The Relationships between the Color of the Nose Area with Milk Yield, Physiological and Blood Paremeters of Holstein Cows in Hot and Humid Conditions

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Abstract: ***The objective of this research was to investigate the correlation between the color of the nasal region and the milk yield, physiological responses, and hematological parameters in Holstein cows under hot and humid conditions. Data were gathered during morning, noon, and evening periods in July, with an average temperature-humidity index of 82 units. Cows with a white nasal region exhibited lower respiration rates breaths per minute during the morning 67.27, 81.53 compared to those with a black nasal region, but higher rates during the noon 96.13, 95.13 and evening 99.33, 98.53 periods. The difference in morning respiration rates between cows with black and white nasal regions was statistically significant $p < 0.05$. Heart rate beats per minute was lower in cows with a black nasal region compared to those with a white nasal region during the morning 69.67, 71.13 and noon 87.27, 90.93 periods, but higher in the evening 88.87, 87.80. the rectal temperature in cows with a black nasal region was 0.14 C higher in the morning and 0.20 C lower at noon compared to cows with a white nasal region. The 305-day milk yield was higher in cows with a white nasal region compared to those with a black nasal region, although this difference was not statistically significant $p > 0.05$. However, the average weekly milk yield kg during the trial period was significantly higher $p < 0.05$ in cows with a white nasal region. While the levels of magnesium mg/dL, iron µg/dL, sodium mmol / L, potassium mmol / L, and calcium mg / dL were higher in cows with a black nasal region, these differences were not statistically significant $p > 0.05$. The study concluded that the average daily milk yield during summer was 21.18±3.68 kg in cows with a white nasal region and 15.02±2.93 kg in cows with a black nasal region, indicating that cows with a white nasal region produced an average of 6 kg more milk per day. Furthermore, physiological data suggested that cows with a white nasal region were better equipped to cope with daytime heat stress during the cooler night hours, which was also reflected in their hematological values.***

Keywords: Nose Area Color, Milk Yield, Blood Metabolites, THI

1. Introduction

Skin color in animal husbandry is among the features used to evaluate its adaptation to high air temperature and solar radiation, as it causes direct heat loss, protects against direct sunlight, encourages convection and heat loss through evaporation (Silva, 2008). Skin properties and skin color play a major role in heat transfer between the skin surface of animals and the surrounding environment. It has been documented that dark skin absorbs more solar radiation than light skin, although the penetration of sunlight into the skin is a function not only of its color but also of the skin's structure (Armitage, 2009; Stuart-Fox et al., 2017). Therefore, climatic conditions were one of the environmental factors that affect the adaptation mechanisms (Demirören, 2005). For the stress level on dairy cattle in hot regions, the temperature humidity index value (THI), which takes into account the common effect of air temperature and humidity, has been developed (Brown-Brandl et al. 2003). When the THI value was higher than 72, moderate stress occurs in dairy cows (Ravagnolo and Misztal, 2000; Dikmen and Hansen, 2009). During this period, feed intake begins to decrease and milk yield to decrease (Bouraoui et al., 2002; Herbut and Angrecka, 2012). Rectal temperature (RT), pulse rate (PR) and respiratory rate (RR), which were among the physiological determinants of adaptation to temperature stress, may cause a decrease in fertility (Indu et al., 2015) and milk yield of animals (Özhan et al., 2001). Although dairy cows were exposed to heat stress during the day, especially in regions where temperature and humidity were very high, they find the opportunity to alleviate the heat accumulated in their bodies during the cool hours of the day. However, when animals do not have the opportunity to relax in cool hours, their stress levels and losses increase further. Especially after calving, Holstein cows were more susceptible to heat stress due to the high metabolic load of milk synthesis due to breastfeeding, the high feed intake required to maintain lactation, and the energy released from the environment, resulting in excessive metabolic heat in their bodies (Dunshea et al., 2013).

