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Effect of LED Lighting Time on Productivity, Blood Parameters and Immune Responses of Dairy Cows*

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LED 점등시간이 젖소의 생산성, 혈액 매개변수 및 면역 반응에 미치는 영향

박진룡 · 윤남진 · 샤메드 · 심관섭

Light is an essential and powerful element to animals. A light-emitting diode (LED) is most efficient in terms of economic benefits. The aim of the present study was to evaluate the effects of LED lighting time on milk production, milk composition, and the immune response of Holstein cows. Forty lactating cows were assigned to four experimental groups: control; natural daylight, treatment; am3-6, pm6-12 and pm6-am6. We found that there was no significant effect on the decrease ratio in milk production among the groups. Milk urea nitrogen (MUN) was significantly decreased in pm6-am6 and pm6-12 than the control. With regard to the hemolytic biochemical analysis, GLU was significantly increased and CRE, T-BIL were significantly decreased in the pm6-12 than the control. IGF-1 levels were significantly increased in pm6-12 compared to other groups. Besides, cortisol was significantly lowered in the pm6-12 than the control, while prolactin, IgA and IgG were not significant among the groups. In addition, catalase and glutathione peroxidase were also significantly increased in pm6-12 than the control. However, antioxidant enzyme activity and superoxide dismutase were not significant among the experimental groups. Therefore, it was concluded that LED lighting time had some impact on blood parameters and immune responses in dairy cows without

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any changes in milk production.

Key words : *blood parameters, holstein cow, immune function, LED lighting time, milk production*

Ⅰ. Introduction

Light is an essential and powerful element to animals and birds. Many previous studies revealed that it influence the growth performance (Rozenboim et al., 2004), reproductive characteristics (Bobadilla-Mendez et al., 2016) and immunity of birds (Xie et al., 2011; Seo et al., 2015). The wavelength, intensity and cycle of light mostly affect the physiological state and activity of animals. Light intensity and spectral quality has also a great effect on plant life such as photo-synthetic performance, differentiation, and flowering (Smith, 1982). Livestock industries have been continuously tried to improve production by applying lighting system to the farm. In poultry production, it is well known that lighting factors (light intensity, color and exposure time) affect the physiology and immune competence of chickens (Foss et al., 1972; Rozenboim et al., 1999; Xie et al., 2008; Blatchford et al., 2009).

In dairy industry, photoperiod is usually used for more milk production. Increased duration of photoperiod increases growth (Rius et al., 2005) and milk production (Dahl et al., 2000) in dairy heifers and lactating dairy cows respectively. Previous studies also showed that expose of 18 h light increased the milk production than the natural daylight in lactating cows (Peters et al., 1981; Dahl et al., 1997). Photoperiod also affected the hormones i.e. melatonin and prolactin revealed the most dynamic changes. The duration of elevated melatonin in turn influences the secretion of a number of other hormones, with prolactin (PRL) and insulin-like growth factor-I (IGF-I) being of greatest relevance to the effects of photoperiod in cattle (Dahl and Petitclerc, 2003). More exposure of light also increased the concentration of IGF-1, which stimulated milk production. Prolactin secretion is very irregular within a given 24-h period and displays increased concentrations during months of $\geq$ 16 h of photo period (Tucker et al., 1984). Furthermore, melatonin increases IGF-I secretion in male Syrian hamsters (Vaughan et al., 1994). Proper pho-toperiod positively influence the immune system of dairy cows could have a substantial impact on cow's health and welfare (Auchtung et al., 2004). There were many studies have been done on the effect of photoperiod in dairy cows, but to our knowledge researches on the effect of LED lighting time are very rare. Therefore, the objective of this study was to evaluate the effect of LED lighting time on milk production, blood parameters and immune responses of dairy cows.

