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Effects of induced heat stress on temperature response and biochemistry: alteration of biochemical constituents in Holstein calves by heat stress

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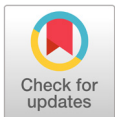
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Abstract

The aim of the present study was to evaluate the effect of the induced heat stress on physiological response and serum biochemical parameters involving glucose, cholesterol, blood urea nitrogen (BUN), non-esterified fatty acids (NEFA), and cortisol in Holstein calves. Ten calves were kept in a climate controlled room (air temperature 37°C and 90% humidity from 09:00 to 19:00) for three days. Those animals were given a one-day adaptation period. During the treatment period, we measured the skin temperature six times. Following the treatment periods, blood samples were collected before the experiment began (09:00) and at the end of the stress period (19:00). To aid analysis of the biochemical parameters, also we monitored the rectal temperature. The results, exhibited that both rectal and skin temperature showed increase in the heat stress-induced animals as compared with un-stressed animals. Moreover, we noticed that the levels of BUN and NEFA increased in the blood serum of heat stress induced animals when compared with un-stressed ones. From these results, we concluded that the physiological and biochemical changes in the calves were induced by heat stress. Hence, the present study findings could be employed as base line data for development of stress reduction techniques in the dairy industry.

Keywords: biochemical constituents, calves, heat stress



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Introduction

In tropical regions, physiological factors, such as solar radiation, air temperature, relative humidity, and wind speed can cause heat stress in dairy cattle (West et al., 2003; Spiers et al., 2004; Tapki and Sahin, 2006). Global warming can also negatively influence the heat exchange between dairy cows and their environment (Baumgard and Rhoads, 2012). Unfavorable temperatures can be considered a chronic stress (Shehab-El-Deen et al., 2010). During heat stress, dairy cows use more energy for circulatory adjustments to

increase the respiratory rate, sweating, and panting (Srikandakumar and Johnson, 2004). Moreover, heat stress is associated with a lower food intake and increased negative energy balance (Igono et al., 1992; Chaiyabutr et al., 2008; Shehab-El-Deen et al., 2010; Choi, 2018). Cortisol is a physiological and stress-associated hormone and is an appropriate biological marker for investigating the hypothalamic-pituitary-adrenal (HPA) axis, and the exogenous administration of adrenocorticotrophic hormone can be used to study the HPA axis activity (Negrao et al., 2004, 2010). The HPA axis is the primary neuroendocrine system involved in stress response. The HPA axis secretes adrenocorticotrophic hormone. However, there are few scientific reports regarding the relationship between heat stress and biochemical parameters in calves. To the best of our knowledge, this is the first evaluation of the biochemical constituent profile in Holstein calves after heat stress.

Materials and methods

Animals and experimental design

The selected animals and the experimental protocol were approved by the Institutional Animal Ethical Committee of the National Institute of Animal Science (NIAS), South Korea. The animals registered in the national database originated from the standard breeding program described in the NIAS guidelines. Animals were fed a formulated diet to meet nutrient requirements according to the NRC (1987). These requirements include 68% total digestible nutrients (TDN), 15% crude protein, and hay. During the heat stress treatment, water and fresh hay were provided *ad libitum*. Ten calves, weighing an average of 126 ± 5 kg, aged 4 - 6 months, were housed for 3 consecutive days in a climatic room. The animals were given a 1-day adaptation period to adapt to the environmental conditions, then the animals were placed in an open barn for 3 days without restrictions on hay consumption. During the experimental period, the behavior of individual animals was monitored. After medical checks for registered diseases and of the respiratory systems, the animals were placed in an environmentally controlled building at NIAS.

