NOTE

Evaluation of heat stress response in crossbred dairy cows under tropical climate by analysis of heart rate variability

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ABSTRACT. The present study aims to examine the effect of tropical temperatures on autonomic nervous activity in Cambodian dairy cattle by analyzing heart rate variability (HRV). Holter-type electrocardiograms were recorded in adult crossbred cows (Cambodian native × Holstein) either in a sheltered area or under direct sunlight. Rectal temperatures and heart rates increased in animals under direct sunlight as compared to those in the shelter. The power spectral analysis of HRV revealed that three out of the five cows studied underwent a decrease in parasympathetic nervous activity under direct sunlight with the remaining two cows showing no apparent change. The HRV analysis would prove to be a useful tool to reveal information about heat tolerance in dairy cows.

KEY WORDS: autonomic nervous system, dairy cattle, electrocardiogram, heart rate variability, heat stress

Excessively hot climate functions as heat stress, which has a negative impact on the productivity of cattle, such as a decrease in milk yield in Holstein cows [18] and reduced meat productivity in feedlot heifers with decreased growth rates [13]. One of the causes of these negative effects of heat stress on cattle productivity is a drop in average daily feed consumption [13]. Furthermore, a decrease in reproductive performance due to insufficient secretion of reproductive hormones, such as progesterone, estradiol, and luteinizing hormone, in Holstein cows is another major cause behind reduced productivity [7, 8, 19]; as a result, feasible estrus is hardly detected, which leads to a decrease in conception rates [8]. To overcome this challenge and attain better productivity from cattle in the face of increasing temperature due to global climate change, it is vitally important that the negative effects of hot climates, such as heat stress, on the physiological responses in cattle should be investigated. Such information can be used to select individuals adapted to hot climates.

Two major physiological pathways have been reported to be involved in stress responses in mammals. First, stress increases the activity of the hypothalamo-pituitary-adrenal (HPA) axis, which leads to an elevation in the blood circulation levels of cortisol [22]. Blood cortisol level is thus one of the parameters to evaluate activity of the HPA axis and is, therefore, used to estimate stress intensity in farm animals [15]. Measurement of blood cortisol levels needs blood sampling, which requires restraining the animals and that may give additional stress to the subjects. In addition, cortisol assay usually requires a couple of days to get the final data. A better method is thus needed to evaluate the intensity of stress in free-moving farm animals without imposing additional stress.

The second physiological response is the change in activity of autonomic nervous system under stress: stress increases sympathetic nervous activity and decreases parasympathetic nervous activity [22]. Heart rate variability (HRV) is a parameter that reflects the balance between sympathetic and parasympathetic nervous activity [4, 9, 23]. In the power spectrum of HRV, the high frequency (HF) power corresponds to the respiratory frequency and is influenced by the vagus nerve activity [2]; thus, HF power is an index of the parasympathetic nervous activity. The low frequency (LF) power is closely associated with the fluctuations of blood pressure [2], and LF power is proposed to be a reflection of both sympathetic and parasympathetic nervous activity. Previous

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studies have suggested that an analysis of HRV with a Holter-type electrocardiograph helps evaluate stress caused by sickness conditions in dairy cows [25]. In calves with external stress caused by relatively high temperature and insect harassment or illness from diarrhea, the HF power decreased and LF/HF ratio increased, indicating a reduction of parasympathetic nervous tone during stress load [14]. Analysis of HRV in another study [21] revealed that change in the sympathovagal balance was clearly affected in bull calves following surgical castration with or without local anesthesia. Therefore, the power spectral analysis of HRV allows researchers to measure stress levels in cattle without handling or restraining them, thus making it a reliable indicator to evaluate heat stress in farm animals.

The present study aims to evaluate heat tolerance in crossbred dairy cows in Cambodia. An electrocardiogram (ECG) of crossbred dairy cows was recorded under sunlight and in shelter, continuously for three days under each condition. Heart rates, time domain indices (R-R interval, and standard deviation [SD] and coefficient of variation [CV] of R-R interval), and frequency domain indices (HF and LF powers, and the LF/HF ratio), which respond and change with autonomic nervous activity [10], were analyzed as an indicator of balance of autonomic nervous system.