Ⅱ. Materials and Methods

1. Animals and Experimental Design

The animals were cared for according to procedures approved by the Institutional Animal Care and Use Committee, Chonbuk National University, South Korea and in accordance with the Korean National Law on Animal Care and Use. The animals were obtained and maintained in the Gochang District, Jeollabuk Province, South Korea (35°25' N, 126°41' E). The cows were housed under hygienic conditions (22-24°C) in barns. The subjects were 40 multiparous Holstein cows in lactation during the experimental period from November 2016 to June 2017. After acclimatization for a period of two weeks, the animals were randomly divided into the four experimental groups (Table 1). Forty lactating cows were assigned to four experimental groups: control; natural day light (average 6:49~18:17) group, am 3-6; natural light + LED (03:00~06:00), pm 6-12; natural light + LED (18:00~00:00) and pm 6-am 6; natural light + LED (18:00~06:00). The supplemental LED light intensity was adjusted to 150 lux at eye level and controlled by an automatic timer. The cows were allowed free access to a Total Mixed Ration (TMR) feed program and water (Table 2). The TMR moisture, crude protein, fat, ash and fiber were determined according to the procedures of AOAC (1990). The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by using an Ankom^{2000} Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA).

Table 1. Experimental Design of LED treatment

Group	Number of cows	Treatment
Control	10	Exposed only natural daylight (average 6:49~18:17)
am 3-6	10	Exposed natural daylight and 3 h LED light (03:00~06:00)
pm 6-12	10	Exposed natural daylight and 6 h LED light (18:00~00:00)
pm 6-am 6	10	Exposed natural daylight and12 h LED light (18:00~06:00)

Table 2. Diet composition (%)

Item	Value	Item	Value
Ingredients (%)		Chemical analysis (%)	
Corn silage	18.5	Moisture	32.6
Corn grain	29.2	Crude protein	11.0

Item	Value	Item	Value
Soybean meal	3.1	Crude fat	1.9
Alfalfa hay	13.8	Crude ash	14.7
Ryegrass	9.2	Crude fiber	5.9
Sudan grass	15.4	NDF	28.0
Oat	9.1	ADF	17.2
Mineral and vitamin premix	1.6		

2. Milk production and composition

Milk production was recorded at every milking with the Lely automatic milking system (Model Astronaut A4 milking robot LH 2014, Lely Industries N.V., Maasluis, the Netherlands) and collected during one day in total, but the time and opportunities for milking were different every day and for each Holstein cow. The composition of the milk was analyzed for fat, protein, lactose, solids-not-fat (SNF), somatic-cell-count (SCC) and milk-urea-nitrogen (MUN) by using a Milko-Scan FT 500 (Foss Electric, Denmark).

3. Blood sample collection and assay procedure

Blood samples from each group (10 mL) were taken from the jugular vein, using evacuated tubes with and without EDTA on the experiment day. Both serum and plasma were harvested by centrifugation at 15,000 rpm for 15 min at 4℃ and stored at -20℃ until analysis. The biochemistry levels of the following were assayed, using an Hitachi 7180 automatic analyzer (Hitachi Ltd., Tokyo, Japan): albumin (ALB), creatinine (CRE), creatine phosphokinase (CK), guanosine triphosphate (GTP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), total cholesterol (T-CHO), total protein (T-PRO), triglycerides (TG), blood urea nitrogen (BUN), non-esterified fatty acid (NEFA),as well as beta-hydroxybutyrate (β-HB), calcium (CA), glucose (GLU), magnesium (MG), and phosphorous (PHO) in the blood.

4. Measurement of antioxidants and hormones in blood

Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and total antio-

xidant capacity (TAC) of activity were analyzed in plasma using ELISA kit (catalog no. 706002, 703102, 707002 and 709001, respectively; Cayman Chemical Company, Ann Arbor, MI, USA). Hormones such as prolactin in plasma was determined using ELISA kit (catalog no. EKU08580; Biomatik, Wilmington, USA), IGF-1 in plasma was measured using ELISA kit (catalog no. CSB-E08893b; Cusabio Biotech Co. Ltd. Wuhan, China). Cortisol in plasma was assayed using the ELISA kit (catalog no. CSB-E13064B; Cusabio Biotech Co. Ltd. Wuhan, China).

