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Circadian changes in physiological responses and blood ionized sodium and potassium concentrations under thermal exposure in Tharparkar and Karan Fries heifers

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To explore the circadian changes in physiological responses and blood ionized sodium, potassium concentrations under thermal exposure in Tharparkar (TP) and Karan Fries (KF) heifers, 12 animals, six each from two breeds were taken and kept first in control thermoneutral (21–26°C and 50–60% RH) and in heat exposed (38°C and 50–60% RH) conditions thereafter. Throughout a 24-hour period physiological responses (rectal temperature, respiration rate and pulse rate) and blood samples were taken at 4-hour intervals, then blood samples were analyzed for sodium (Na^+) and potassium (K^+) levels. Significant circadian variations in physiological responses and blood Na^+ and K^+ within 24 hours have been observed. Significant ($P < 0.01$) variation in physiological responses and blood Na^+, K^+ concentrations were observed in both Tharparkar and Karan Fries heifers under control and heat exposed conditions. Values of physiological responses were lower in the morning and showed an increasing trend and reached the highest value in the afternoon. Blood Na^+ and K^+ concentrations decreased from 11.00 hours to 19.00 hours due to heat exposure. The decreases in blood electrolyte levels were greater in Karan Fries in comparison to Tharparkar heifers that expressed less heat tolerance capacity in crossbred than indigenous cattle. This finding suggests that it is important to standardize the time of sample collection in comparative studies, in which more discrete differences may be identified in physiological responses and blood electrolyte concentrations.

Keywords: circadian rhythms; physiological responses; blood sodium; blood potassium; Tharparkar; Karan Fries; thermal exposure

Introduction

A variety of biological variables oscillate in organisms, including behavior, physiological functions and biochemical factors. If any event within a biological system recurs at approximately regular intervals, it is called biological rhythm. A circadian rhythm is a biological rhythm that persists under constant environmental conditions (light–dark and temperature) with a period length of 24 hours. Circadian rhythmicity is a ubiquitous property of the physiology and behavior of all organisms.

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Among the many variables that exhibit circadian rhythmicity, physiological responses have received considerable attention. The circadian rhythm of physiological responses have been studied in a variety of mammalian organisms including farm animals, but most investigations have been conducted in small animals with body size under 10 kg (Refinetti and Menaker 1992). Greater stability of physiological responses is to be expected in large mammals because of their small surface/volume ratio, which contributes to thermal inertia (Aschoff 1982). In tropical and subtropical regions, cattle coming from temperate climates fail to adapt and become unproductive. McDowell (1972) reported that a small increase in core temperature influenced tissue and neuroendocrine functions, which reduced fertility, growth, lactation and ability to work. The heat tolerance capacity of crossbred cattle is poor mainly due to less developed and low density of sweat glands. Blood electrolytes are important variables and values can be affected by the time of sample collection. Rhythmicity in blood electrolytes has been extensively studied in humans and laboratory animals but much less in farm animals. Skotnicka et al. (2007) reported that the blood plasma electrolyte concentration in adult livestock is relatively stable. El-Nouty et al. (1980) found that both Na^+ and K^+ serum concentrations were reduced in Holstein cows during prolonged heat stress. Extending the study of rhythmicity of physiological responses and blood electrolytes to farm animals, the effect of heat stress on electrolyte levels in blood is important not only from a comparative perspective but also from an economic perspective, as greater knowledge of this process can lead to improvements in livestock production. So the present investigation was carried out to explore circadian variation in physiological responses and blood electrolytes (blood ionized sodium and potassium) and effect of heat stress on those healthy heifers. Rhythmicity in physiological reactions is an important physiological process both as a convenient and reliable marker of the operation of the biological clock and as an indicator of the general health of an animal and of its energy metabolism.

