at 05.00-07.00 a.m. in the morning and decreases to basal levels

after 07:00 a.m. (Claustrat et al. 2005; Castro et al. 2011). The pineal gland synthesizes approximately 80% of the amount of MLT in the bloodstream. On the other hand, MLT synthesis is not limited to the pineal gland. The presence of hydroxyindole-O-methyltransferase (HIOMT), known to be involved in the synthesis of melatonin, in organs other than the pineal gland suggests that MLT synthesis also occurs outside the pineal gland. It has been reported that some cells in the Harder gland, lacrimal gland, retina, erythrocytes, platelets and gastrointestinal tract synthesize MLT (Cardinali and Pévet 1998). There are no literature data on MLT secretion from the mammary gland. Although there is information that milk and serum MLT levels follow a parallel course (Milagres et al. 2014), it is reported that this parallelism changes according to the lactation and non-lactation periods of animals. While milk MLT levels were 7±2 pg/ml during the lactation period in Ayrshire cows, it was 15±1 pg/ml at night and did not show any correlation with serum MLT levels (7±2 pg/ml during the day, 27±7 pg/ml at night), milk and serum MLT levels changed in parallel in the non-lactation period (Erikkson et al. 1998). This study was conducted on Holstein cows that were on the 110th day of lactation on average. The fact that the milk MLT level did not change at the same rate as serum MLT is consistent with the study of Erikkson et al. (1998). On the other hand, it differs from the studies of Vanecek (1998) and Castro et al. (2011) who suggested that there is a relationship between blood and milk melatonin concentrations. In addition, it was observed that milk MLT levels decreased on the days 5th, 10th and 15th days following the red light application, and the statistical difference was detected on the 10th day. Statistically significant correlations between milk and serum MLT levels were detected only at day 15 in the control and on day 10 of the trial. In the light of these findings, it can be thought that milk MLT levels respond to red light application differently from serum MLT levels, serum and milk MLT levels do not always follow a parallel pattern, and there may be other mechanisms controlling milk MLT levels.

It is thought that there are some relationships between milk production, milk content and MLT. MLT applied externally (implanted) to dairy cows grazing in the pasture by Auldist et al. (2007) decreased milk yield, decreased milk lactose levels, and increased fat, protein and casein levels. Externally applied MLT implant in Holstein cows decreased the number of milk somatic cells and also increased serum albumin alaninetransferase and lactatedehydrogenase levels while decreasing cortisol levels (Yang et al. 2017). In his study, it was noted that after MLT administration, the concentration of IgG and IgM increased transiently, followed by a significant decrease in white blood cells and lymphocytes. In conclusion, MLT treatment improved immune activity in cows, reduced SCC, and improved milk quality.

Milk fat is a complex mixture of different types of fat, which is the body's main source of energy. As the milk fat content increases, the quality of the milk improves (Romero Velarde et al. 2019). Molik et al. (2011) showed that MLT application and exposure to short day photoperiod in different seasons in sheep had significant effects on protein, fat, and lactose levels and fatty acid content of sheep milk ($P<0.01$). In this study, a high positive correlation ($r=+0.94$, $p<0.01$) was observed between serum MLT and milk fat levels in the control group on the 5th day. In addition, high positive correlations ($r=+0.89$, $p<0.05$) were determined between morning and evening serum MLT and milk fat levels in the 5th day red light group. This correlation between serum MLT and milk fat levels was not detected in the control group, although the red light was also seen on the 10th day. It can be thought that the fat content in milk is positively affected by light application. On the other hand, endogenous MLT and exogenous MLT may have different interactions with milk parameters, and the dose of MLT is one of the issues that may be important in this interaction.

In their study on cow's milk, they found the relationship between milk fat (%) and milk SCC and milk protein (%) levels as $r=0.37$ and $r=0.63$, respectively. Similarly, in this study, correlations were calculated as $r=0.60$ between evening milk SCC and milk fat in the experimental group on the 15th day, and $r=0.61$ between milk fat and milk protein levels in the morning group. The correlation between milk fat and milk SCC was slightly higher than that observet in this study ($r=0.62$) (Ikonen et al. 2004). In both studies, correlations were not found to be statistically significant ($P>0.05$). It was determined that the correlation level, which was $r=-0.81$ $p<0.05$ on the 5th day in the evening of the experimental group, was $r=+0.36$ on the 10th day and $r=+0.12$ on the 15th day, and may have changed by being affected by red light. SCC with serum MLT was calculated as $r=+0.87$ ($p<0.05$) on the 5th day, $r=+0.44$ on the 10th day and $r=-0.72$ on the 15th day. This trend of change in serum MLT levels can be considered as an important finding in terms of preventing mastitis formation and improving milk quality.

On the 10th day of the study, a negative correlation (r=-0.81, p<0.05) was determined between evening serum MLT levels and milk SCC in the control group. In addition, a negative correlation was determined between serum MLT levels and milk SCC in the 10th day of red light application (r=-0.81, p<0.05). Yang et al. (2017) as reported, it is thought that this negative correlation between MLT and SCC obtained in our study may be important in preventing mastitis. The fact that serum and milk MLT levels did not decrease statistically can be considered as an important finding in our study, although a nighttime illumination of 50 lx was used with red light.

Conclusion and Recommendations

As a result of the data obtained in the study, it was observed that serum and milk MLT levels and milk parameters were not adversely affected by 15-day-old red LED bulbs (652 nm) and night (12 h) illumination in Holstein dairy cows.

In this study, in which we tried to measure the effect of red light, no statistical difference was found in terms of serum MLT, milk MLT and milk SCC measured in the morning control and morning trial groups (P>0.05). In the evening control and evening trial groups, a statistical difference was obtained for the same values on the 10th day of only the milk MLT group (P=0.004).

When the correlations of milk components were examined, it was determined that FAT and SCC were affected more in red light groups than in the control group. No significant changes were detected between serum MLT, milk MLT and milk parameters in the day,morning and evening samples. Only a very high positive correlation was determined between serum MLT and milk MLT at day 15. In addition, a strong positive correlation was observed between serum MLT and FAT in the morning and evening, while a negative correlation was found between MLT and DEN.

It was observed that 12h of illumination with red LED bulbs did not have any negative effect on milk parameters. As it can be understood from the relations between milk MLT levels and milk SCC on the 5th, 10th and 15th days of the study, it can be argued that red LED bulbs can be used in night lighting for quality milk production without causing any serious problems.

As a result, suitable red light applications in compulsory night lighting will make it possible to obtain rich quality milk without causing significant decreases in MLT levels. It can be claimed that lighting with a wavelength of 652 nm for 12h can be used in night-time shelter lighting without causing significant decreases in blood MLT levels, as in the dark.

Ethics committee for the use of experimental animals: The study was conducted at the Experimental Medicine Research and Application Center (SUDAM) of Selçuk University and the ethical approval (Year 2020 - No: 202002) was obtained from SUDAM.

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