5. Statistical analysis

All values were expressed as the means and standard errors of the mean (SEM) and the differences among the experimental groups were statistically evaluated by Analysis of Variance (ANOVA) procedures, followed by Duncan's Multiple Range Test for post-hoc comparisons. The milk production data were analyzed by regression analyses using the of SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). The formula for the regression analysis used to identify determinants of milk production was as follows.

$$Y = aX + b \quad (1)$$

a = slope (decrease ratio), b = intercept

Ⅲ. Results and Discussion

1. Milk Production

Dairy farming was generally known as one of the most profitable farming sectors in Korea. Fig. 1. shows the rate of decrease in milk production. Here, the slope is the decrease ratio and the intercept b represents the milking potential of each cow. Exposure of short day photoperiod (8 h of light : 16 h of dark) on cows had greater milk yields than long day photoperiod (16 h of light : 8 h of dark) cows (Mikolayunas et al., 2008). This is accordance with our current experiment that the 6h photoperiod had a lower decrease ratio of the milk production compared to others (Table 3). The pattern of milk production has measured by lactation curve that provides valuable information during lactation period. Moreover, biological efficiency of the cows determined by the pattern of milk yield (Scott et al., 1996).

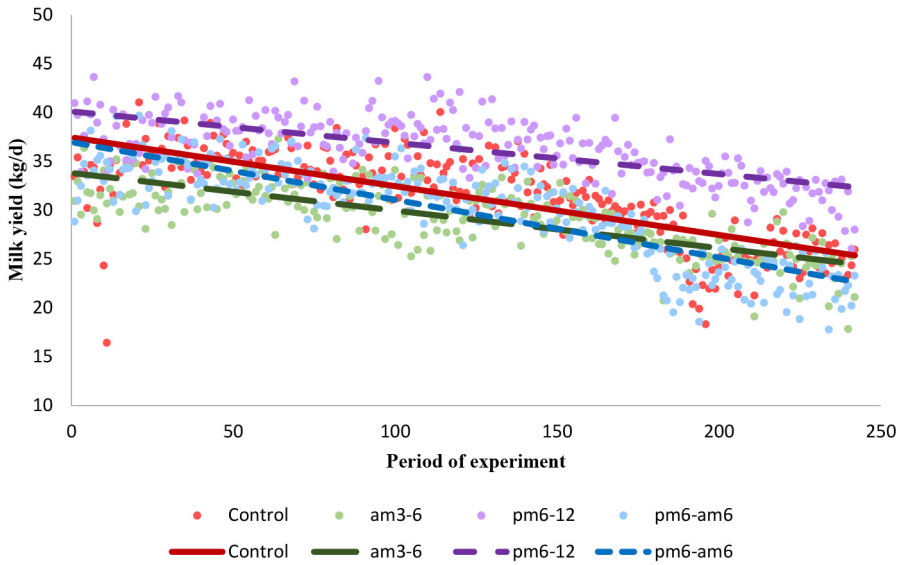

Fig. 1. Effects of different time photoperiod (3 h, 6 h and 12) on milk production.

Table 3. Effect of different time photoperiod (3 h, 6 h and 12) on milk yield

Item	Treatment			
	Control	am3-6	pm6-12	pm6-am6
Lactation slope	-0.05672±0.0097	-0.04084±0.0115	-0.03556±0.0123	-0.06604±0.0081

Mean values are presented as mean ± SE.

