



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 3763-3767
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www.thepharmajournal.com
Received: 09-05-2022
Accepted: 12-06-2022

Pradeep Godwal
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

Meenaxi Sareen
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

Amit Kumar Chotiya
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

NS Rathore
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

Anil Moolchandani
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

RA Legha
ICAR-National Research Centre on
Equines, Equine Production
Campus, Bikaner, Rajasthan, India

Yash Pal
ICAR-National Research Centre on
Equines, Hisar, Haryana, India

Vijay Kumar
ICAR-National Research Centre on
Equines, Equine Production
Campus, Bikaner, Rajasthan, India

Corresponding Author
Pradeep Godwal
Department of Veterinary
Biochemistry, Rajasthan
University of Veterinary and
Animal Sciences, Bikaner,
Rajasthan, India

Impact of heat stress on diurnal physiological responses of sun exposed Martina Franca jacks in semitropical desert climate

Pradeep Godwal, Meenaxi Sareen, Amit Kumar Chotiya, NS Rathore, Anil Moolchandani, RA Legha, Yash Pal and Vijay Kumar

Abstract

The effect of heat stress on the diurnal rhythmic changes in physiological indices was studied in Martina Franca donkeys (n=6) in the semitropical arid desert climate in the two periods i.e. i) 'High THI (Thermal heat index) period in October 2016 with solar exposure from 10.00 h to 14.00h in the afternoon and ii) 'Thermoneutral THI period' in winter (28 Dec, 2016) under same period of solar exposure in open sheds. Physiological responses were recorded at 0.700h, 10.00h, 14.00h & 16.00h for 7 consecutive days in the heat stress period which were compared with that on one single day in winter. All physiological responses respiration rate (RR), rectal temperature (RT), heart rate (HR), skin temperature (ST) increased significantly due to heat stress in the afternoon than in the morning hours during October 2016 in heat stress climate of high thermal heat index (THI). Overall, the study concluded that the impact of climatic heat stress due to solar radiation in the afternoons in exotic Martina Franca donkeys was significant in terms of increased physiological stress indices. The impact showed diurnal rhythmicity which whose magnitude reduced with the decline in climatic THI with the diurnal time point in day and season.

Keywords: Martina Franca (Poitou) jacks, diurnal rhythm, physiological responses, heat stress, solar exposure

Introduction

Thermal stress refers to the impact of meteorological parameters on an animal which either interfere with the dissipation of body heat to the environment (high ambient temperature and relative humidity) or which impose an external heat load on the animal (solar radiation). The more heat an animal produces internally by its metabolism, the less its ability to tolerate external heat (Bianca., 1976) [3]. Thermal stress is one of the most important stressors in the hot regions of the world (Altan *et al.*, 2003) [1].

In Rajasthan, heat stress is one of the important factors affecting the working capacity of all animals, including equines. Heat stress is known to affect the physiological indices in animals, including goats, lamb and equines (Singh and Saxena., 1995). During 2009-10, an attempt was made to study and compare the physiological adjustments of indigenous and exotic donkeys and mules at ICAR-National Research Centre on Equines, Bikaner. The exotic donkeys (Poitou) were received as gift under Indo-UK collaborative research project through Overseas Development Authority (ODA, UK) at the centre for breed improvement and better quality mule production (Pal *et al.*, 2000) [10]. However till date no other comprehensive report describes the physiological stress changes occurring in these donkeys in response to climatic stress and their tolerance levels after having kept at the subtropical desert climate for more than a decade. Only a single study has described their heat tolerance in response to solar radiation (Pal *et al.*, 2000) [10]. Therefore, this study was planned to study the heat tolerance in Marina Franca (Poitou) Donkeys in the subtropical climate. In this region, even a short solar exposure during most part of the year other than peak winter is going to induce a heat stress in these animals as they have black coat and dense hairs being native of cold and temperate region.

Material and Methods

Experimental animals and their source, feeding and management

Six adult and apparently healthy, male exotic *Martina Franca* (Poitou) donkeys weighing between 270 to 350 kg, maintained at Equine Production campus (EPC), Bikaner of ICAR-National Research Centre on Equines, Hisar were selected for experimentation at the campus.