Materials and methods

The experiment was conducted on 12 heifers aged between 1.5 to 2 years, six each from Tharparkar and Karan Fries (HF × Tharparkar) breeds, selected from the herd of the National Dairy Research Institute (NDRI), Karnal, India. The study was carried out in the month of February. All experimental procedures were reviewed and approved by the animal ethics committee at NDRI (Karnal, Haryana, India). The experimental animals were maintained as per the existing management schedule specific to the age and physiological requirements of the animal. The feed consisted of green fodder (berseem and oat) and concentrate mixture supplemented with minerals and salts. Clean water was available ad libitum round the clock to these experimental animals. All the experimental animals prior to the start of actual experimental work were kept in the shade for adaptation to experimental conditions for 15 days to avoid variations in data recordings due to the handling stress of these animals. Animals were first kept in control conditions (thermoneutral zone) at 21–26°C and 50–60% RH for one week. Then animals were exposed to heat stress in a climatic chamber at 38°C temperature and 50–60% RH from 9 am to 2 pm. Provision for feeding and water to the animals were available inside the chamber during the experimental period. In the climatic chamber temperature and humidity were controlled automatically with the desired air flow. Temperature and humidity were displayed digitally through an LCD monitor. Changes in physiological responses were monitored continuously and blood samples were taken at 4-hour intervals throughout a 24-hour period, beginning at 7 am. The rectal temperature (RT) was recorded by a clinical thermometer inserted about 5 cm deep into the rectum of animals so that it remained in contact with the mucous membrane for at least 1–2 minutes. The observations were recorded in degrees Celsius (°C). The respiration rate (RR) was recorded by observing the flank movement for one minute in which each inward and outward movement of the flank was counted as one complete respiration. The respiration rate was expressed as breaths per minute. The pulse rate (PR) of the animals was counted by observing the pulsation of the middle coccygeal artery at the base of the tail and expressed as beats per minute. Each blood sample collection procedure which included all cows lasted $\leq$10 minutes. Blood samples were collected aseptically from the jugular vein using indwelling catheters. Blood sodium and potassium were estimated with the help of an atomic absorption spectrophotometer (model PU9100X atomic absorption spectrophotometer, Philips) described in the AAS (1988) manual. Statistical analysis for estimation of physiological responses and blood electrolytes (blood ionized sodium and potassium) and effect of heat stress on those parameters in healthy heifers was performed using the standard methods as suggested by Snedecor and Cochran (1967). The results were analyzed using ANOVA (Table 3) and the correlation coefficient (Table 2).

Results and discussion

Physiological responses

The changes in physiological responses (rectal temperature, respiration rate and pulse rate) over a period of 24 hours in Tharparkar and Karan Fries heifers under control and thermal exposed conditions are presented in Table 1. The rectal temperature of both breeds showed a circadian variation in control as well as thermal exposed conditions which is presented in Figure 1. Under thermoneutral conditions, the lowest rectal temperature observed was 37.99 ± 0.07°C in Tharparkar and 37.83 ± 0.14°C in Karan Fries heifers at 7.00 hours and which increased up to the highest values of 38.74 ± 0.11°C in Tharparkar and 38.84 ± 0.06°C in Karan Fries at 15.00 hours in the afternoon. In heat exposed conditions the rectal temperatures were higher than under control condition (thermoneutral condition). The minimum rectal temperatures observed in heat exposed conditions were seen at 7.00 hours (38.37 ± 0.16°C in TP and 38.39 ± 0.14°C in KF) and the maximum at 15.00 hours (38.96 ± 0.05°C in TP and 39.19 ± 0.24°C in KF). This observation supports the observations of Singh and Singh (2006). Significant differences ($P < 0.01$) were observed between different times and also between control and heat exposed conditions. During the period of thermal exposure, the rectal temperature of both the breeds increased. The respiration rates (control condition) at the start i.e. at 7.00 hours were 19.67 ± 0.61 and 20.83 ± 1.49 breaths per minute in Tharparkar and Karan Fries heifers, respectively. The values reported by Singh and Singh (2006) corroborate the values found in the present study. The RR increased progressively in the afternoon in relation to thermal exposure to a peak level of 30.33 ± 1.61 and 32.83 ± 1.05 breaths per minute in KF and TP heifers, respectively in control conditions and 34.00 ± 1.00 and 38.83 ± 0.92 breaths per minute in heat exposed conditions at 15.00 hours (Table 1). Soly and Singh (2001) also reported higher RR during the afternoon hours than morning hours in crossbred calves in the summer

Table 1. Mean (± SE) of physiological responses and blood ionized electrolytes (Na^+ and K^+) of Tharparkar and Karan Fries heifers at different time periods in a day under control and thermal exposed conditions.

