BIODIVERSITAS ISSN: 1412-033X
Volume 23, Number 1, January 2022 E-ISSN: 2085-4722
Pages: 533-539 DOI: 10.13057/biodiv/d230157

Behavioral, physiological, and blood biochemistry of Friesian Holstein dairy cattle at different altitudes in West Java, Indonesia

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Manuscript received: 9 October 2021. Revision accepted: 30 December 2021.

Abstract. *Tanuwiria UH, Susilawati I, Tasrifin DS, Salman LB, Mushawwir A. 2021. Behavioral, physiological, and blood biochemistry of Friesian Holstein dairy cattle at different altitudes in West Java, Indonesia. Biodiversitas 23: 533-539.* For dairy cows, the study of physiological aspects and those related to it is very important based on altitude. The data of this study are the main considerations to determine the ability of homeostasis, prediction of production and appropriate feed management. This study aims to examine the behavior, physiological abilities, and blood plasma biochemistry of sixty dairy cows. It was conducted using three locations in West Java Province based on topography, namely: (i) location with topography 350-500 meters above sea level (masl): Sukabumi Regency; (ii) location with topography 550-750 masl: Sumedang Regency; and (iii) locations with topography > 800 masl: Bandung Regency. Furthermore, 5 mL of blood samples were taken accurately, using a syringe and a 5 mL tube containing EDTA, respectively. Blood sampling was taken carefully from the jugular vein of dairy cows at the beginning of every month for six sampling periods. After each collection, the whole blood was separated from the plasma directly using a centrifuge, with a speed of 4500 ppm for 7 minutes. The plasma obtained was used to measure the value of blood biochemistry related. The plasma analysis was conducted using a Kenza 240TX model spectrophotometer. The procedure for analyzing blood samples followed the instructions listed in the randox and biolabo kits. Furthermore, behavioral and thermoregulation measurements were performed every week during this research. The results showed an increase in dairy cows' time spent lying and drinking at low altitudes compared to dairy cows at high altitudes. In contrast, rumination and feeding activities were decreased, as shown in the blood's physiological response and biochemical profile. Dairy cows appear to be more challenging to adjust physiologically at lower altitudes.

Keywords: Body function, cow, metabolism, topography

INTRODUCTION

West Java province is known for developing dairy cows and is a milk producer in Indonesia. The dairy cattle are spread across various rearing locations with a wide range of altitudes from low to high. During its development, the macrophysical condition of the environment is no longer a primary consideration, even though the thermoneutral zone is in the range of 18-23°C (Ippolito et al. 2014; Ammer et al. 2018). Dairy cattle breeding has been developed with a direct effect on its adaptability.

The temperature and humidity of the maintenance location, in macro terms, are strongly influenced by altitude (Hetti et al. 2014; Lomb et al. 2018). A low altitude has a higher environmental temperature than a location at a higher altitude than sea level. This condition is exacerbated by an increase in the average temperature of the environment (Loyau et al. 2014).

The profile of physical environmental factors such as topography, temperature, and humidity directly affects the physiological performance of dairy cattle. For example, the maintenance location with high temperature causes heat exchange difficulty from the animal body to the atmosphere or surrounding environment. This is because the heat radiation to the livestock body is getting higher. Another fact shows that the heat adjustment effort is getting more complicated when the maintenance location and high temperature are also accompanied by high humidity.