The high ability of animals to adapt to changing environmental conditions in hot climates was due to the animals' adaptation mechanisms to changing conditions (Demirören, 2005). Other studies in subtropical (Becerril et al., 1993) and tropical (Maia et al., 2005) environments have also reported higher milk yield in Holstein cows with white hair than animals with black hair (Hansen, 1990). Among the thermoregulation mechanisms in some breeds, it has been reported that there was an increase in skin vascularization with a larger skin surface, shorter hairs, lighter hairs, both larger diameter and more sweat glands (Landaeta-Hernández et al., 2011; Riley et al., 2012). Animals under heat stress provide physiological adaptation by increasing sweating, respiration and pulse rate (Indu et al., 2015). The number and distribution of sweat glands also different. The structure, distribution and sizes of sweat glands in 21 different body regions in cattle were different

from each other. Most sweat glands were located in the neck region (Özkütük, 1990). Although the average number of sweat glands per square cm in the cattle body was 1871, it can reach 1000 in the lower parts of the body, and up to 2500 in the length and where the leg joins the body (Findlay and Yang, 1950). In addition, the forehead, back (sacral) and waist were the regions with the least sweat glands. However, the area between the upper lip and nose (nose area) in cattle was always moist and it differs from other body parts with its color change and pigmentation. The chance of breeding and survival of animal breeds in certain regions depends on the adaptation mechanisms. Although Holstein cattle were bred almost everywhere in the world today, there were important differences between breeds raised in tropical regions and breeds raised in temperate regions in terms of anatomical, physiological, morphological, behavioral and performance. Using the parameters related to the adaptation mechanism in animal breeding programs will provide a great advantage to make early selection. For this purpose, it became necessary to include performance and physiological parameters as well as blood components in the region where the animals were located.
For this reason, this study aimed to examine the relationships between the color of the nose area, which was always humid in the hot conditions, and milk yield, physiological parameters and blood values in Holstein cows.

2. Materials and Methods

This study was conducted with Holstein cows in Çukurova University Faculty of Agriculture, Dairy Cattle Research and Production Unit. The Faculty of Agriculture Dairy Cattle Research and Production Unit has a total of 240 Holsteins, 180 of which were dairy cow. These animals were housed in free-stall semi-open barns where fans and shower heads were used for cooling in hot weather.Adana, in southern Turkey, located in the Eastern Mediterranean Section of the Mediterranean region, at an altitude of 23 m, was located between latitudes 34-46 and 35-38 east longitude. In 2020, the average annual temperature was 28.6 °C, and the average annual precipitation was 673 mm. During the operating period, the maximum ambient temperature ranges from 22 °C to 39 °C; The relative humidity was between 18% and 99% (Table 1).The temperature-humidity index (THI), which was used as a practical indicator of the degree of stress on animals caused by weather conditions in hot regions, was calculated according to the formula reported by Broucek et al. (2006).

THI = (0,8 x maximum ambient temperature) + [% relative humidity / 100 × (average ambient temperature -14,4)] + 46,4.

The average temperature and relative humidity values in the morning, noon and evening during the 1st, 2nd and 3rd weeks in which the experiment was conducted in the study conducted were given in Table 1.

Table 1: During the experiment the ambient temperature (°C) and relative humidity (%) values

Days	Hours	Temperature (°C)			Humidity (%)			
		$\bar{x} \pm Sx$	Min.	Max.	$\bar{x} \pm Sx$	Min.	Max.	THI
1-7	07.00	26.29±0.76	25	27	85.43±8.52	74	94	78.16
	13.00	34.57±1.40	32	36	41.43±11.91	23	55	83.56
	17.00	31.86±2.34	27	34	50.00±17.58	32	79	82,33
8-14	07.00	27.29±0.76	26	28	68.14±22.98	30	94	77,77
	13.00	34.14±1.57	31	36	40.00±15.77	18	62	83.10
	17.00	33.29±1.38	32	36	42.43±14.95	20	59	83.22
15-21	07.00	24.43±1.81	22	26	87.57±12.82	69	99	75.98
	13.00	35.86±2.34	33	39	37.00±12.34	22	53	85.54
	17.00	33.86±2.67	31	38	46.00±16.99	27	67	85.75