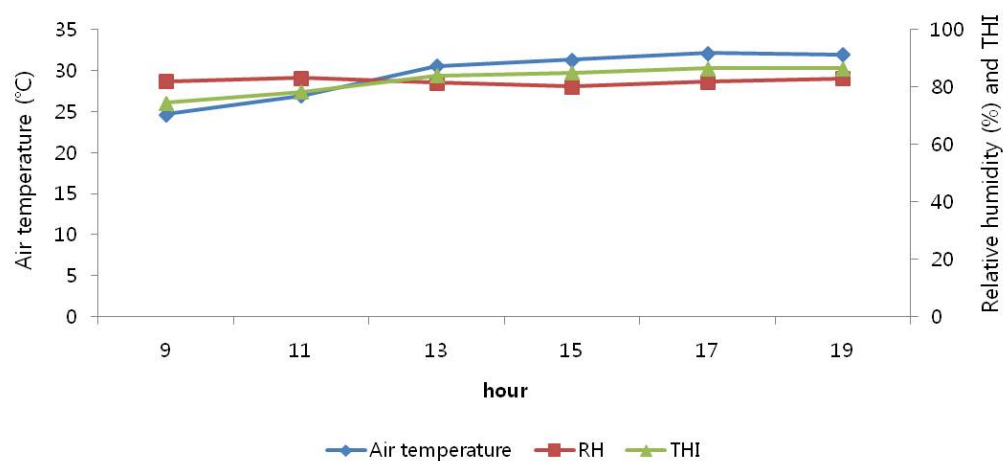


Fig. 1. Air temperature, relative humidity (RH), and temperature and humidity index (THI) during heat stress.

Heat stress treatment

Calves were exposed to high air temperatures and humidity in a climatic room for 3 consecutive days. We maintained the air temperature at 37°C and the humidity at 90% from 09:00 to 19:00 (10 h). Following the experimental period, the air temperature and humidity were reduced gradually to 20°C over 14 h. The ambient air temperature and relative humidity were recorded using a data logger and the temperature and humidity index (THI) was calculated (Fig. 1).

Sample collection

Throughout each experimental period, the skin temperature was monitored every 1.5 h using a temp-gun (Hitachi, USA) on the left side of the shoulder and neck for a total of three measurements, with a distance of approximately 60 cm between the humans and animals to prevent any human influence on the skin temperature. The rectal temperature was measured before and after heat stress (BHS and AHS). Blood samples were collected before the experiment began (09:00) and at the end of the experimental period (19:00). The collected blood samples were centrifuged at 3,000 rpm for 15 min at 4°C to obtain plasma. The obtained plasma was stored at -80°C until further analysis. Plasma concentrations were analyzed photometrically (Abx Pentra 400, Horiba, Kyoto, Japan) using a kit from Wako Chemicals (Neuss, Germany).

Statistical analyses

The collected data were subjected to a generalized linear model procedure (PROC-GLM) of the Statistical Analysis System (SAS Institute, Cary, USA). Differences among treatment means were determined using Duncan's multiple range tests. Statistical significance was established at $p < 0.05$.

Results

Experimental cows were kept in a free stall under heat stress for 3 days. During the experimental period, the air temperature ranged from 24.7°C to 32.1°C with a mean of 29.6 ± 3.1 °C. The relative humidity had a mean of 82.0 ± 1.1 % and the THI was above 72 (Fig. 1). The rectal temperature (RT) and skin temperature (ST) values were significantly lower ($p < 0.05$) at 09:00 than at 19:00 h (Table 1). During the experimental periods, the skin temperature of the calves increased from time 09:00 to 19:00. Calves showed higher temperatures AHS than BHS. In addition, the blood constituents reflected the nutritional and physiological status of the heat stress induced in the animals. Table 2 shows the changes in the biochemical parameters before and after heat stress. No significant changes were observed in the levels of albumin, Ca, blood glucose, aspartate transaminase (AST), alanine transferase (ALT), Mg, P, total protein, cholesterol, and cortisol in the calves with heat stress. However, the serum levels of blood urea nitrogen (BUN) and non-esterified fatty acid (NEFA) were significantly ($p < 0.05$) higher AHS than BHS.

Table 1. Means and standard error (SEM) of the rectal temperature (RT) and skin temperature (ST) measured in the experimental calves under heat stress.

Time sampling data	Mean \pm SEM					
	09:00 h	11:00 h	13:00 h	15:00 h	17:00 h	19:00 h
RT ($^{\circ}$ C)	37.13b \pm 1.18	-	-	-	-	39.90a \pm 0.11
ST ($^{\circ}$ C)	32.54d \pm 0.12	34.49c \pm 0.16	35.33bc \pm 0.11	36.54ab \pm 0.14	35.92ab \pm 1.12	36.94a \pm 0.14

a - d: Values within a row with differing superscripts denote significant differences ($p < 0.05$) between periods.

Table 2. Biochemical values (mean \pm SEM) in the calves.