As homothermic livestock, the homeostatic state maintains normal cell metabolism, even in minimal conditions (Mingoti et al. 2016; Mushawwir et al. 2021). This homeostasis is achieved through the mechanism of action of the organ systems (Hetti et al. 2014), especially maintaining average body temperature (Allen et al. 2015; Fabris et al. 2017). This physiological phenomenon greatly influences livestock behavior, both exterior and interior (metabolism) (Gray et al. 2015; Carrol et al. 2016)

The heart rate, respiration organs, and kidneys regulate and evaporate body heat through conserving extracellular body fluids, namely blood, either through sweat or urine. Excessive mechanism of action of the organs causes increased inflammation to the death of the heart (Adriani and Mushawwir 2020) and kidney cells, also the other tissue. In addition, damage to tissue cells causes a decrease in the function of enzymes (Adriani et al. 2015; Slimen et al. 2016; Hernawan et al. 2017), hormones, and other proteins that are unable to work optimally (Sang-Ho et al. 2018). This condition simultaneously triggers changes and stimulation of antioxidants (Mushawwir et al. 2020) and biochemical profiles in blood plasma (Hecker and McGarvey 2011; Tanuwiria and Mushawwir 2020).

Environmental macro physical factors also directly influence antioxidant activity. The tense situation that livestock can tolerate causes the stimulation of antioxidant enzymes or endogenous antioxidants. Therefore, the physical conditions of the environment affect the biochemical profile of plasma as antioxidants and buffer for extracellular fluids. Previous studies have shown increased stress for livestock in low topographies with associated environmental temperature (Gehrke et al. 2013; Kamil et al. 2020; István et al. 2020). Its biological effects have been widely reported, especially the low productivity (Mushawwir et al. 2010; Hohenbrink and Meinecke-Tillmann 2012) and reproduction (Khan et al. 2015; Slimen et al. 2016; Ammer et al. 2018; Hidayat et al. 2019). Very few studies reported on the impact of altitude differences on dairy cows, including behavioral responses, physiological and biochemical responses, and growth and productivity. Hence, this investigation was conducted to examine the impact of variations in altitude on the behavior of dairy cows, their physiological abilities, and blood plasma biochemistry.

MATERIALS AND METHODS

Research area

Sixty dairy cows aged 3-5 years were used in three locations of West Java Province, Indonesia based on topography (Figure 1), namely: (i) Sukabumi District (350-500 meters from sea level); (ii) Sumedang District (550-750 meters above sea level (masl); (iii) Bandung District (> 800 masl).

Before the research, the altitude was determined by measurement (altitude of the place). Meanwhile, the experimental animal sample consisted of 20 animal samples at each type of topographic location and the experimental cattle were reared intensively during two months. All animal samples were imported from the Dairy Cattle Breeding Center, Baturaden (BBPTUHPT Baturraden), Banyumas, Indonesia. All of the experimental animals were ear tagged for easy observation.

All animal samples are fed forages and concentrate, while drinking water is given ad-libitum. Forage used in this experiment was *Pennisetum purpureum* with nutrients contents: crude protein 6.28%; crude fiber 31.55%; dry matter 21.52%, crude fat 2.02%, Calcium 0.47% and Phosphor 0.42%. The concentrate consists of a mixture of tofu dregs, rice bran and soybean meal, with crude protein, crude fiber, dry matter, crude fat, calcium and Phosphor content were 13.5%; 5.56%; 65.08%; 4.57%; 1.20% and 0.93%, respectively.

Experimental design

Treatment and animal samples

The height of the FH dairy cattle breeding site was determined using the EK-Fan type altimeter before determining the sample cattle. The altitude is measured using an EK-Fan type altimeter, with a measurement capability of -700 to 9000 meters (-2300 ~ 2950 ft) and an accuracy deviation of 1 to 3 meters.

A total of sixty, 3-5 years old female FH dairy cows were used for the six months of this observation. Twenty samples of FH dairy cows were randomly selected from the respective FH dairy populations based on their altitude location. Each altitude location consists of four open system cages, each of which five samples of dairy cows were selected.

The average housing temperature ware 24-26^{0}C (Bandung; > 800 masl), 27-29^{0}C (Sumedang; 550-750 masl), 29-32^{0}C (Sukabumi; 350-550 masl). Furthermore, the percentage of air humidity was measured (range 75-87%, with an average of 82%) using a wet and dry bulb thermometer placed in each dairy housing, respectively.