			Hours					
Parameters	Breed	Condition	7.00	11.00	15.00	19.00	23.00	3.00
Rectal temperature (°C)	TP	C	37.99 ± 0.07	38.31 ± 0.05	38.74 ± 0.11	38.66 ± 0.11	38.19 ± 0.09	38.14 ± 0.11
		E	38.37 ± 0.16	38.45 ± 0.04	38.96 ± 0.05	38.76 ± 0.10	38.67 ± 0.09	38.42 ± 0.13
	KF	C	37.83 ± 0.14	38.40 ± 0.12	38.84 ± 0.06	38.68 ± 0.09	38.33 ± 0.11	38.27 ± 0.19
		E	38.39 ± 0.14	38.62 ± 0.09	39.19 ± 0.24	38.95 ± 0.08	38.59 ± 0.14	38.50 ± 0.07
Respiration rate (breaths/minute)	TP	C	19.67 ± 0.61	24.83 ± 0.48	30.33 ± 1.61	26.33 ± 0.67	20.83 ± 0.75	16.00 ± 0.73
		E	21.50 ± 0.76	28.17 ± 1.38	34.00 ± 1.00	25.83 ± 0.65	27.33 ± 1.23	21.17 ± 1.08
	KF	C	20.83 ± 1.49	23.67 ± 1.56	32.83 ± 1.05	28.17 ± 1.82	19.33 ± 0.33	15.67 ± 0.76
		E	22.50 ± 0.92	28.00 ± 0.92	38.83 ± 0.92	33.67 ± 0.92	26.17 ± 0.92	23.00 ± 0.92
Pulse rate (beats/minute)	TP	C	53.17 ± 1.08	63.50 ± 1.06	71.50 ± 0.85	73.83 ± 0.70	70.33 ± 0.61	60.00 ± 0.86
		E	52.17 ± 1.35	73.17 ± 0.83	79.17 ± 1.92	81.83 ± 0.75	72.33 ± 0.67	63.17 ± 0.91
	KF	C	55.17 ± 1.08	65.67 ± 0.92	76.67 ± 1.28	71.50 ± 0.92	68.67 ± 0.80	57.00 ± 1.41
		E	58.17 ± 2.20	74.17 ± 1.90	89.50 ± 0.85	87.67 ± 1.80	77.50 ± 1.12	65.67 ± 1.17
Blood ionized sodium (meq/lit)	TP	C	136.25 ± 1.75	137.99 ± 2.33	143.13 ± 1.18	140.87 ± 1.51	141.88 ± 1.49	137.46 ± 1.70
		E	136.53 ± 1.52	132.88 ± 0.77	133.80 ± 2.28	136.05 ± 1.75	140.31 ± 1.80	133.63 ± 1.36
	KF	C	139.67 ± 1.52	140.38 ± 2.14	146.10 ± 0.79	143.96 ± 1.98	142.72 ± 0.47	133.33 ± 2.44
		E	137.72 ± 1.71	133.39 ± 3.16	129.13 ± 1.02	128.39 ± 2.00	135.46 ± 3.29	137.23 ± 2.00
Blood ionized potassium (meq/lit)	TP	C	4.06 ± 0.17	3.43 ± 0.06	3.50 ± 0.09	4.45 ± 0.07	4.33 ± 0.07	4.59 ± 0.09
		E	4.02 ± 0.27	3.35 ± 0.23	3.15 ± 0.24	3.56 ± 0.26	3.44 ± 0.15	4.17 ± 0.14
	KF	C	4.05 ± 0.11	3.74 ± 0.16	3.57 ± 0.06	4.45 ± 0.13	4.14 ± 0.25	4.66 ± 0.07
		E	3.38 ± 0.29	3.17 ± 0.38	3.06 ± 0.23	2.97 ± 0.25	4.01 ± 0.24	4.39 ± 0.11

