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Diurnal rhythm of blood haematological parameters of sun exposed *Martina franca* jacks in semitropical desert climate

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Abstract

Diurnal rhythm of blood haematological parameters was studied in adult and apparently healthy, male *Martina franca* (Poitou) jacks (n=6). The jacks weighing between 270 to 350 kg, and were fed with standard ration for maintenance as per NRC (2007) between 10:00h to 14:00h the donkeys remained exposed to solar radiation. Blood was collected for haematological parameters at 07:00 h, 10:00 h, 14:00 h and 16:00 h on 1st, 4th and 7th day in a seven day experimental period during October 2016 heat stress period (HS). Thermal heat index (THI) =69.90 at7.00h and 80.14 at 14.00h) and on a single day in peak winter in December 2016 Control 'C', THI: 63.93 at 7.00h and 80.14 at 14.00h). Haematological indices were studied on 1st, 4th and 7th day in the heat stress period which were compared with that on a single day in winter. WBC count increased significantly from 7:00h to 10.00h in both the seasons. Maximum significant increase was observed from 07:00h to 14.00h in heat stress period on 7th day of exposure. RBC, Hb and Hct concentrations increased in the afternoon on day 1 and 4 only in the heat stress period. Higher WBC, HCT, Hb and RBC were observed due to heat stress in the afternoons in the heat stress period and the significance varied.

Keywords: *Martina franca* (Poitou), diurnal rhythm, haematological profile, heat stress, RBC, WBC, Hb and HCT

1. Introduction

In Rajasthan, heat stress is one of the important factors affecting the working capacity of all the animals, including equines. Heat stress is known to affect the physiological indices in animals, including goats, lamb and equines (Karim and Patnayak., 1999). During 2009-10, an attempt was made to study and compare the physiological adjustments made by indigenous and exotic donkeys and mules at ICAR-National Research Centre on Equine, 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) [19]. However till date no comprehensive report describes the physiological stress including hematological changes occurring in these donkeys in response to thermal 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) [19]. However this study did not evaluate the hematological responses and major responses during the recovery period. Therefore, this study was planned to determine the effect of heat tolerance on physiological reactions and haematological parameters in exotic donkeys (*Martina Franca*).

2. Materials and Methods

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 in accordance with the ethical treatment of experimental animals as per Institute Animal Ethics Committee of Rajasthan University of Veterinary and Animal Sciences, Bikaner. These animals were provided with standard ration for maintenance as per NRC (2007) once in the morning at 8 AM and water *ad-Iibitum* 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 10:00h to 14:00h in the open sun in their sheds.

2.1 Collection of blood samples

4 ml blood samples were collected from jugular vein with negligible pain and minimal disturbance to the animals. Blood was collected with all aseptic precautions in sterilized tubes containing heparin as anticoagulant @ 2 IU/ml of blood. Blood was immediately mixed gently with the anticoagulant by inverting the tubes four to five times.

2.1.1 Hematological parameters

Total leukocyte counts (TLC, units x1000/cu mm), total erythrocyte counts (TEC, units x millions/cu mm), Packed cell volume (%) and Hemoglobin (g%) were estimated in automated five parts blood cell counter (MS4, France). Prior to analysis of the samples, the analyser was calibrated by ascertaining the accuracy of analysis through manual hematology of representative equine samples as per standard hematological methods (Jain, 1975) [9].

2.1.2 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 $p<0.05$.

3. Results and Discussion

The various blood haematological 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

Table 3.1: Effect of heat stress on white blood cell counts in *Martina Franca* donkeys (Poitou Jacks)

	WBC (counts x1000/cu mm)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	9.14 ±1.31	8.85 ±0.76	7.78 ±0.64^{a}	9.70 ±0.76^{a}
10:00 h	8.10 ±1.00^{A}	7.81 ±0.46^{A}	9.79 ±1.30^{abA}	13.44 ±1.50^{Bb}
14:00 h	9.97 ±0.73^{B}	7.62 ±0.70^{A}	14.82 ±0.83^{cC}	11.57 ±0.84^{abB}
16:00 h	7.96 ±0.73^{A}	9.96 ±0.30^{AB}	12.33 ±0.58^{bcB}	10.49 ±1.10^{abB}

A, B, C: 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, 4, 7d: days of exposure from 10AM-2PM; TN: animals kept in similar condition in thermoneutral climate in winter