The average air temperature calculated during the time of the experiment was measured as 26 °C in the morning, 35 °C in the afternoon, and 33 °C in the evening. It was seen that there was an increase in air temperature during the day. It was seen in Table 1 that the difference in air temperature measured in the working environment is 9 °C between morning and noon and 7 ° C between morning and evening. Igono et al. (1992) determined that at temperatures below 21 ° C, cows create a safety margin to reduce the effects of temperature stress on production, and that the minimum, average and maximum values of critical temperatures for milk production were 21 °C, 27 °C, 32 °C and THI values were 64, 72, 76. In addition, relative humidity in the working environment was found to be quite higher in the morning hours (80.38) compared to noon (39.48) and evening (46.14) hours. The daily average THI 72 was considered to be the critical point where milk yield decreases (Johnson, 1987). During the experiment, the THI values of the environment were found in the range of minimum 75 and maximum 85, and the average THI value was calculated as 82. The calculated THI values in experiment days were higher than 72, which was the critical threshold for heat stress in cows (Table 1) (Moran, 2005).

In order to create homogeneous experimental groups within the nose area color groups, a selection was made to be similar in terms of live weight, lactation order, lactation period and service period characteristics. Information about these cows in the study was summarized in Table 2.

Table 2: Characteristics of animals to be used in the study

	Black	White
Number of cows	7	7
Lactation order	2. ve 3 lactation	2. ve 3. lactation
Live weight	591±27,48	597±28,64
Service period	84,20±22.72	86,60±12.90
Body condition score (BCS: system of 5)	2,5-3	2,5-3
Clinical illness	No	No

Experimental group cows were fed with Total Mix Ration (TMR) (concentrated feed: roughage ratio = 60:40). TMR; It consists of corn silage, alfalfa, wheat straw and concentrate (18% crude protein and 2650 kcal / metabolic energy (ME) / kg). The cows were fed and milked three times a day. Water was given to the animals ad libitum.Milk yield of these animals was milked daily and recorded electronically. In addition, milk yield records arranged according to 305 days were also used in the study.

Clinical-physiological parameter measurements such as respiratory rate (breath / minute), rectal temperature (° C), pulse rate (beats / minute) of animals used in the study were recorded three times a day at 07.00, 13.00 and 17.00 hours.Rectal temperature was measured by placing a digital thermometer (DeltaTrak, Pleasanton, CA, USA) at a depth of approximately 20 cm for 60 seconds into the rectum wall of the cow. Respiratory rate was recorded by visual observation of abdominal movement. An outward movement was counted as a breath and the respiratory rate was expressed as breaths per minute.Pulse rate (beats / minute) was counted by observing the pulse of the middle coccygeal artery at the base of the tail. Both respiration and heart rate were evaluated using a stopwatch.

Blood samples from animals were taken from the jugular vein. 10 ml of blood taken from the animal was placed in plastic tubes containing heparin and centrifuged at 2800 x g for 30 minutes to remove their serum. It was stored at 120 ° C until analyzed. Plasma concentrations of 10% Ca, Mg, Na, K were measured by atomic absorption spectrophotometry (model 5000; Perkin-Elmer, Inc., Norwalk, CT) after deproteinization (Bauer et al., 1974).

The milk yield, physiological parameters and blood values of the experimental groups were analyzed in the random blocks design using the SPSS (Ver 16) computer program. Statistical significance between the mean values was tested at p<0.05 level.

3. Results

Climate information during the trial period was given in Table 2. The THI value, which measures the stress level in dairy cattle, was above 72, which was the stress threshold reported for dairy cattle. According to the literature reports, it was understood that the climatic conditions in the period in which the experiment was conducted were at a level that creates stress on animals.

Within the scope of this study, milk yield of Holstein cows with white or black nose areas were investigated and the data obtained were shown in Table 3.