Experiments were carried out in accordance with the ethical treatment of experimental animals as per Institute Animal Ethics Committee. These animals were provided with standard ration for maintenance as per NRC (2007) once in the morning at 8 AM and water *ad-libitum* during the course of study except during the period 10:00h to 14:00h. All experimental animals were housed individually in partially open shed with sufficient height, sunlight, ventilation and space for animal movement. Animals were in the habit of staying exposed to sun during the period 10AM-2.00PM (10.00h to 14.00h) in the open sun in their sheds.

Experimental Design

The climate induced a moderate heat stress on the animals during the period 10-2 PM as was evident from the high temperature humidity index (THI) in October 2016. Therefore, the experimental design included the comparison of physiological and biochemical response of the solar exposed animals in the October 2016 with the responses in the similar condition during winter season. All six donkeys were weighted before experimentation for a week during October 2016. On all the experimental days, the animals were weighed immediately after each of the diurnal time period (07.00h, 10.00 h 14.00h and 16.00h). The data of physiological responses (rectal temperature, heart rate, respiration rate and skin surface temperature) were recorded at 07:00h, 10:00h, 14:00h and 16:00h on each day of the seven day experimental period during October. During mid winter (28th December 2016), the physiological responses recorded on one day in each animal at the respective time points. Only a single day response from six donkeys was chosen during winter as there was no sufficient heat stress during winter when the animals remained in the same sheds. This day served as control (Thermoneutral period) to compare with the moderate heat stress during October.

Meteorological variables

The meteorological variables such as ambient temperature, humidity, wind velocity, atmospheric pressure were recorded at the centre and also corroborated with the online data from the website www.timeanddate.com. The selection of this website was decided based on the accuracy of its data ascertained through dry bulb and wet bulb temperature recorded at the campus before start of the trial. The wet bulb temperature and dew points were subsequently calculated from the primary data by the online calculator Relative Humidity (by Time Brice and Todd Hall) of National Weather Service, National Oceanic and Atmospheric Administration, USA, available at https://www.weather.gov/epz/wxcalc_rh.

These parameters were used in the calculation of Temperature Humidity Index (THI) = $0.72 \times (T_{db} + T_{wb}) + 40.6$ (Johnson *et al.*, 1963; McDonald, 1972). These meteorological variables and THI values are presented in the table below

Physiological parameters

All the physiological responses were recorded as per the standard procedures on all the experimental days as given under.

- 1) Rectal Temperature:** Rectal temperature was recorded by inserting a digital thermometer about 3 cm deep in the rectum until the beep sound was produced when it sensed a stable temperature and represented in °C.
- 2) Heart Rate:** Heart rate was recorded by hearing heart beats per minute using stethoscope.
- 3) Respiration rate:** Respiration rate was recorded by observing the flank movements per min. Each inward and outward moment was counted as one respiration and recorded as respirations/min.
- 4) Body Weight:** Body weight (kg) was recorded in a weigh-bridge at EPC, Bikaner at four time points during the day (07:00h, 10:00h, 14:00h & 16:00h).

Statistical analyses

The data was subjected to descriptive analysis for deriving group means, standard error, analysis of variance (ANOVA) and post ANOVA pair-wise comparison of means by Fisher's Least Significant Test, and correlations between various parameters in statistical software SYSTAT 7.0. Significance was considered at $p < 0.05$.

Results

Climatic elements during the experimental period

The various the various climatic elements and THI is presented in Table 1. The climatic variable dry bulb temperature, varied significantly between the two study periods- 'heat stress period' in October and 'thermoneutral period' in winter. The variation in the climatic variables was also significant when observed at different diurnal time points of the day (i.e. 07.00h, 10.00h, 14.00h & 16.00h). The THI as calculated by formulas of McDowell (1972) also showed that diurnal time points from 10.00h to 16.00h exhibited substantial thermal stress as THI was greater than 80 (from 10:00h to 14:00h). On the other hand, the thermoneutral period in winter did not exhibit THI greater than 70.93 (from 10:00h to 14:00h) which showed that during the thermoneutral day, donkeys were exposed to a highly comfortable climate.