season. Circadian rhythms in RR are presented graphically in Figure 2. Changes in RR within 24 hours were significant ($P < 0.01$) in both Tharparkar and Karan Fries heifers and these are in agreement with the findings of Singh and Singh (2006). Significant ($P < 0.01$) variations were also observed in both Tharparkar and Karan Fries heifers in control and heat exposed conditions and between two breeds. The pulse rate also showed an increasing trend in both the breeds from a minimum value of 53.17 ± 1.08 and 55.17 ± 1.08 beats per minute in TP and KF breeds, respectively in control conditions to a maximum of 73.83 ± 0.70 and 71.50 ± 0.92 beats per minute in TP and KF breeds, respectively. During thermal exposure PR increased from a minimum value of 52.17 ± 1.35 and 58.17 ± 2.20 beats per minute in TP and KF breeds, respectively to a maximum of 81.83 ± 0.75 and 87.67 ± 1.80 beats per minute in TP and KF breeds, respectively. The increasing pulse rate was greater in KF than TP animals, which may be due to adaptation differences of breeds. Das et al. (1999) have also reported circadian changes in the pulse rate of Murrah buffalo calves exposed to direct solar radiation during the summer season. Circadian rhythms in PR are presented graphically in Figure 3.

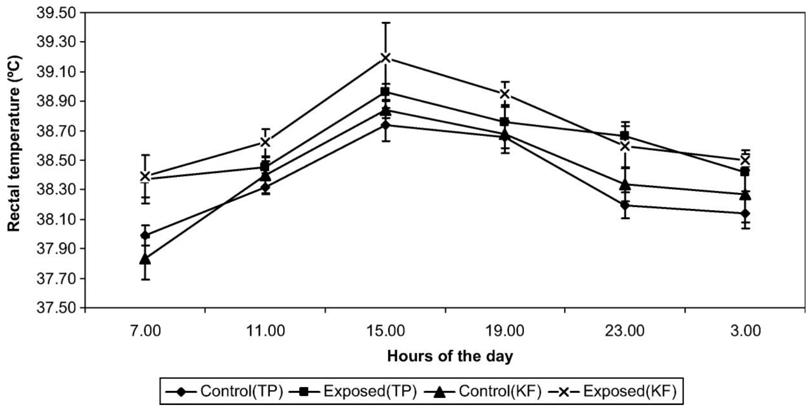

Figure 1. Mean circadian fluctuations in rectal temperature (°C) in control and thermal exposed conditions in Tharparkar and Karan Fries heifers.

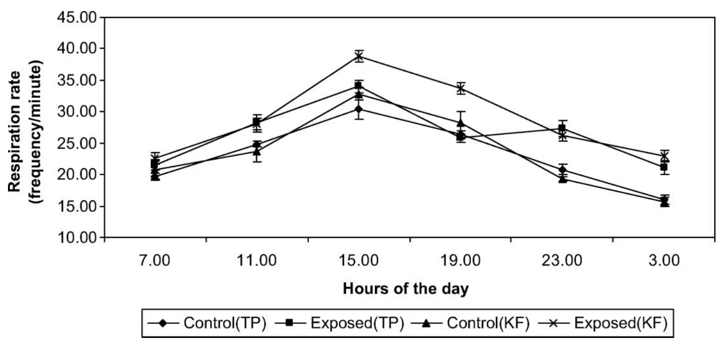

Figure 2. Mean circadian fluctuations in respiration rate (breaths/minute) in control and thermal exposed conditions in Tharparkar and Karan Fries heifers.

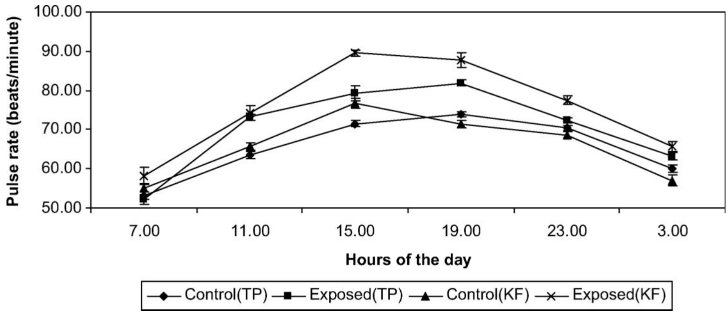

Figure 3. Mean circadian fluctuations in pulse rate (beats/minute) in control and thermal exposed conditions in Tharparkar and Karan Fries heifers.