Table 3: Milk yield of Holstein cows with white or black nose area in warm environment (Mean ± Standard error)

Features		Black	White	P value
Milk yield	Milk yield adjusted for 305 days (kg)	7566.2±772.66	8498±1296.98	0,198
	Average weekly milk yield during the trial (kg)	15,02±2.93	21.18±3.68	0,019

In Table 3, when the milk yield (kg) of cows with black and white nose area, arranged according to 305 days, was examined, it was seen that white nose area cows have higher milk yield than black nose area cows (8498±1296.98 and 7566.2±772.66), but the difference of 932 kg was insignificantly (p> 0.05). However, the difference of 6 kg between the average daily milk yield level of the white nose area cows (21.18±3.68 kg) and the average daily milk yield level of the black nose area cows (15,02±2.93 kg) in the conditions when the experiment was conducted was significantly (p <0.05).

Within the scope of this study, the average and standard errors of pulse rate, respiratory rate and rectal temperature detected in the morning, noon and evening measurements of the experimental groups and the results of variance analysis were given in Table 4.

Table 4: Pulse rate, respiratory rate and rectal temperature values and variance analysis results measured in the morning, noon and evening of the trial groups

Parameters	Time	Black	White	P value
Respiratory rate (breaths / minute)	Morning	81.53±18.36	67.27±10.00	0,016
	Noon	95.13±15.62	96.13±17.21	0,669
	Evening	98.53±10.03	99.33±10.71	0,310
Pulse rate (beats / minute)	Morning	69.67±11.49	71.13±7.43	0,681
	Noon	87.27±12.10	90.93±9.59	0,365
	Evening	88.87±7.14	87.80±8.33	0,709
Rectal temperature (° C)	Morning	38.07±0,75	37.93±1.66	0,807
	Noon	39.80±0,75	40.00±0.54	0,410
	Evening	40.07±0,48	40.07±0.52	1,000

As seen in Table 4, stressful hot and humid conditions changed the physiological data of the experimental material animals in the morning measurements (P <0.05) but performed at a similar level at other hours. Respiratory rate of a cow according to the ambient temperature was 26 - 50 pieces / minute per minute, body temperature 37.5-39.5 ° C; pulse rate was 40-60 pieces / minute. There were changes in physiological adaptation parameters in cattle raised in places with high THI values. In Table 4, the pulse rates (beats / minute) of the animals with black nose area were found to be lower than those with white nose area in the morning and noon hours (morning; 69.67±11.49, 71.13±7.43, noon; 87.27±12.10, 90.93±9.59, respectively) and higher in the evening hours (88.87±7.14, 87.80±8.33, respectively). In addition, the rectal temperatures (° C) of the white nose area animals were lower than the black nose area animals in the morning (37.93±1.66, 38.07±0,75, respectively) and higher in the noon (40.00±0.54, 39.80±0,75, respectively) (Figure 2). In the evening, rectal temperatures (°C) of both groups were found to be equal (Table 4).

In the case of heat stress, the blood mineral values detected in the serum of the animals were given in Table 5.

Table 5: Blood mineral values of Holstein cows with white and black nose area in hot and humid conditions

Minerals	Black	Minimum	Maximum	White	Minimum	Maximum	P value
Magnesium (mg/dL)	2.43±0.47	2.10	3.25	2.09±0.18	1.86	2.24	0,170
Iron (µg / dL)	120.60±19.42	102	148	99.00±25.65	73	128	0,172
Sodium (mmol / L)	139.40±2.61	138	144	138.20±1.10	137	140	0,371
Potassium (mmol/L)	4.82±0.19	4.50	5.00	4.66±0.32	4.40	5.10	0,367
Calcium (mg / dL)	9.26±0.49	8.40	9.60	9.04±0.59	8.10	9.70	0,540

When Table 5 was examined, it was seen that the average blood mineral average (magnesium, iron, sodium, potassium and calcium) of the animals with the white nose area was lower than the animals with the black nose area. However, this difference was not significantly (p> 0.05).