Table 1: Mean \pm S.E. Climatic elements and THI during the experimentation period

TIME	DBT(°C)	WBT(°C)	THI	RH%
Heat stress climate*				
07.00Hrs	23.57 \pm 0.48	17.12 \pm 0.65	69.90 \pm 0.77	52.00 \pm 0.50
10.00Hrs	33.00 \pm 0.38	20.56 \pm 0.73	79.17 \pm 0.72	31.29 \pm 2.59
14.00Hrs	35.43 \pm 0.30	19.49 \pm 0.23	80.14 \pm 0.18	21.00 \pm 1.25
16.00Hrs	34.43 \pm 0.48	19.22 \pm 0.35	79.23 \pm 0.59	22.00 \pm 0.85
Thermoneutral climate (control)				
07.00Hrs	14.00 \pm 0.00	8.6	56.87 \pm 0.00	47.00 \pm 0.00
10.00Hrs	26.00 \pm 0.00	14.16	69.52 \pm 0.00	24.00 \pm 0.00
14.00Hrs	29.00 \pm 0.00	14.36	71.82 \pm 0.00	16.00 \pm 0.00
16.00Hrs	27.00 \pm 0.00	13.99	70.11 \pm 0.00	20.00 \pm 0.00

*: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM); Thermoneutral climate (control): animals kept in similar condition in thermoneutral climate on one day in winter (Dec 2016)

Table 1: Diurnal changes in body weight (Kg) of sun exposed Martina Franca donkeys (Poitou Jacks)

Diurnal Time Points	1d	2d	3d	4d	5d	6d	7d	TN (control)
0700Hrs	293.67±8.27	296.67±8.22	298.33±7.71	299.00±7.40	295.33±7.31	297.67±7.86	296.17±7.92	297.00±7.90
1000Hrs	291.67±8.07	295.00±8.22	296.50±7.54	297.17±7.52	293.50±7.14	295.83±7.89	294.50±8.00	295.17±8.02
1400Hrs	290.33±7.91	293.67±8.29	293.33±7.88	296.50±7.56	292.67±7.21	294.67±8.07	293.33±7.88	293.50±8.11
1600Hrs	294.83±8.09	295.67±8.24	297.33±7.46	300.00±7.21	295.17±7.69	296.67±7.85	296.83±7.89	296.50±8.37

1, 2, 3, 4, 5, 6, 7d: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM);
 TN (control): animals kept in similar condition in thermoneutral climate as control on one day in winter (Dec 2016)

Body weight changes

The mean body weight changes are presented in table 2. The body weight exhibited a diurnal change from 07.00 to 180.00h and no significant changes were observed either between the two seasons or within a day. However all the days exhibited a lower bodyweight at 14.00h than at 07.00 and 16.00h.

Physiological responses

The physiological parameters studied to quantify the effect of heat stress on donkeys during the heat stress period characterized by high solar radiation during the afternoons in

comparison to the thermoneutral period in winter are presented in the following Tables (2-5). All the physiological responses exhibited significant increases in magnitude in October than in the winter (Thermoneutral period). The responses also exhibited significant increases at 14.00h after exposure to sun in the October when compared to the values observed in the morning and 10.00h before exposure to solar radiation in sun. Skin temperature and respiration rates exhibited maximum change in the magnitude after exposure to sun at 14.00h in the heat stress period in October than in winter.

Table 2: Diurnal changes in respiration rate (breaths/min.) of sun exposed Martina Franca donkeys (Poitou Jacks)

Diurnal Time Points	1d	2d	3d	4d	5d	6d	7d	TN (control)
0700Hrs	34.83± 0.83 ^{aC}	35.17±0.40 ^{aC}	32.83±0.65 ^{aBC}	33.33±1.02 ^{aBC}	32.00±0.89 ^{aB}	33.33±1.02 ^{aBC}	31.00±1.13 ^{aB}	24.50±0.76 ^{aA}
1000Hrs	36.67±0.61 ^{aDE}	36.50±0.62 ^{aCDE}	35.33±0.42 ^{bBCD}	37.67±1.41 ^{bDE}	34.17±0.54 ^{aBC}	38.00±1.26 ^{bE}	33.67±0.61 ^{bB}	26.83±0.65 ^{bA}
1400Hrs	40.33±0.61 ^{bBC}	41.00±0.86 ^{bBCD}	40.00±0.73 ^{bC}	43.33±1.12 ^{cD}	42.50±1.54 ^{bED}	42.67±1.23 ^{cD}	39.00±0.86 ^{cB}	29.00±0.51 ^{cA}
1600Hrs	38.67±0.67 ^{bB}	39.67±0.61 ^{bBC}	39.33±0.67 ^{bC}	41.67±1.50 ^{cC}	41.67±0.95 ^{bC}	40.00±1.46 ^{bcBC}	37.50±0.62 ^{cB}	29.00±0.36 ^{cA}