Four non-lactating and one lactating crossbred (Cambodian native zebu × Holstein-Friesian) dairy cows were used. All cows were 1.5–4 years old and in healthy condition. Animals were maintained under natural conditions at the experimental dairy farm on the Asian Satellite Campus in Cambodia (Royal University of Agriculture), Nagoya University. The animals were fed *ad libitum* with mixed feeds (fresh Mulato II grass and fresh brewers grains), and had free access to water. They were provided areas for free movement in a sufficiently quiet experimental setup either in the cow shelter or paddock without shade. All the experimental procedures were approved by the Committee of the Care and Use of Experimental Animals at the Graduate School of Bioagricultural Sciences, Nagoya University (Approval numbers 2016030102 and 2017030276). The experiment was conducted from February to May 2016 and April 2017, which are the hottest months in Phnom Penh, Cambodia. Holter ECG recordings were obtained consecutively during a 3-day period from each cow. On the first day of recording, all cows were kept in the shelter for 24 hr as a pre-treatment period. On the second day of recording, all cows were kept in the shelter for 12 hr (18:00–06:00 hr) and then transferred to the paddock without shade for exposure to direct sunlight for 12 hr (06:00–18:00 hr) as a heat-treatment period. On the third day of recording, all cows were kept in the shelter again for 24 hr as a post-treatment period.

The ambient temperature and humidity in the experimental area were recorded every minute using a data recorder (MHT-381SD, MotherTool Co., Ltd., Ueda, Japan) during the 3-day experimental period. The temperature–humidity index (THI) was calculated using the following formula: THI=(1.8T + 32)–(0.55–0.0055RH) (1.8T–26), where T is temperature in degrees Celsius and RH is relative humidity as a percentage [5]. Rectal temperature of cows was recorded with a standard digital thermometer (TV-714J, Astec Co., Ltd., Tsukuba, Japan) inserted into the rectum at 06:00, 09:00, 12:00, 15:00 and 18:00 hr.

Electrocardiograms were recorded with apex-base lead using a Holter-type electrocardiograph (QR2500; Fukuda M-E Kogyo Co., Ltd., Nagareyama, Japan) as previously described [25]. The ECG electrodes (Vitrode G-600; Nihon Kohden Corp., Tokyo, Japan) were attached to the skin of the animals after shaving the fur. Negative, positive, and neutral electrodes were attached to the left shoulder blade. Two additional positive electrodes were attached to the left olecranon. The Holter-type electrocardiograph was attached to the body with medical adhesive tape. The recorded ECGs, excluding artifacts, were analyzed using an ECG processor analyzing system (SRV-2W, Softron, Co., Ltd., Tokyo, Japan) as described previously [11, 25]. Datasets comprising 512 points were resampled at 200 msec and each dataset was applied to a Hamming window and fast Fourier transform so as to obtain the power spectrum of HRV. Heart rates, time domain indices (R-R interval, and SD and CV of R-R interval), and frequency domain indices (absolute and normalized power of the LF (0.04–0.1 Hz) and HF (0.1–1.0 Hz) bands, and the LF/HF ratio) were calculated. These parameters were analyzed every hour and then calculated for 6 hr on average during nighttime (21:00–03:00 hr) and daytime (09:00–15:00 hr) in each individual cow: the daytime period was selected based on the increase in rectal temperature of cows kept under direct sunlight on Day 2 (Fig. 1C); the corresponding period at night was selected as nighttime period. Statistical differences in the rectal temperature, heart rates, and HRV indices were determined using one-way ANOVA followed by Holm's multiple comparison at each time point.