2. Milk Composition

Milk urea nitrogen (MUN) is an inexpensive tool to indicate the balance between dietary protein and energy, also how efficiently the cow is utilizing the protein or nitrogen to produce milk. The higher MUN concentrations are tightly correlated to plasma urea nitrogen(PUN) concentrations, and both support the observed high dietary protein and nitrogen intake which are related to milk production (Tacoma et al., 2016). Lactation curve of MUN did not affected by the milk yield curve and thus did not change much with stage of lactation (Mucha and Strandberg, 2011). Wood et al. (2003), who reported an increase of MUN concentration after the peak of lactation. Some authors reported that positive relationship between milk yield and MUN (Stoop et al., 2007) / MUN was lowest at the beginning of lactation and reached peak value and it was significantly decreased until end of lactation period. However, in our study the MUN was

significantly increased in exposure of 3 h photoperiod compared to 6 h and 12 h photoperiod. There was no significant difference in milk fat, milk protein, SNF, lactose and SCC among the treatment groups (Table 4). In this study milk yield was not related with MUN secretion, perhaps MUN can be influenced by expose of 3 h photoperiod. Normally, bovine milk contains about 3.2% protein, 3.4% fat, and 4.8% lactose, as well as various minerals and vitamins (Guo and Wang, 2016).

Table 4. Milk composition of experimental groups

Item	Treatment			
	Control	am3-6	pm6-12	pm6-am6
Milk fat (%)	2.98±0.31	4.01±0.11	3.85±0.86	3.84±0.23
Milk protein (%)	3.26±0.09	3.49±0.14	3.54±0.19	3.45±0.2
SNF (%)	8.68±0.1	8.76±0.17	8.96±0.18	8.78±0.21
Lactose (%)	4.85±0.03	4.66±0.07	4.83±0.08	4.77±0.07
SCC (x 1000)	155.6±43.66	288.83±71.07	212.33±104.48	241.86±81.07
MUN (mg/dL)	20.28±0.5^{a}	19.88±1.65^{a}	15.77±0.88^{b}	14.89±1.14^{b}

Mean values are presented as mean ± SE.

$^{a-b}$ Means within a row with different superscript letters are significantly different ($p<0.01$)

3. Blood biochemical parameter analysis

However, farmers face a practical problem in that they are unable to predict which cows are likely to succumb to metabolic disorders or efficiency of milk production (Sundrum, 2015). AST and ALT are cell integrity markers for cell membrane, cell functional and cell morphological damages in liver and blood of dairy cattle (Zhang et al., 2017). Based on this, we are determined to analyze the blood parameters of white color LED exposed animal at different exposure time. In our results there were not significant differences in the AST and ALT levels in all experimental group. Additionally, both 3 h and 12 h photoperiods cows showed significantly ($p<0.02$) increased the creatinine (CRE) level as compared to 6 h exposed photoperiod cows. Additionally, the level of glucose (GLU) was significantly ($p<0.001$) increased in 6 h exposure of photoperiod compared to other group, whereas there were no significant differences among the control, 3 h and 12 h exposure of photoperiod (Table 5). Oral administration of propylene glycol increases plasma glucose and insulin, and modestly increases milk yield (Zhang et al.,

2017). Glucose and total protein during lactation were also influenced by age, presumably associated with an increase in milk production with age (Blum et al., 1983). It may accordance with our current results that increased the level of glucose in 6h could be related with increased the milk production but not significantly (Table 5). Increases total bilirubin values of the cattle diagnosed with subclinical and clinical ketosis may indicate the existence of a functional disorder or liver damage. The level of total bilirubin (T-BIL) were significantly higher in control cows, exposure of 3h, 6h and 12h photoperiod did not produce any significant alteration in T-BIL levels (Table 5). Kappen (2012) reported that T-BIL was not significant difference between short day (8 h light : 16 h dark) and long day (16 h light : 8 h dark) photoperiods of cats. Our results also indicate that there was no significant difference between each other but, it has significantly ($p<0.01$) increased in control cows. Thus, the exposure of different time photoperiod on cow does influence the T-BIL. T-CHO and triglycerides are taken up by the mammary gland which could explain the negative relationship between milk yield and plasma T-CHO level. In contrast, Schwalm and Schultz (1976) reported positive correlations between milk yield and plasma T-CHO concentrations. Increasing TG levels are described as an indicator of stress condition (Odihambo Mumma et al., 2006). There was an interaction between photoperiod length and light intensity for cholesterol level. The continuous photoperiod length and dim light increased cholesterol level.