A, B, C,D,E: Values with different superscripts within a row (within days) vary significantly with each other ($p < 0.05$)
 a,b,c: Values with different superscripts within a column (within time) vary significantly with each other ($p < 0.05$)
 1, 2, 3, 4, 5, 6, 7d: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM);
 TN (control): animals kept in similar condition in thermoneutral climate as control on one day in winter (Dec 2016)

Table 3: Diurnal changes in pulse rate (beats/min.) of sun exposed Martina Franca donkeys (Poitou Jacks)

Diurnal Time Points	1d	2d	3d	4d	5d	6d	7d	TN (control)
0700Hrs	33.67±1.31 ^{aB}	32.50±0.62 ^{aAB}	33.50±1.54 ^{aB}	33.00±1.13 ^{aAB}	32.67±0.80 ^{aAB}	33.33±0.99 ^{aB}	34.83±0.31 ^{aB}	30.50±0.22 ^{aA}
1000Hrs	38.67±2.51 ^{abB}	37.00±1.84 ^{abB}	35.67±1.74 ^{abAB}	36.83±0.91 ^{bB}	34.50±0.89 ^{aAB}	38.33±2.16 ^{bB}	37.67±.74 ^{aB}	31.83±0.40 ^{bA}
1400Hrs	45.00±2.35 ^{bB}	44.67±2.40 ^{bcB}	43.67±2.33 ^{cB}	41.67±1.67 ^{cB}	43.83±1.80 ^{bB}	43.33±1.98 ^{cB}	44.33±2.03 ^{bB}	33.66±0.21 ^{cA}
1600Hrs	42.00±1.46 ^{bB}	41.50±1.52 ^{bcB}	39.83±1.56 ^{bcBC}	39.83±0.98 ^{bC}	40.50±1.41 ^{bC}	40.17±0.98 ^{bcBC}	41.50±1.50 ^{bB}	31.66±0.49 ^{bA}

A, B, C,D,E: Values with different superscripts within a row (within days) vary significantly with each other ($p < 0.05$)
 a,b,c: Values with different superscripts within a column (within time) vary significantly with each other ($p < 0.05$)
 1, 2, 3, 4, 5, 6, 7d: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM);
 TN (control): animals kept in similar condition in thermoneutral climate as control on one day in winter (Dec 2016)in winter

Table 4: Diurnal changes in rectal temperature (°C) of sun exposed Martina Franca donkeys (Poitou Jacks)

Diurnal Time Points	1d	2d	3d	4d	5d	6d	7d	TN (control)
0700Hrs	36.51±0.12 ^{aB}	36.30±0.19 ^{aAB}	36.54±0.10 ^{aB}	36.57±0.14 ^{aB}	36.53±0.23 ^{aB}	36.49±0.10 ^{aB}	36.37±0.12 ^{aAB}	36.06±0.08 ^{aA}
1000Hrs	37.16±0.11 ^{bB}	37.17±0.08 ^{cB}	37.16±0.09 ^{bB}	37.03±0.05 ^{bB}	37.03±0.12 ^{bB}	36.96±0.09 ^{bB}	36.97±0.15 ^{aB}	36.37±0.08 ^{bA}
1400Hrs	38.16±0.11 ^{dB}	38.01±0.11 ^{bB}	38.11±0.17 ^{cB}	38.06±0.11 ^{cB}	37.87±0.08 ^{cB}	37.92±0.11 ^{cB}	37.95±0.11 ^{cB}	36.86±0.05 ^{cA}
1600Hrs	37.73±0.05 ^{cB}	37.71±0.06 ^{bB}	37.72±0.09 ^{dB}	37.76±0.06 ^{dB}	37.56±0.08 ^{cB}	37.79±0.04 ^{bcB}	37.63±0.09 ^{cB}	36.86±0.13 ^{cA}

A, B, C,D,E: Values with different superscripts within a row (within days) vary significantly with each other ($p < 0.05$)
 a,b,c: Values with different superscripts within a column (within time) vary significantly with each other ($p < 0.05$)
 1, 2, 3, 4, 5, 6, 7d: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM);
 TN (control): animals kept in similar condition in thermoneutral climate as control on one day in winter (Dec 2016)

Table 5: Diurnal changes in skin temperature (°C) of sun exposed Martina Franca donkeys (Poitou Jacks)