Ambient temperature and THI during days of pre-, heat- and post-treatments are shown in Fig. 1. The ambient temperature (Fig. 1A) and THI (Fig. 1B) appeared to decrease from 18:00 to 06:00 hr and increase from 06:00 to 15:00 hr on all days. Both the ambient temperature and THI tended to be higher when cows were present under the sunlight (Day 2) than inside the shelter (Days 1 and 3). As shown in Fig. 1C, the rectal temperature tended to increase from 06:00 to 15:00 hr in cows kept under the sunlight (Day 2), whereas it appeared relatively stable throughout the daytime on the pre-treatment day (Day 1). After the heat treatment, the rectal temperature returned to be stable on the post-treatment day (Day 3). The rectal temperature of cows kept under sunlight was significantly higher at 12:00 and 15:00 hr than that of the animals kept inside the shelter on both pre- and post-treatment days at the same time points (*P*<0.05, analyzed by Holm's multiple comparison). Rapid breathing was observed in all five cows exposed to sunlight.

Changes in heart rate and HRV indices in cows during pre-, heat-, and post-treatments are summarized in Table 1. The heart rate of cows under the sunlight (daytime on Day 2) was significantly higher than in the period when cows were inside the shelter (Days 1 and 3) (*P*<0.05). The mean R-R interval of cows exposed to sunlight was significantly lower than that of cows inside the shelter (*P*<0.05). Other HRV indices, such as the SD and CV of the R-R interval, the absolute LF and HF power values, and the LF/HF ratio, showed large individual differences. Alteration of rectal temperature, heart rate, R-R interval, SD and CV of R-R interval, LF/HF ratio and normalized LF and HF powers in individual animals during the daytime of pre-, heat- and post-treatment days are shown in Fig. 2. The rectal temperature (Fig. 2A) and heart rate (Fig. 2B) increased, and the R-R interval (Fig. 2C) decreased in all five animals on Day 2, although one animal (#4) showed less remarkable fluctuation of the heart rate and R-R interval. The
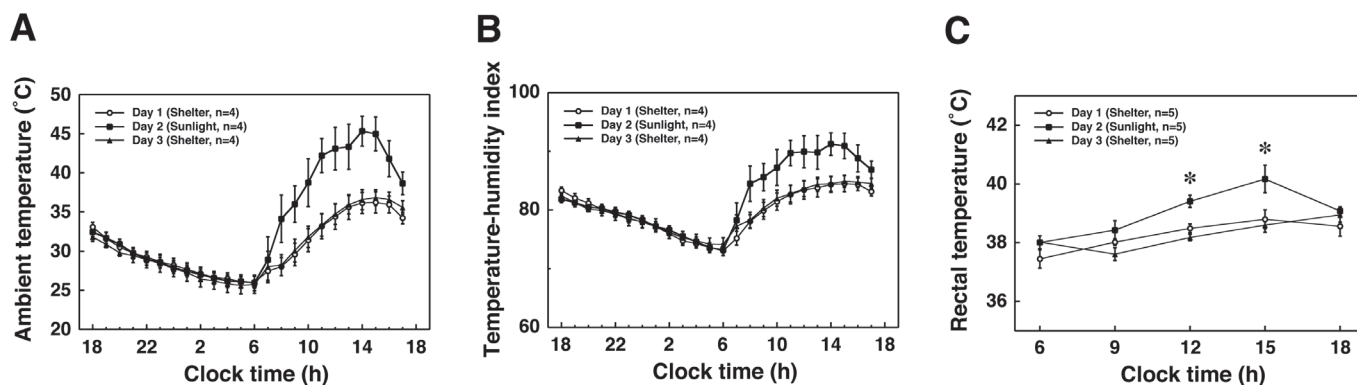
Fig. 1. (A and B) Changes in ambient temperature (A) and temperature-humidity index (B) during 3-day experimental period (n=4). (C) Changes in rectal temperature of cows during daytime (06:00–18:00 hr) of 3-day experimental period (n=5). Values are means ± SEM. *on Day 2, $P<0.05$ vs Day 1 and Day 3.

SD (Fig. 2D) and CV (Fig. 2E) of R-R interval of cows exposed to sunlight decreased, except for one animal (#6), which showed the most drastic increase of heart rate under the sunlight (Fig. 2B). Little is known why this animal showed different response to the exposure to direct sunlight. On the other hand, as a result of the power spectral analysis of HRV, three out of the five animals studied showed an increase in the LF/HF ratio and normalized LF power (Fig. 2F and 2G), and a decrease in the normalized HF power (Fig. 2H), when exposed to sunlight (Day 2). The remaining two animals, including one lactating cow (#1), kept under sunlight did not show any apparent changes in frequency domain indices. To summarize these results, among the five examined, none of the individuals showed consistent responses in the three indices (R-R interval and SD and CV of R-R interval) based on the time domain analysis, whereas the animals were divided into two groups in accordance to the different response in indices (LF/HF ratio and normalized LF and HF powers) based on the power spectral analysis of HRV.