Table 5. Blood biochemical parameters of experimental groups

Item	Treatment			
	Control	am3-6	pm6-12	pm6-am6
ALB (g/dl)	4.08±0.13	4.25±0.14	4.49±0.09	4.47±0.08
CA (mg/dl)	11.43±0.32	12.07±0.18	11.81±0.26	12.08±0.25
CK (IU/L)	138.89±25.56	154.20±11.70	172.60±26.24	127.00±7.03
CRE (mg/dl)	24.21 ± 0.56^{A}	22.15 ± 0.95^{AB}	20.69 ± 0.69^{B}	23.76 ± 1.12^{A}
GTP (IU/L)	32.30±2.00	28.60±1.80	31.20±1.74	29.33±1.75
GLU (mg/dl)	51.10 ± 3.28^{b}	52.00 ± 1.81^{b}	64.00 ± 2.35^{a}	51.00 ± 2.21^{b}
AST (IU/L)	92.70±15.87	80.90±3.64	82.20±4.97	80.56±3.94
ALT (IU/L)	29.30±1.77	29.70±1.65	31.70±1.71	32.67±1.72
LDH (IU/L)	886.60±77.63	854.20±78.33	861.80±46.16	825.33±67.12
MG (mg/dl)	3.00±0.11	3.12±0.09	3.32±0.06	3.20±0.12

Item	Treatment			
	Control	am3-6	pm6-12	pm6-am6
PHO (mg/dl)	6.21±0.35	6.25±0.22	5.71±0.30	6.34±0.33
T-BIL (mg/dl)	0.10 ± 0.02^{A}	0.06 ± 0.01^{B}	0.05 ± 0.01^{B}	0.06 ± 0.01^{B}
T-CHO (mg/dl)	199.90±16.94	200.10±12.24	238.40±16.06	231.56±19.32
T-PRO (g/dl)	26.65±1.01	26.97±0.41	27.52±0.51	28.10±0.62
TG (IU/L)	8.90±1.27	9.30±0.79	8.80±0.63	11.11±0.96
UN (mg/dl)	16.15 ± 1.05^{b}	20.05 ± 1.15^{a}	15.50 ± 0.69^{b}	11.91 ± 0.32^{c}
NEFA (uEq/L)	171.50±63.34	88.20±7.56	78.80±6.22	99.44±4.10
β-HB (mmol/L)	69.50 ± 4.61^{b}	85.30 ± 4.87^{b}	72.50 ± 6.25^{b}	109.78 ± 7.04^{a}

Mean values are presented as mean ± SE.

$^{a-c}$ Means within a row with different superscript letters are significantly different ($p<0.01$)

$^{A-B}$ Means within a row with different superscript letters are significantly different ($p<0.05$)

In table 5 shows the urea nitrogen (UN) levels was significantly ($p<0.001$) increased in 3h photoperiod when compared to 12 h. the levels in 12 h photoperiod was significantly lower than 3 h and 6 h photoperiod. Lazarin et al. (2012) found an increase in the concentration of UN in cows consuming crude protein and energy at different times on days 6, 9 and 12 of the estrous cycles. Extending photoperiod with artificial light each increased efficiency of feed nitrogen utilization by 4.2 to 6.9% (Jonker et al., 2002). Dahl et al. (1997) reported use of an artificial photoperiod to increase milk production by 8 to 10%. Management factors that may increase FCM (bovine somatotropin, 3× milking, increased photoperiod) should increase nitrogen efficiency and reduce urinary and fecal nitrogen excretion per unit of milk produced. Due to increased milk production and nitrogen efficiency, a decrease in nitrogen excretion per unit of milk production of 8, 7, and 5% is predicted from using bovine somatotropin, 3× milking, and increased photoperiod, respectively (Dunlap et al., 2000). From our study, many of the pathological changes were observed in hormones and urea nitrogen levels which might not have influenced the milk production. Increased UN levels in blood, when the kidney function slow and may be altering the antioxidant status (Table 5). More recent studies on circadian rhythms of some liver functions showed a robust daily rhythmicity in goats maintained under a 12L:12D light – dark cycle and fed a single meal, which vanished when animals were food-deprived, revealing that digestive processes, not feeding, act as a circadian zeitgeber on the blood concentration of urea (Piccione et al., 2007). Daily rhythmicity of liver functions is widely documented