Table 2. Correlation coefficient among physiological responses and blood ionized electrolytes (Na^+ and K^+) of Tharparkar and Karan Fries heifers at different time periods in a day under control and thermal exposed conditions.

Parameters	RT	RR	PR	Na	K
Control condition					
RT	1				
RR	0.544**	1			
PR	0.654**	0.661**	1		
Na	0.512**	0.422**	0.467**	1	
K	−0.173	−0.502**	−0.146	−0.196	1
Thermal exposed condition					
RT	1				
RR	0.658**	1			
PR	0.586**	0.744	1		
Na	−0.285*	−0.305**	−0.294*	1	
K	−0.252*	−0.469**	−0.360**	0.198	1

*$P < 0.05$, **$P < 0.01$.

Table 3. Analysis of variance of physiological responses and blood ionized electrolytes (Na^+ and K^+) of Tharparkar and Karan Fries heifers at different time periods in a day under control and thermal exposed conditions.

		Mean sum of squares				
Source of variation	DF	Sodium	Potassium	RT	RR	PR
Between breed	1	0.0261	0.0560	0.209	69.444*	275.007**
Between treatment	1	1304.173**	9.933**	3.022**	667.361**	1914.063**
Between time	5	51.007*	4.032**	1.994**	709.694**	2259.729**
All effect interaction	5	43.606	0.430	0.0343	7.378	15.696**
Error	120	20.989	0.223	0.0815	7.283	8.710

*$P < 0.05$, **$P < 0.01$.

Changes in PR within 24 hours were significant ($P < 0.01$) in both Tharparkar and Karan Fries heifers and these are in agreement with the findings of Singh and Singh (2006). Significant ($P < 0.01$) variations were also observed in both Tharparkar

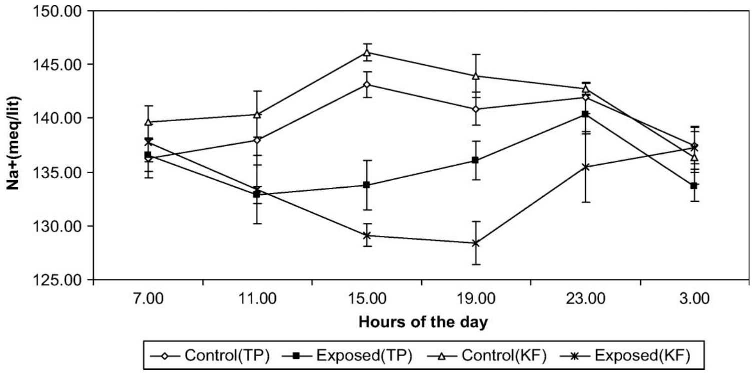

Figure 4. Mean circadian fluctuations in blood ionized sodium (Na^+) levels in control and thermal exposed conditions in Tharparkar and Karan Fries heifers.

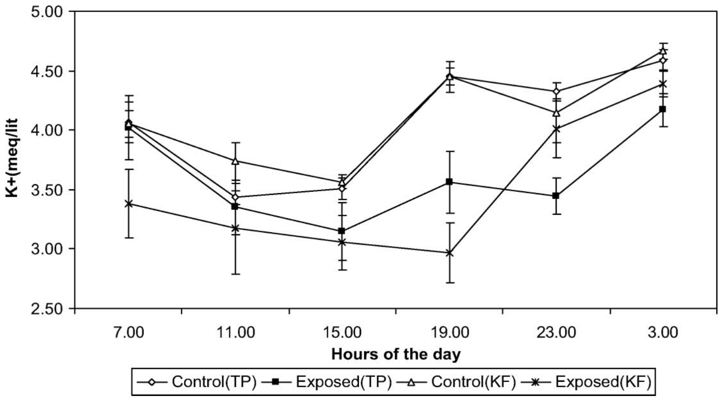

Figure 5. Mean circadian fluctuations in blood ionized potassium (K^+) levels in control and thermal exposed conditions in Tharparkar and Karan Fries heifers.

and Karan Fries heifers in control and heat exposed conditions and between two breeds.