4. Discussion

Studies have reported that white-skinned cows have higher milk yield than black-skinned cows (Lee et al. 2016; Becerril et al. 1993; Maia et al. 2005). The results of this study were similar to the results of the literatures on skin color (Lee et al. 2016; Hansen, 1990; Becerril et al. 1993; Maia et al. 2005). Skin properties and skin color play a major role in heat transfer between the skin surface and the surrounding environment. It has been reported that dark skin absorbs more solar radiation than light skin (Armitage, 2009; Stuart-Fox et al., 2017), shorter and lighter hairs, larger diameter and more sweat glands and the level of skin vascularization (Landaeta-Hernández et al., 2011; Riley et al., 2012) were effective in the color of the skin. When the milk yield values of this study were examined, the milk yield of white nose area cows in summer was 21.18 ± 3.68 kg, and black nose area cows were 15.02 ± 2.93 kg. The fact that white nose area animals yield 6 kg more milk than black nose area animals can be explained by the better adaptability of white colored ones.

Gaughan et al. (2000) suggested that there was a difference in the respiratory rate of animals between day and night (Mundiave Yamamoto, 1997), and that animals can cope with heat stress by storing heat during the day and distributing it at night (Kabunga,1992). In their study, Osei-Amponsah et al. (2020) calculated the respiratory numbers of animals as 66.0, 81.8, 113.1, respectively, in an environment where THI values were 72, 73-82, ≥83. Alkoyak and Çetin (2016) determined that the respiratory rates (breaths/minute) of Holstein cows were 27.6, 64.0 ve 60.7 and the body temperatures were 38.62 ºC, 39.76 ºC, 39.76 ºC in different environmental conditions where temperature and humidity were 18 ºC - 60%, 28 ºC - 40% and 28 ºC - 80%. Anzures-Olvera et al. (2019) stated that the rectal temperature (° C) value of black-skinned cows was slightly higher.

Decreased respiratory rates do not always indicate that an animal copes with hot conditions, some animals may do slower but deep open-mouth breathing rather than frequent breathing. However, in this study, although the average respiratory rate of the animals with the white nose area was 99.33 ± 10.71, determining it as 67.27 ± 10.00 in the morning can be interpreted as they can overcome the heat load that accumulates in the cool evening hours. It has been reported that cattle can increase their respiratory rate during the colder night hours in order to reduce the heat load and increase heat dissipation due to high temperatures in their bodies during the daytime (Gaughan et al., 2000). The reason for the increase in the respiratory rate observed in the afternoon was that the high ambient temperature increases the energy expenditure of the animals and causes an increase in the oxygen demand in the tissues (Garner et al., 2017).

Bun et al. (2018) stated that Holstein cows exposed to extreme heat without shadows had a higher heart rate than those kept in shaded area. Bouraoui et al. (2002) stated that the heart rate of the animals was 66, 74, 69 respectively, in the morning, noon and evening, during the summer when the THI value increased from 68 to 78 and the air temperature increased by 0.5 ° C per day. When Table 4 and Figure 3 were examined, the pulse rate (beats / minute) n the morning, noon and evening was found to be 69.67, 87.27 and 88.87 for black nose area animals, and 71.13, 90.93 and 87.80 for white nose area, respectively. Pulse rate of black and white nose area cows was found to be higher than Bouraoui et al. (2002)'s study results. It can be said that the pulse rate (beats / minute) numbers of animals with white nose area in the study were higher than animals with black nose area in the morning and noon hours, because such reactions were the stimulation mechanisms of the body initiated by the cow to restore temperature balance (Bouraoui et al., 2002). When the physiological data of this study were evaluated in general, it was understood that cows with white nose area were more advantageous than those with black nose area in terms of adaptation to hot and humid conditions.