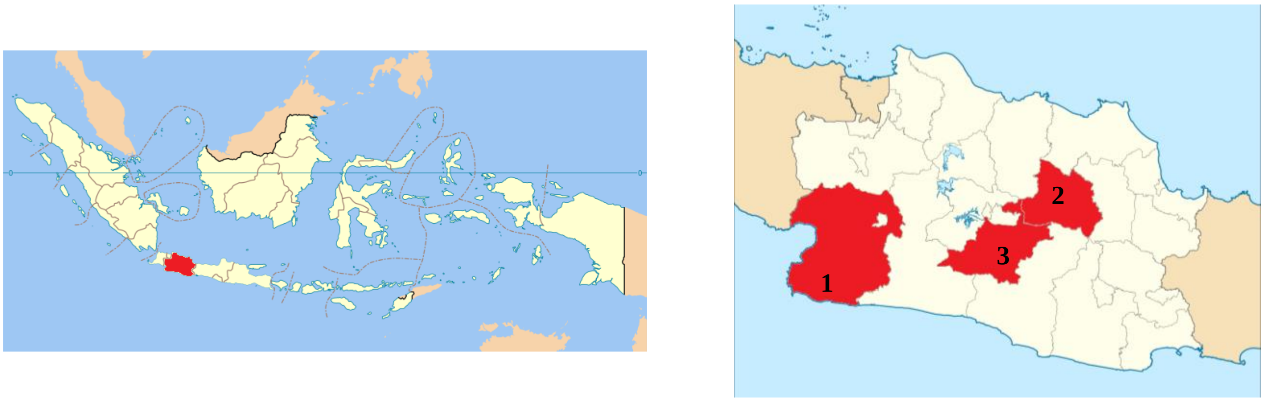

Figure 1. Research location in West Java Province, Indonesia: 1. Sukabumi; 2. Sumedang, and 3. Bandung

Data/blood sample collection and data analysis

Data/blood sample collection

In this investigation, approximately five mL of animal blood samples were taken accurately, using a syringe and a 5 mL tube containing EDTA, respectively. Blood samples were obtained from the jugular vein of dairy cows at the beginning of every month for 6 sampling periods. After each collection, the whole blood was separated from the plasma directly using a centrifuge, with a speed of 4500 ppm for 7 minutes. The plasma obtained was used to analyze and measure the blood biochemistry level related to liver function, heat stress, and reproduction. Plasma analysis was conducted using a Kenza 240TX model spectrophotometer. The procedure for analyzing blood samples followed the instructions listed in the randox and biolabo kits.

Behavioral variables were observed every Monday, Wednesday, Friday and Sunday during the experiment, using a CCTV camera, type of Sricam SP014. All observations on animal behavior were carried out from the feeding (07.00 a.m.) to 04.00 p.m. Sample numbers have been marked on the left and right backs for easy observation. The recording behavioral observations were saved automatically in the computer and analyzed manually. Two CCTV cameras were placed at each study location. The dairy cow's behavior was observed for each animal, and the results were averaged.

Feeding behavior was determined by calculating the amount of time spent to take the feed until it was swallowed. Drinking behavior was calculated based on time and how often to drink water. Rumination behavior was calculated how much time was spent re-digesting the ingested feed (rumination) and how often the feed bolus was regurgitated and swallowed after eating until 04.00 p.m. Lying behavior was calculated based on the amount of time spent lying down without rumination activity while standing behavior was determined based on the amount of time needed to stand without eating, drinking and rumination activities.

Physiologic responses related to thermoregulation measurements were measured every week. Finally, the physiological response was measured using a digital thermometer and a stethoscope. Physiologic responses were measured every Monday, Wednesday, Friday and Saturday. Data were collected in the morning (05.00 a.m), afternoon (02.00 p.m) and evening (09:00 p.m). In addition, heart rate was measured just behind the left third rib, while skin and mammary surface temperature were measured with an infrared thermometer.