Diurnal Time Points	1d	2d	3d	4d	5d	6d	7d	TN (control)
0700Hrs	33.37±0.24 ^{aB}	33.88±0.44 ^{aB}	34.08±0.50 ^{aB}	34.64±0.83 ^{aB}	34.54±0.56 ^{aB}	33.41±0.50 ^{aB}	34.16±0.42 ^{aB}	20.57±0.40 ^{aA}
1000Hrs	40.53±1.43 ^{bBC}	40.95±1.66 ^{bBCD}	37.76±1.14 ^{bBC}	39.38±0.85 ^{bBC}	39.39±0.42 ^{bBC}	44.49±0.67 ^{bC}	44.42±1.53 ^{bBC}	21.83±0.56 ^{bA}
1400Hrs	49.20±0.46 ^{cCD}	48.03±0.66 ^{bB}	45.99±0.99 ^{bB}	46.07±1.26 ^{cB}	47.26±1.05 ^{cBCD}	49.52±0.43 ^{cD}	47.13±0.84 ^{cBC}	23.54±0.31 ^{cA}
1600Hrs	40.34±1.72 ^{bB}	34.08±0.50 ^{bB}	39.16±1.11 ^{cB}	38.34±1.48 ^{bB}	40.63±1.95 ^{bB}	37.44±1.48 ^{bB}	37.78±1.65 ^{bB}	21.17±0.34 ^{bA}

A, B, C,D,E: Values with different superscripts within a row (within days) vary significantly with each other ($p<0.05$)
a,b,c: Values with different superscripts within a column (within time) vary significantly with each other ($p<0.05$)
1, 2, 3, 4, 5, 6, 7d: Heat stress climate (7 consecutive days of heat stress period in October 2016 with solar exposure from 10AM-2PM);
TN (control): animals kept in similar condition in thermoneutral climate as control on one day in winter (Dec 2016)

Discussion

Climatic elements

The climatic elements exhibited substantial and significant diurnal changes between the two seasons. The THI also revealed that the period of solar exposure between 10.00h and 14.00h during October was indeed a heat stress period with THI consistently in the highly severe heat stress zone for livestock (McDowell, 1972) while the same during the winter was under thermoneutral zone. This justified our selection of the heat stress period in the experiment.

Body weight changes

The body weight and physiological indices were studied in the Martina Franca (Poitou) donkeys during two different climatic conditions in the subtropical arid desert. These responses have already been presented in Table 2. The body weight of the *Martina Franca* donkeys recorded at four diurnal time points in heat stress period and thermo neutral day in winter did not vary significantly between each other. However, we observed that the BW decrease from 07:00h to 10:00h and then to a lowest at 1400h on all days in both seasons. The first decline from 07:00h to 10:00h was due to faecal losses that occurred between 07:00h to 10:00h. The second decrease from 10:00h to 14:00h occurred due to restriction on feed and water intake for the animals. The body weight increased at 16.00h again as the adlib feed and water intake was again restored after 14.00h. The body weight of the *Martina Franca* donkeys in this study was higher than that reported for Martina Franca broodmares by Chiofalo *et al.* (2006) [4, 13] and lower than those by Salimei *et al.* (2006) [4, 13].

Effect of heat stress on physiological responses in Martina Franca donkeys

To the best of our knowledge, no such report is available which has provided diurnal change in the body weight in two seasons. Rectal temperature increased significantly in the 'heat stress period' due to solar exposure between 10.00h and 14.00h when compared with that during the winter season as well as to that during the morning hours (07:00h). While, rectal temperature didn't decline significantly from 14:00h to 16:00h during winter season, it declined significantly during the 'heat stress period'. Overall the corresponding values were significantly higher in the heat stress period when compared with the thermoneutral period in winter. Similar trend was observed for respiration rate, pulse rate and skin surface temperature. To the best of our knowledge, this is the first report which has described the changes in these responses when compared at different diurnal times during the two different seasons in a subtropical desert climate in Martina Franca donkey. Only one previous report (Pal *et al.*, 2000) [10] has reported changes in rectal temp and respiration