The normal physiological serum magnesium level of cows was determined by Altıntaş and Fidancı (1993) as 0.65-1.23 mmol / L (1.58-2.99 mg / dl). In addition, in studies conducted by various researchers, they determined the serum total magnesium concentration in the blood at the rates of 1.91-3.32 mg/dl (Stancioiu and Constantinescu, 1983); 1.75-2.84 mg/dl (Bari et al., 1996) 1,20 mmol/L (2,92 mg/dl) (Yıldız and Balıkçı, 2004). When Table 5 was examined, it was seen that the magnesium ratios of animals under heat stress were at levels similar to those determined by other researchers. The ability of animals with both white and black nose area to keep their blood magnesium levels at normal levels in hot conditions (average THI during the sampling month: 82 units) was expressed as a high indicator of the mechanism of adaptation to thermal stress (Hammond et al., 1996). Anzures-Olvera et al. (2019) stated that blood variables did not differ between white and black skinned cows, and the results were among the accepted standard values for cows (Jain, 1993). Thus, they stated that the skin color of the animals did not cause any physiological imbalance or worsening of the health status of the cows. When Table 5 was examined, it was seen that the difference between blood metabolites was not significantly ($p > 0.05$) as stated by Anzures-Olvera et al. (2019).

Iron has important functions in the animal body such as growth, energy production, a healthy immune system and the transport of oxygen in the blood by hemoglobin. Utlu et al. (2005) stated the serum iron level as 120-186 µg / dl for healthy cattle. When these data were examined, it was seen that the average iron level of black nose area animals was close to the lower limit (120.60 µg / dl), but the iron ratio of white nose area animals was much lower than the normal level (99.00 µg / dl) (Table 5). Fidan (2006) determined the iron rate in summer as 128.56 µg / dL. The iron ratio in Table 5 was lower than the value found by Fidan (2006). Iron deficiency is associated with growth retardation, poor immune function, weakness, and anemia (Graham, 1991; Puls, 1994). Especially, the reason for the lower blood iron ratio of white nose area animals was that the animals were moderately and severely stressed during the weeks of the experiment. During this period, respiratory rate, respiratory depth and heart rate increase in order to get the oxygen needed by the body. The animal in stress conditions tries to meet the increase in oxygen demand in the tissues by breathing frequently. The low iron level in both color groups during this period can be explained by the change in respiratory rate.

The most important effect on osmotic pressure and acid-base balance to remain within normal limits in animals was due to Na, K and Cl. In the study, blood calcium levels of cows were found to be within normal physiological limits (2-3 mmol/L (8-12 mg/dl) (Altıntaş and Fidancı, 1993)), but lower than the results of Yıldız and Balıkçı (2004) (Table 5). The low rate of calcium may be caused by the decrease in feed consumption of animals under heat stress and the removal of calcium from the body due to sweating (Kennedy, 2011). Kennedy (2011) stated in study that the animals' blood and sweat calcium ratios were on average 8-10 mg / dL and 8.5-9.9 mg / dl. Normal serum potassium concentration of cows was between 3.6-5.6 mmol / L (Altıntaş and Fidancı, 1993) and serum sodium concentration was between 135-155 mmol / L (El-Zahar, 2015). In Table 5, it was seen that the blood sodium and potassium levels of both white and black nose area animals were within normal physiological limits. However, sodium and potassium values were found at higher levels in the study than other researchers (Yıldız and Balıkçı, 2004; Ferreira et al., 2009).

The reason for the different levels of minerals in animals was that the organisms need for minerals was affected by various factors. One of these factors was the increase in sodium and potassium excretion from the body through sweat (Montain et al., 2007). Soil structure and geography of the region, mineral content of water and feed materials, storage conditions in the body and animal characteristics reduce or increase the need. It was reported that the mineral ratios determined for animals were not fixed, but may vary depending on yield, live weight, environment and various feed related factors (Boğa and Filik, 2011; Kreplin and Yaremcio, 2009). Increasing calcium need of animals with high milk yield and excessive excretion of calcium with urine and sweat under heat stress will reduce the calcium rate from the body.

5. Conclusions

As a result of this study, it was determined that the average daily milk yield of white nose area cows in the hot conditions during the weeks of the experiment was 6 kg more than the daily milk yield of black nose area cows. From the physiological data, it was understood that the white colored ones were able to overcome the heat load that occurs during the day more comfortably during the cool hours of the night and that the struggle with hot conditions was also effective in their blood values. In addition to the precautions to be taken in terms of animal breeding in hot conditions, selection of animal’s resistant to heat shows the importance.

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