in laboratory animals (Furukawa et al., 1999). Other authors clearly showed a circadian rhythm of urea, with daily peaks at 11.00 a.m. (Lefcourt et al., 1999). This was in agreement with previous studies (Gustafsson and Palmquist, 1993), which relate mean blood urea concentration with diet. Circadian rhythm of blood urea could be obscured by frequent restricted feeding, as shown in previous studies, where high frequency or continuous feeding showed unchanging concentrations of serum urea (Folman et al., 1981). This is confirmed by our results, the exposure of 3h photoperiod shows significantly ($p<0.001$) higher UN than 6 h and 12 h. Even though between the 6h and 12h, the level of UN was higher in 6h light exposed cows.

Sun light used to boost in several species, especially in horses a positive effect in erythrocytes and hemoglobin (HB) concentration (Stendel, 1980). Although, the HB levels were significantly increase ($p<0.001$) in 12 h when compared to other treatment groups. Thus, the longtime expose of photoperiod could be positive effect in β-HB (Table 5). Many researchers reported that HB level of blood indicates the deficiency of protein level in food because circadian fluctuations that are closely related to food intake (Kato et al., 1978). It was sure that ration was not properly balanced; the animals were suffering from malnutrition and anemia. Penev et al. (2014) have reported light intensity increase from 5 to 50 lx was associated with higher hemoglobin, calcium, bicarbonates, leukocyte and erythrocyte counts. Egena and Alao (2014) were reported that HB did not significantly affect milk yield and butterfat percentage in dairy cattle. The same results have been observed in our current results that increased HB did not affect the milk yield in all treatment of cows.

4. Enzymatic antioxidant

Cortisol is a stress hormone and could affect the antioxidant capacity; the higher level of cortisol leads to lower antioxidant levels. Decreased the cortisol level could have chance to reduce the antioxidant status (Limberaki et al., 2011). This is accordance with our study showed that the decreased the cortisol level in 6 h and 12 h photoperiod could have increased the glutathione peroxide (Gpx) in same hour and increased the catalase level only in 6h photoperiod (Fig. 2. and Fig. 4). Hence, there were negative co-relation between cortisol and antioxidant status. The basis for these differential effects of short photoperiod are not yet clear (Kott et al., 1986). The short photoperiod during the dry period enhances the immune system of cows (Auchtung et al., 2004).