rate. Pal *et al.* (2000) [10] reported rectal temperature of only 3 exotic Martina Franca (Poitou) donkeys during hot dry season when they were kept in shade for the first 9 days followed by six days under solar exposure from 08:00h to 06:00h. The significant increase in these responses in the afternoon hours in this study is in agreement with the findings of these authors. The mean post exposure values were similar to our finding when considered at 14:00h at 16:00h the value reported by (Pal *et al.*, 2000) [10]. The post exposure recovery of these responses studied by Pal *et al.* (2000) [10] is also similar to that in our study for rectal temperature but not for respiration rate, although the trend was similar. Similar findings were also reported by Pal *et al.* (2000) [10] in indigenous donkeys and mules. The respiration rates of these donkeys in our study was about 10 respirations /min higher than those reported by Pal *et al.* (2000, 2012) [10]. A previous study by same authors (Pal *et al.*, 1998) [9] has also reported a similar trend for ponies and donkeys exposed to solar radiation for 6h for six days in the same experimental location. Pal *et al.* (2012) [10] reported the rectal temperature and pulse rate to resting control donkeys (adult female Indian) in the similar range as reported in the present study at 07:00h. The present study has additionally reported the skin surface temperature of these donkeys which has not been reported earlier. The increase in the skin surface temperature was much higher in comparison to the other physiological responses. This is due to the fact that Martina Franca donkeys have dark coloured skin and hair which make them less tolerant to heat than indigenous donkeys of light coloured hair and skin (Pal *et al.*, 1998) [9]. Therefore, when exposed directly to solar radiation, the skin is the first organ to experience the heat load. Respiration rate, pulse rates and rectal temperature arise later. The skin absorbs the maximum heat and when the heat load is excessive, the temperature of internal organs start increasing elevating the core (rectal temperature). This increase in skin temperature and internal organs is sensed by higher neuronal centre of thermoregulation in the hypothalamus which activates the heat loss mechanisms by increasing the pulse rate and respiration rate. Such increases help dissipating heat and keeps the core temperature within the thermoregulatory limit (Upadhyay and Aggarwal, 1997; Hahn, 1999; Das *et al.*, 1999; Silanikove, 2000; Beatty *et al.*, 2006, Kumar and Kumar 2013) [15, 6, 5, 14, 2, 7]. The overall effect of these increased responses resulted in an increase of RT from 36 to 38°C. The increased pulse rate provides cooling by increasing the blood flow from core to peripheral organs, skin and appendages so that greater heat is dissipated via blood to skin by sweating and other mechanisms (Upadhyay and Aggarwal, 1997; Hahn, 1999; Das *et al.*, 1999; Beatty *et al.*, 2006; Kumar and Kumar 2013) [15, 6, 5, 2, 7]. The increased respiration rate is heat dissipation mechanism. It not only helps in removing excess heat through respiratory

tract to environment, but also cools the blood running from lungs to the heart (Upadhyay and Aggarwal 1997; Hahn, 1999; Das *et al.*, 1999; Beatty *et al.*, 2006; Kumar and Kumar 2013) [15, 6, 5, 2, 7]. In another study on working horses and donkeys, heat stress behaviour was found to be significantly correlated to increased rectal temperature (Pritchard *et al.*, 2006) [12]. High rectal temperature and behavioural response in working equines can be used to make judgments regarding rest and cooling requirements, precluding the need to seek veterinary attention (Pritchard *et al.*, 2006) [12].

Conclusions

The heat tolerance in the *Martina Franca* donkeys has been studied in the subtropical desert climate in Rajasthan. When kept exposed to climate in the afternoon, these animals have shown specific and diurnal stress responses which remained increased even after 2 hours of being exposed to sun. These responses exhibited a diurnal rhythmic pattern with the maximum magnitude at 14:00h and 16:00h in the afternoon in both the seasons. Climatic heat stress on exotic *Martina Franca* donkeys exposed to the sun in the afternoon hours during heat stress period as compared to peak winter in the same housing caused significantly increased physiological responses. The most significant increase was observed in the skin surface temperature which was not seen in the thermoneutral climate in winter. These observations would be helpful in developing suitable management and housing strategies for equines in the region so as to optimize their production potential

Acknowledgement

The authors are highly thankful to the Director, ICAR-National Research Centre on Equines Hisar, for extending all the facilities for carrying out this work as part of the M. V. Sc. Dissertation of Dr. Pradeep Godwal at the Equine Production campus, Bikaner under the project 'Assessment and optimization of equine management in an intensive system'. The authors also highly acknowledge the academic support to Dr. Pradeep Godwal by Hon'ble Vice-Chancellor, Rajasthan University of Veterinary and Animal Sciences, Bikaner.

Conflict of Interest

Authors have no conflict of interest

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