Blood electrolytes

Blood ionized sodium (meq/lit) and potassium (meq/lit) levels of Tharparkar and Karan Fries heifers at different time periods in the day under control and thermal exposed conditions are presented in Table 1. Mean values for the measured variables i.e. Na^+ and K^+ concentrations were within reported reference ranges (Radostits et al. 1994). In control conditions, individual maximal values for Na^+ concentrations were highest at 15.00 hours (143.13 $\pm$ 1.18 meq/lit for TP and 146.10 $\pm$ 0.79 meq/lit for KF). Minimum values for Na^+ concentration (136.25 $\pm$ 1.75 meq/lit) were seen in TP at 7.00 hours and in the case of KF (133.33 $\pm$ 2.44 meq/lit) at 3.00 hours. K^+ maximum values were found at 3.00 hours (4.59 $\pm$ 0.09 meq/lit for TP and 4.66 $\pm$ 0.07 meq/lit for KF) and minimum values 3.43 $\pm$ 0.06 meq/lit for

TP at 11.00 hours and 3.57 $\pm$ 0.06 meq/lit at 15.00 hours. Similar observations were reported by Bajcsy et al. (1999) in pregnant Holstein cows. In heat exposed conditions maximum blood Na^+ concentration (140.31 $\pm$ 1.80 meq/lit) was seen in TP at 23.00 hours and a minimum value (132.88 $\pm$ 2.09 meq/lit) was seen at 15.00 hours, whereas in KF the maximum value of blood sodium concentration (137.72 $\pm$1.71 meq/lit) was seen at 7.00 hours and the minimum value (128.39 $\pm$ 2.00 meq/lit) was seen at 19.00 hours. In heat exposed conditions the maximum blood K^+ concentration (4.17 $\pm$ 0.14 meq/lit) in TP was seen at 3.00 hours and minimum value (3.15 $\pm$ 0.24 meq/lit) was seen at 15.00 hours, whereas in KF the maximum value of blood potassium concentration (4.39 $\pm$ 0.11 meq/lit) was seen at 3.00 hours and the minimum value (2.97 $\pm$ 0.25 meq/lit) was seen at 19.00 hours. Changes in blood sodium and potassium concentrations within 24 hours were significant ($P < 0.05$ for Na^+ and $P < 0.01$ for K^+) in both Tharparkar and Karan Fries heifers. Circadian rhythms in blood Na^+ and K^+ concentrations are presented graphically in Figures 4 and 5, respectively. Significant circadian variations in the plasma levels of Na^+ and K^+ within 24 hours have been observed in the present study and these are in agreement with the findings of Skotnicka et al. (1997). Significant circadian fluctuations in blood Na^+ and K^+ concentrations with opposing patterns were found in our study; similar findings were reported by Yashiki et al. (1995) and Bajcsy et al. (1999). Fluctuations in measured variables did not correlate with feeding times in our study. Similar observations were made for horses (Greppi et al. 1996) and pregnant Holstein cows (Bajcsy et al. 1999). K^+ concentrations vary independently from feeding periods. If feeding does not affect the concentration of these minerals in monogastric animals, its effect in ruminants, as typical polygastric animals, should be even less apparent. Significant ($P < 0.01$) variation in blood Na^+ and K^+ concentrations were observed in both Tharparkar and Karan Fries heifers in control and heat exposed conditions. Blood Na^+ and K^+ concentrations were decreased from 11.00 hours to 19.00 hours due to heat exposure (Figures 4 and 5). These findings support the observations of El-Nouty et al. (1980). This reduction in plasma Na^+ concentration during heat stress may be caused by an increase in urinary Na^+ excretion due to increased total urinary output and loss of electrolyte output in sweat. The decreases in blood electrolyte levels were greater in Karan Fries in comparison to Tharparkar heifers that expressed lower heat tolerant capacity in crossbred than indigenous cattle. Correlation coefficient showed a significant ($P < 0.01$) relationship among rectal temperature, respiration rate, pulse rate, blood ionized sodium and potassium level (Table 2).

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