Data analysis

The variables were analyzed using a mathematical formula. Before being analyzed using ANOVA, the normal distribution was determined using the normality test. The one-way analysis of variance (ANOVA) was applied to analyze all of the variables. Then, the average difference between responses was calculated and determined using Duncan's multiple range tests (Mushawwir et al. 2011). The data analysis was conducted using the software of SPSS IBM 2015, with a 95% significance level.

RESULTS AND DISCUSSION

Behavior parameters

Table 1 shows the impact of different altitudes on time spent for dairy cows. The locations with different altitudes are closely related to physical environmental factors such as temperature and humidity; where the lower the altitude, the higher the environmental temperature. On the other hand, low or cold ambient temperatures are felt by livestock at rearing locations with higher ground.

The result showed that the feeding behavior occurred more frequently in dairy cattle for the highlands > 800 masl, for instance 123.81 min/d, was longer ($p<0.05$) compared to the group at 550-750 and 350- 500 masl, which were 108.75 and 73.82 min/d, respectively. On the other hand, the drinking behavior of cattle in the lowlands spent more time than those in the dairy cow in the highlands. The proportion of time required to express the behavior of dairy cows in each topography is shown in Figure 2.

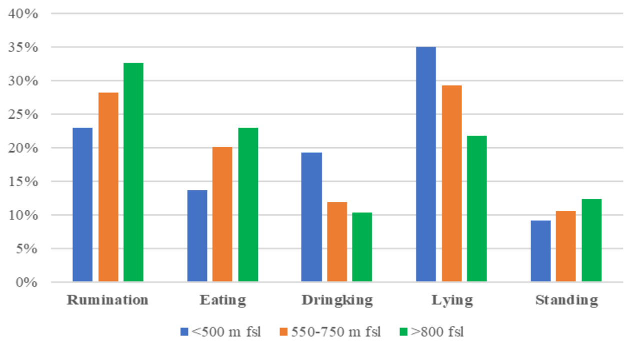

Figure 2. The proportion of time required to express the behavior of dairy cows in different altitude

Table 1. The effect of altitude different on time spent in behavior activity

Parameters	Altitude (masl)			
	-500	550-750	>800	p-values
Rumination (the number of time/d)	35.46±1.58^a	39.74±2.84^b	45.83±2.04^c	0.036
Eating (min/d)	73.82±2.82^a	108.75±3.95^b	123.81±3.84^c	0.041
Drinking (the number of time/d)	25.93±2.81^a	19.18±2.16^b	18.18±2.72^b	0.015
Lying (min/d)	188.85±0.84^a	158.04±0.17b	117.55±0.46c	0.025
Standing (min/d)	49.75±2.31^a	57.03±1.87^b	66.73±1.49^b	0.018

Note: Value in this table are means ± Standard Deviation; ^{a,b} The average in the same line, marked with different superscripts, shows a significant difference

It was emphasized that lower altitude causes an increase in environmental temperature (Hecker and McGarvey 2011; Tanuwiria et al. 2011). Previous studies reported that environmental temperature is an essential factor influencing the consumption of feed and drinking water. For example, Hidayat et al. (2019) showed a decrease in feed consumption along with an increase in housing temperature. Meanwhile, Layau et al. (2014) and Na et al. (2020) showed a report with an increase in drinking water consumption and environmental temperature. These eating and drinking behavior are closely related to the instinct of dairy cows to maintain homeostasis of the body by maintaining a balance between environmental heat radiation and metabolic heat. This condition has an impact on decreasing the time needed to consume feed and rumination, on the contrary increasing the proportion of time for drinking activities (Table 1 and Figure 2). This is also supported by another behavior, namely the lying behavior. Lomb et al. (2018) reported that lying down was an attempt for livestock to evaporate body heat by conduction techniques. Therefore, cattle in the lowlands of 350-500 masl spend more time lying down (Table 1) than theat of > 550 masl. These results have also been shown by Hetti et al. (2014), Gray et al. (2015) and Gleret et al. (2016), as well as in poultry (KKUI, 2038). However, a better adaptation in Pasundan cattle was presented by Mushawwir et al. (2020).