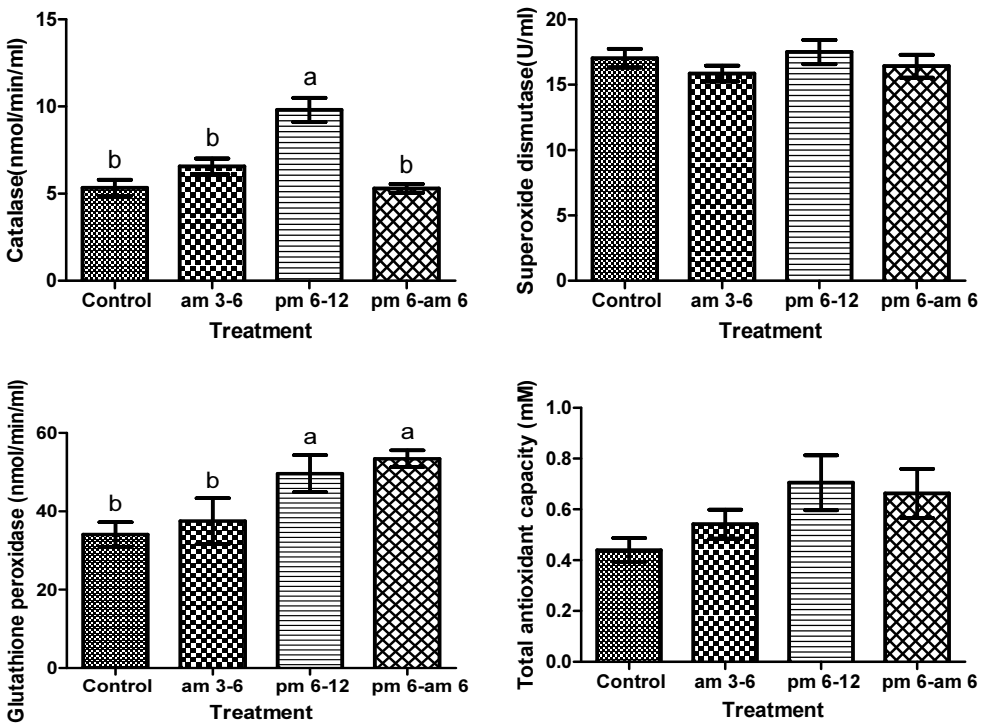

Fig. 2. Levels of enzymatic antioxidant in hemolysate of experimental groups.

5. Activities of Immunoglobulin (IgA & IgG) and Hormones

Concentration of plasma prolactin was the change of the seasonal photoperiod; the others had no effect, neither during milking nor during continuous measuring for 24 h (Kollmann, 2007). In this study we have measured the prolactin level and immunoglobulin levels such as IgA and IgG. Our results show that the level of prolactin, IgA and IgG were not significantly altered all experimental cows (Fig. 3. and Fig. 4). In addition, the 6h exposure of photoperiod can significantly enhance ($p<0.0004$) the level of IGF-1 where it significantly decreased in 12h exposed cows. Prolactin and its receptor have a major role in the regulation of growth hormone that stimulates the mammary gland to produce milk (lactation). In contrast, prolactin induced cells greatly reduced IgG1 by memory gland epithelial cells (Viitala et al., 2006). Long photoperiod in cows produced an increase in the prolactin content and overall milk yield. The lowest concentration of prolactin is observed during the short-day period (Crawford et al., 2015). However, in our current experiment the prolactin level was not significantly altered so, it might not have influence milk production. Changes in the secretion of prolactin during the lactation

period of sheep have an effect on the amount of milk produced and on the synthesis of proteins, fat and immunoglobulins. Long day pattern of melatonin secretion influences the prolactin and insulin like growth factor-1 (IGF-1) concentration in circulation. IGF-I is considered to be responsible for the mammary gland function. Infusion of IGF-I into the pubic artery of lactating goats has been shown to increase blood flow and milk production (Dahl et al., 2002). From our results, the increased level of IGF-1 was observed in 6h expose light exposed cows higher milk production than long term period. Ingestion and absorption of adequate amounts of colostral immunoglobulins (Ig) are essential for establishing passive immunity, which protects neonates from infectious diseases (Weaver et al., 2000).