The present study demonstrated that the power spectral analysis of HRV is a good tool to evaluate heat stress and uncovers the individual differences in heat tolerance in Cambodian dairy cows. Based on the power spectral analysis of HRV, the five cows used in the present study can successfully be categorized into two groups. Three out of the five cows were likely to be more heat stressed than the other two cows. The three cows in the former category showed an increase in the ratio of LF/HF power, which reflects an increase in the sympathetic nervous activity in response to the exposure to direct sunlight. The other two cows, including one lactating, did not show such clear increase in sympathetic nervous activity under exposure. This suggests that the latter two cows are likely to have a higher heat tolerance. Alternatively, these cows may have failed to respond to heat stress appropriately, resulting in no clear alteration of autonomic nervous activities. Further studies are needed to clarify whether the data of power spectral analysis of HRV is correlated with the productivity of dairy cows, such as milk production or reproductive performance. It is unlikely that lactation affects the physiological response to heat, because the individual showed similar patterns of changes in rectal temperature and heart rate to most of the other cows.

It is well established that, in the power spectral analysis of HRV, the HF power reflects parasympathetic activity, whereas the LF power represents both parasympathetic and sympathetic activities [1]. A change in LF/HF ratio has previously been reported to reflect a real-time change in the dominant autonomic nervous system [17]. Keeping this in perspective, HRV has been proposed to be a non-invasive means of the real-time measurement of physical or psychological stresses faced by farm animals [14]. In theory, the increased activity of the HPA axis is well-established to be a response to stress [3]. Historically, circulating cortisol levels have been used to estimate stress, as previously suggested [16]. However, measurement of such peripheral hormonal levels encompasses potential inaccuracy in evaluation of heat stress responses, because the measurement method itself may cause stress in animals by holding them for blood collection procedures. In this point of view, analysis of HRV based on ECGs recorded with a Holter-type electrocardiograph may have an advantage, because the stress caused by the experimental procedure itself is minimized. There have been several reports on the relationship between heat exposure and HRV in humans [6]. Several studies have been performed to evaluate stress using HRV in different animal species as well. Heart rate variability in dogs and calves has also been studied to reflect stress, as in the case of humans [12]. The present study also demonstrates the usefulness of HRV to estimate heat stress in each individual dairy cow under natural high temperatures. We cannot eliminate the possibility that oxidative stress caused by ultraviolet (UV) components of direct sunlight might affect the fluctuation of autonomic nervous activity, because strong UV irradiation induces the excessive production of reactive oxygen species (ROS) [20], and ROS affects autonomic nervous activity [24].

In conclusion, the present study suggests that the power spectral analysis of HRV would be useful to evaluate heat tolerance in cows, and the data from this analysis would guide researchers and livestock farmers to breed high-performance dairy cows adapted to tropical climates.

Table 1. Changes in heart rate and heart rate variability indices in the five cows during pre- (Day 1), heat- (Day 2) and post- (Day 3) treatments