Environmental temperature dramatically affects the physiological conditions of livestock. This condition can be exacerbated when there is an interflow of high air humidity and ambient temperature. Therefore, dairy cattle should adjust their body temperature to the ambient temperature as homothermic livestock. Regulation of the body in adjusting the physical conditions of the environment with temperature involves all organs (Monteiro et al. 2016; Mushawwir et al. 2018; Na et al. 2020).

Physiological responses

Table 2 shows the physiological responses related to dairy cattle thermoregulation at different altitudes. An increase in physiological responses indicated the ability of animals to maintain the condition of their bodies through respiration, heart rate, and body temperature (rectal and skin surface temperature). For ruminants, heat dissipation was mainly carried out through evaporation by sweating, while a small part was achieved through evaporation by breathing. Subsequently, environmental heat radiation triggers hormonal work, especially epinephrine. Several previous studies have shown that cows in the lowlands have higher daily epinephrine concentrations than those in the highlands. Mushawwir et al. (2021) reported that epinephrine increases vascular vasodilation. The results of other studies showed that epinephrin also increases blood flow rate (Ippolito et al. 2014; Khan et al. 2015; Adriani and Mushawwir 2020). The increased blood flow rate was necessary to evaporate body heat through the sweat glands and partly through water vapor from the respiration system.

Involvement of body organs in achieving body heat homeostasis, the body organs respond with different degrees of activity. This thermoregulation effort seems to be responded to by the organs more intensively, through an increase in heart rate and a higher working frequency of the respiratory system (Wang et al. 2007), then manifested by body surface temperature and higher rectal temperature (Mushawwir et al. 2010; 2011). Physiological status illustrates that the interaction of organs in regulating heat and maintaining the body is indispensable since it is a cumbersome physiological and biochemical mechanism. As an impact, the need for more energy (Tian et al. 2015) and nutrients in the basal ratio should be more complex (Grelet et al. 2016; Suryaningsih et al. 2019; Hidayat et al. 2019). In addition, decreased digestion of crude fiber (Renaudeau et al. 2012) and the rate of conversion of non-carbohydrate precursors to pyruvate (gluconeogenesis) has increased (Mushawwir et al. 2010; Pickler et al. 2013; Gray et al. 2015; Mushawwir et al. 2021). There are also changes in the profile of carbohydrate metabolism (Dhanasekaran et al. 2011; Mushawwir et al. 2020) and increased levels of free radicals such as ROS (Burdick et al. 2011; Eyng et al. 2015; Siskos et al. 2017).

Blood biochemistry

Table 3 shows the biochemical profile of dairy cow blood plasma as a result of different altitudes.

Specific responses related to cardiac function and metabolism change are associated with maintenance locations with different altitudes. For example, in Table 3 the levels of gamma-glutamyl transpeptidase (γ-GT) were higher ($P < 0.05$) in the group reared at lower altitudes than those raised at higher altitudes. This phenomenon occurs in all groups of cows with different ages of lactation.

Table 2. Effect of altitude different on thermoregulation of FH dairy cattle

Response	Altitude (masl)			
	-500	550-750	>800	p-values
Heart rate (beat/minute)	49.46±1.58^a	47.74±1.84^b	46.83±2.04^b	0.024
Rectal temperature (^{0}C)	40.05±0.05^a	39.01±0.03^b	39.02±0.02^b	0.028
Skin surface temperature (^{0}C)	41.15±0.03^a	39.25±0.05^b	39.15±0.07^b	0.025
Mammae surface temperature (^{0}C)	41.37±1.74^a	42.83±1.96^b	42.72±2.58^b	0.037

Note: Value are means±Standard Deviation; ^{a,b} The average in the same line, marked with different superscripts, shows a significant difference