Parameter	Before heat stress	After heat stress
Albumin (g/dL)	3.9 \pm 0.2	3.9 \pm 0.1
Ca (mg/dL)	10.6 \pm 0.5	10.7 \pm 0.5
Glucose (mg/dL)	84.2 \pm 6.5	87.6 \pm 9.5
ALT (IU/L)	20.4 \pm 2.3	20.4 \pm 2.4
Mg (mg/dL)	2.3 \pm 0.2	2.3 \pm 0.2
P (mg/dL)	8.3 \pm 0.7	8.1 \pm 0.6
Cholesterol (mg/dL)	75.9 \pm 9.1	77.0 \pm 9.8
Total protein (g/dL)	6.5 \pm 0.2	6.6 \pm 0.3
BUN (mg/dL)	18.7 \pm 2.4*	16.7 \pm 3.4*
NEFA (μ Eq/L)	167.4 \pm 107.5*	88.7 \pm 44.6*
Cortisol (μ g/mL)	0.6 \pm 0.6	0.8 \pm 0.6

AST, aspartate transaminase; ALT, alanine transferase; BUN, blood urea nitrogen; NEFA, non-esterified fatty acids.

* $p < 0.05$.

Discussion

Heat stress in dairy cows is a result of all the high temperature-related forces that can induce alterations at the sub-cellular level to assist the cows in avoiding physiological dysfunction and remaining healthy (Kadzere et al., 2002). The present study examined the effect of heat stress on the temperature and biochemical changes in calves.

Cattle are homeotherms and maintain a constant body temperature at a particular level. Normal body temperature for cattle ranges from 36.0 $^{\circ}$ C to 38.4 $^{\circ}$ C (Hansen et al., 1992). The rectal temperature is an indicator of thermal balance and may be used to assess the adversity of the thermal environment, which can affect dairy cows' growth, lactation, and reproduction (Johnson, 1980a). A rise of less than 1 $^{\circ}$ C in the rectal temperature is enough to reduce performance in most livestock species (McDowell et al., 1976). Thus, the body temperature is a sensitive indicator of the physiological response to heat stress in cows, as it is usually constant under normal conditions. A rise in body temperature of only 4.4 $^{\circ}$ C above normal is often quickly fatal, and most mammals die at a core temperature of 42 - 45 $^{\circ}$ C (Brody, 1948; Silanikove, 2000). Table 1 shows that the calves exposed to heat stress had higher temperatures compared with BHS. The same trend has been observed in Friesian cows (Muller and Botha, 1993). Therefore, changing the body temperature patterns may provide an opportunity to characterize physiological actions that may be related to adaptations for heat stress.

We found that heat stress did not increase plasma NEFA levels in the calves; these results agreed with the

results from other heat-stressed ruminant models (Sano et al., 1983; Itoh et al., 1998; Ronchi et al., 1999; Rhoads et al., 2009; Schwartz et al., 2009). The lack of an elevated NEFA response is especially surprising as acute heat stress causes a marked increase in circulating cortisol, norepinephrine, and epinephrine levels (Collier et al., 2005), which are catabolic signals that normally stimulate lipolysis and adipose mobilization. The calculated energy balance (EBAL) is known to be closely associated with circulating NEFA levels. The fact that heat-stressed cows fail to enlist this 'shift' in post-absorptive energy metabolism (despite inadequate nutrient intake) may indicate that heat stress directly affects the energetics and is not just mediated by the feed intake.

Cortisol is a stress-related hormone; plasma cortisol concentrations have been used as physiological markers of stress. Hormonal responses to heat stress include a rise in plasma cortisol with short-term exposure to heat, maybe because of the 'stress' reaction. Cortisol does not consistently increase when animals are exposed to moderate heat (Johnson, 1980b). In the present study, a small increase in the cortisol levels was observed AHS. Acute heat stress has been shown to significantly increase the cortisol concentration (Abilay et al., 1975), whereas prolonged heat stress was accompanied by slight decreases in the plasma levels of cortisol (Marple et al., 1972; Alvarez and Johnson, 1973; Rhynes and Ewing, 1973; Vanjonack and Johnson, 1975; Ingraham et al., 1979; Adeyemo et al., 1981). Urea is a good indicator of dehydration, but the concentration of urea may be affected by additional factors, mainly nutritional factors. In the present study, heat stress decreased blood urea nitrogen concentrations (18.7 ± 2.4 vs. 16.7 ± 3.4 mg/dL). This study confirmed the findings of Elmasry et al. (1989) and Ronchi et al. (1995) that chronic heat exposure in cattle and buffaloes results in decreased urea levels.

Conclusion

Heat stress is a cause of great concern among livestock owners. Under the climate change scenario, elevated temperature and relative humidity will impose heat stress on all the species of livestock, and will adversely affect their productive and reproductive potential especially in dairy cattle. The immediate need for livestock researchers aiming to counter heat stress impacts on livestock is an understanding of the biology of the heat stress responses. This will provide researchers with a basis for predicting when an animal is under stress.

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