	HR (bpm)	R-R interval (msec)	SD (msec)	CV (%)	LF power (msec^2)	HF power (msec^2)	LF/HF ratio
Day 1 (pre-treatment)							
Nighttime	81.71 ± 4.97	738.52 ± 44.56	39.47 ± 13.10	5.36 ± 1.70	60.44 ± 39.75	95.02 ± 158.77	2.23 ± 1.52
Daytime	82.79 ± 3.47	728.28 ± 31.97	39.30 ± 5.53	5.36 ± 0.73	54.07 ± 23.41	85.70 ± 111.47	1.52 ± 0.69
Day 2 (heat-treatment)							
Nighttime	81.55 ± 5.91	739.49 ± 53.65	40.57 ± 7.33	5.49 ± 0.97	55.47 ± 7.49	53.94 ± 45.91	1.57 ± 0.68
Daytime	95.45 ± 6.80[a]	635.06 ± 49.73[a]	32.81 ± 18.00	5.26 ± 3.21	88.55 ± 129.18	50.83 ± 51.15	2.56 ± 1.94
Day 3 (post-treatment)							
Nighttime	82.86 ± 7.11	732.14 ± 65.34	53.22 ± 42.08	7.62 ± 6.69	64.25 ± 41.44	61.83 ± 43.57	1.48 ± 0.64
Daytime	81.62 ± 5.31	739.53 ± 49.28	46.92 ± 22.73	6.36 ± 3.15	66.23 ± 18.44	75.90 ± 37.31	1.30 ± 0.70

Values are mean ± S.D. of data obtained from five cows. Mean values of nighttime and daytime on each day for 6-hr period were calculated during 21:00–03:00 and 09:00–15:00 hr, respectively. HR, heart rate; SD, standard deviation of the R-R interval; CV, coefficient of variation of the R-R interval; LF, low frequency; HF, high frequency. a) $P<0.05$ vs. the same period on Day 1 and Day 3 (Holm's multiple comparison).
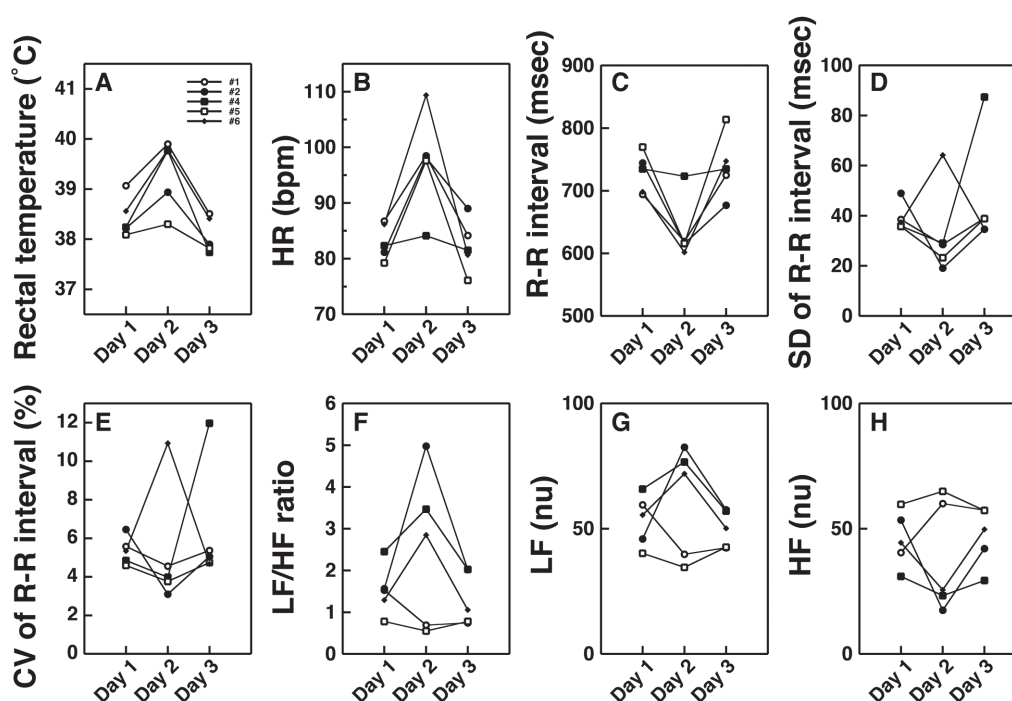

Fig. 2. Individual changes in rectal temperature (A), heart rate (B), R-R interval (C), SD (D) and CV (E) of R-R interval, LF/HF ratio (F), and normalized LF (G) and HF (H) powers in the five cows on pre- (Day 1), heat- (Day 2) and post- (Day 3) treatment days during daytime (09:00−15:00 hr). HR, heart rate; SD, standard deviation: CV, coefficient of variation; LF, low frequency; HF, high frequency; nu, normalized unit.

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