Table 3. The biochemical profile of dairy cow blood plasma on the different altitudes

Responses	Altitude (masl)			
	300-500	550-750	>800	p-values
γ-Glutamyl Transpeptidase (IU/L)	48.46±1.32[a]	45.62±2.73[b]	43.66±1.26[b]	0.021
Glutamate Oxaloacetate Transaminase (IU/L)	75.53±1.25[a]	68.42±1.54[b]	53.53±1.72[b]	0.028
Glutamate Pyruvate Transaminase (IU/L)	35.68±1.17[a]	29.22±1.82[b]	21.52±1.66[b]	0.032
Non-esterified Fatty Acid (NEFA) (mg/dL)	135.17±1.31[a]	219.52±1.81[b]	221.64±1.13[b]	0.027
Soluble Transferrin Receptor (mg/dL)	0.87±0.01[a]	1.73±1.58[b]	2.65±1.74[c]	0.041
Total Iron Binding Capacity (mg/dL)	2.36±0.23[a]	2.62±3.56[a]	3.04±3.52[b]	0.023
Transferrin (mg/dL)	1.06±0.10[a]	1.63±0.21[b]	2.34±0.13[b]	0.037
Ferritin, nmol/mg	0.57±0.13[a]	0.63±0.12[a]	0.61±0.11[a]	0.024
Ceruloplasmin (mg/dL)	1.52±0.11[a]	1.41±0.12[b]	1.31±0.15[b]	0.036
Albumin (mg/dL)	1.19±1.01[a]	2.21±1.13[a]	3.18±0.05[b]	0.028
Total Protein (mg/dL)	14.74±1.34[a]	18.31±1.14[a]	21.42±0.15[b]	0.033
Carbonic acid (H_2CO_3) (mg/dL)	9.63±1.42[a]	12.62±0.73[b]	14.67±0.83c	0.046
Follicle Stimulating Hormone/FSH (ng/mL)	7.85±1,76[a]	12.73±0.78[b]	13.69±0,07[c]	0.021

Note: Value in this table are means±Standard Deviation; [a,b] The average in the same line, marked with different superscripts, shows a significant difference

It is known that the γ-GT enzyme has a high concentration in heart tissue cells. As previously described, lower altitudes have higher temperatures. Therefore, homeostasis efforts that involve the work of the heart (Seok et al. 2019; Mushawwir et al. 2020) in the context of providing energy (Tanuwiria et al. 2011; Roland et al. 2016) can increase the death of cardiac cells (necrosis). Several previous studies have shown that necrosis increases with enhancing the temperature in the housing cattle (Xu et al. 2015; Tian et al. 2016; Lomb et al. 2018).

Increased death of cardiac cells with increasing temperature impacts the migration of λ-GT enzymes to the vessel system to increase the levels of this enzyme in the blood plasma. The results of previous studies indicate that cell death (necrosis) causes an increase in metabolite migration to the vessel system (Ippolito et al. 2014; Valle et al. 2017; Tanuwiria and Mushawwir 2020). The γ-GT enzyme levels appeared to increase in the dairy cow group with increasing lactation age at the rearing location with a low altitude, namely 49.46 IU / L in the III lactation group. It showed higher (P <0, 05) in all cattle groups with the 5th lactation age, 51.75 IU / L. Although this phenomenon does not occur in groups of dairy cattle kept at altitudes > 700 masl. Slimen et al. (2016) have reported that cell death (necrosis and apoptosis) increases following the metabolism and age.

The livestock kept in a temperature zone that corresponds to the thermoneutral zone of dairy cattle causes no stimulation of the activation of the hormone epinephrine from nor-epinephrine (Vizzotto et al. 2015; Mushawwir et al. 2018). Instead, these hormones were triggered by neurotransmitters due to the stimulation of nerve receptors (Ippolito et al. 2014). Epinephrine is a terminal compound for alpha-epinephrine receptors in the blood vessel walls (Gehrke et al. 2013; Cai et al. 2014; Mushawwir et al. 2020). As a result, it causes increased vasodilation or dilation (Gray et al. 2015; Siskos et al. 2017) and increases the heart rate (Hecker and McGarvey 2011; Mingoti et al. 2016).