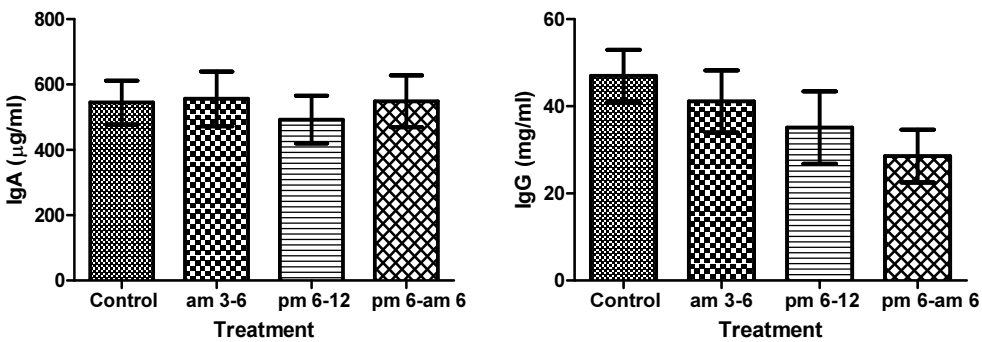

Fig. 3. Immunoglobulin (IgA & IgG) in plasma of experimental groups.

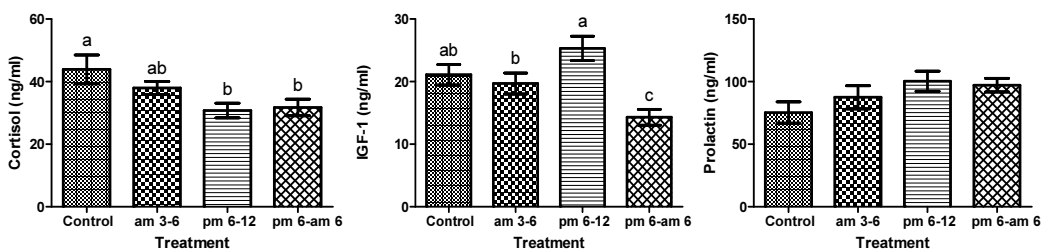

Fig. 4. Cortisol, IGF and Prolactin levels of experimental groups.

Colostrum Ig concentrations are influenced by many factors, delayed milking after parturition decreases Ig concentrations, due to dilution effects of increasing colostrum volumes. A similarly decrease in IgG concentrations was reported in experiments with a small number of animals where only 2 quarters were continuously milked (Pritchett et al., 1994; Moore et al., 2005). Verweij et al. (2014) proposed that continuous milking decreased average colostrum Ig concentrations by almost 50%, compared to cows with a conventional dry period of at least 42 days.

However, in our results there were no significant difference in IgA and IgG among the treatment group, it has not been related with milk production.

Cortisol is a primary stress steroid hormone secreted from the zona fasciculata of the adrenal cortex. It can be accelerating protein degradation (protein damage) leads to produced oxidative stress (Hackney and Walz, 2013). Cortisol was not affected by a variation of photoperiod and illumination with artificial sunlight. Different cortisol levels were observed at morning and evening milking under natural conditions, but did not exist when lighting with artificial sunlight around milking was performed (Kollmann, 2007). In our study, the level of cortisol was significantly decreased in 6 h and 12 h white color exposed LED cows compared to 3h, whereas in control group have significantly increased compared to all photoperiod group.

Ⅳ. Conclusion

In conclusion, the 6h photoperiod of white color LED exposed animal showed more antioxidant levels due to lower level of cortisol. The level of UN, 3-HB and IGF-1 were increased in 3 h, 12 h and 6h respectively in white color exposed animals which might have related to high milk production. The level of MCV and MCH were increased in 6 h and 12 h photoperiod of cows. Moreover, the level of T-BIL was significantly reduced in all experimental cows compared to control results, liver metabolism might be occurring properly. The level of glucose was significantly increased in 6 h than 3 h and 12 h light exposed cows. Additionally, the MUN was significantly increased in 3 h photoperiod when compared to 6 h and 12 h. The increased MUN level in 3 h photoperiod did not affect the milk production. Our results suggest that the different times of photoperiod could influence of external stimuli, such as feeding time, on rhythmic pattern (metabolites) involved in alteration of hormones function, milk composition, biochemical parameters and antioxidant status. Additionally, these results of the study are considering the widespread use of photoperiod in dairy animal industry to increasing incidence of antioxidant levels.

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