The results of this investigation (Table 3) also showed an increase (P <0.05) in the concentration of Soluble Transferrin Receptor and Total Iron Binding Capacity of all groups of dairy cows reared at different altitude locations. Increased levels of both types of protein occur with increasing altitude. This increase in levels is caused by lower oxygen concentrations at high altitudes, to increased expression of these two types of proteins and support oxygen distribution throughout the tissue. These changes can also be caused by continued tissue growth to increase the capacity for lipid synthesis (Tian et al. 2015). In addition, it increases metabolism due to tissue mass (Carrol et al. 2016; Ammer et al. 2018). It increases the liver mass and causes gluconeogenesis (Adriani and Mushawwir 2020; Tanuwiria and Mushawwir 2020; Mushawwir et al. 2021).

Subsequently, the reproductive potential was lower in low altitude dairy cows (300-500 masl) compared to those in higher altitudes. This potential was shown by circulating FSH levels in the blood, and the FSH levels in dairy cows (Table 3) for the lowlands to the highlands were 7.85; 12.73, and 13.69 ng/mL, respectively. This increase indicates the activity of follicular growth and egg cells in dairy cows. Several previous studies (Mingoti et al. 2016; Monteiro et al. 2016) have shown a very high correlation of FSH levels with folliculogenesis activity. This potential showed that high FSH levels support high milk production (Roland et al. 2016; Tian et al. 2016; Siskos et al. 2017; Mushawwir et al. 2020).

Several studies have shown that the altitude factor plays an essential role in milk synthesis in dairy cows, but it is also strongly determined by feed factors (Fabris et al. 2017; Sisay et al. 2018; Hidayat et al. 2019). However, altitude is very decisive and closely related to temperature and humidity (Mushawwir et al. 2020). It is also closely related to heat regulation and radiation (Burdick et al. 2011); other studies have shown that the rate of energy use is only to maintain heat balance in and outside the body (Ippolito et al. 2014; István et al. 2020). The quality feed contributed to the ability of dairy cows at low altitudes to produce high milk. The research by Renaudeau et al. (2012) and Seok et al. (2019) declared that adding methionine and lysis with glutamic acid can support dairy cattle production under exposed heat stress. Another measure showed that chitosan can be affected in regulation and avoid tissue damage and support protein synthesis in growth by giving to livestock (Mushawwir et al. 2021).

Related to the increase in the biosynthetic capacity of milk by alveolar cells in the mammary tissue, it is, of course, related to the precursors of milk formation (Seok et al. 2019). The high need for precursors can intensify glucose formation from various sources, such as protein (Mushawwir et al. 2011; Pickler et al. 2013; Khan et al. 2015). It can also promote escalated metabolism rate of rumen cavities and villi in the dairy cow (Tanuwiria et al. 2011; Fabris et al. 2017, Ammer et al. 2018; Hidayat et al. 2019; István et al. 2020; Adriani et al. 2021).

Based on the results of the current study, the proportion of time spent expressing their behavior is a compensation for the physical environmental conditions (temperature and humidity) in each topography. Likewise for thermoregulation to maintain normal physiology. Changes in blood metabolism, is a metabolic profile that shows the hemostatic condition it has achieved.

ACKNOWLEDGEMENTS

The authors are grateful to the Bioscience/Biolab Analysis Laboratory and Animal Physiology and Apply Biochemistry Laboratory, Faculty of Animal Husbandry, Universitas Padjadjaran and Rector of Universitas Padjadjaran, Indonesia with Academic Leadership Grant, (ALG), for their financial support in this experiment. Many awards were given to Adang Sudrajat for their assistance in animal sampling.

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