Table 3.2: Effect of heat stress on Red Blood Cell counts in *Martina Franca* donkeys (Poitou Jacks)

	RBC(millions/cu mm)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	7.32 ±0.05^{B}	7.78 ±0.55^{B}	6.07 ±0.30^{A}	5.87 ±0.38^{Aa}
10:00 h	7.52 ±0.59^{B}	7.41 ±0.42^{B}	6.12 ±0.22^{A}	6.57 ±0.32^{aAB}
14:00 h	7.76 ±0.31^{B}	7.64 ±0.49^{B}	6.21 ±0.24^{A}	7.11 ±0.52^{bAB}
16:00 h	8.02 ±1.03^{B}	7.81 ±0.33^{B}	6.01 ±0.31^{A}	6.00 ±0.23^{aA}

A, B, C: 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, 4, 7d: days of exposure from 10AM-2PM; TN: animals kept in similar condition in thermoneutral climate in winter

Table 3.3: Effect of heat stress on Hemoglobin in *Martina Franca* donkeys (Poitou Jacks)

	Hemoglobin (g/dl)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	13.00 ±0.82^{B}	12.43 ±0.57^{AB}	10.42 ±054^{A}	10.72 ±0.82^{abA}
10:00 h	13.47 ±1.12^{B}	13.25 ±20.82^{B}	10.68 ±0.47^{A}	11.45 ±0.70^{abAB}
14:00 h	13.80 ±0.56^{B}	13.80 ±0.90^{B}	9.73 ±0.54^{A}	12.82 ±1.09^{aAB}
16:00 h	14.67 ±1.9^{B}	14.12 ±0.47^{B}	11.00 ±0.53^{A}	10.10 ±0.27^{bA}

A, B, C: 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, 4, 7d: days of exposure from 10AM-2PM; TN: animals kept in similar condition in thermoneutral climate in winter

Table 3.4: Effect of heat stress on Hematocrit in *Martina Franca* donkeys (Poitou Jacks)

	Hematocrit (%)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	40.32 ±1.13^{AB}	43.07 ±3.29^{B}	35.18 ±1.19^{aA}	36.67 ±1.61^{aAB}
10:00 h	41.63 ±2.89	41.33 ±2.08	37.27 ±1.52^{ab}	42.98 ±2.38^{ab}
14:00 h	42.83 ±1.39	42.80 ±2.76	41.62 ±1.70^{b}	46.23 ±3.98^{b}
16:00 h	44.92 ±5.63	43.63 ±1.37	37.93 ±1.91^{ab}	39.15 ±2.11^{ab}

A, B, C: 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, 4, 7d: days of exposure from 10AM-2PM; TN: animals kept in similar condition in thermoneutral climate in winter

The heat stress period with THI above 80 in the afternoon during the October with substantial exposure of the animals to solar radiation in open sheds induced several hematological changes as compared to the thermoneutral period in winter with THI less than 70 (Tables 3.1. to 3.4). The significant increase in the total leukocyte count with the diurnal progression of day was a result of heat stress during the afternoon hours combined with hemoconcentration as the feed intake and water intake was restricted between 10:00h to 14:00h. The levels at 14:00h in the heat stress period on 7th day remained higher than the corresponding values in the thermoneutral day. There was an increase in WBC due to heat stress on 7th day. Such a response was not visible on the first day. Moreover, the WBC counts initially increased from 7:00h to 10:00h but later on declined during the thermoneutral period as the day progressed. Since there was no substantial solar radiation in the winter, cold stress coupled with the mild wind chill continuing in winter, which is supposed to have increased WBC levels during the early hours of the day. The heat stress itself results in increased leukocyte counts in the horses and donkeys (Alrhman, 2015) [1]. Very few studies have reported hematological values of donkeys. Most of these reports are concentrated on providing the reference values of different breeds of donkeys. No report has described the hematological changes at diurnal time points in donkeys due to climatic effect. This study is therefore so far the first of its kind to report such values. As the diurnal change in the ambient temperature is significantly large in tropics, particularly, in the arid climate, during summers, such information is important from the clinical and diagnostic point of view. Most of the clinicians refer the hematological changes observed in donkeys with the reference values of horses. This is not considered a valid approach (Jordana *et al.*, 1998; Mori *et al*., 2003; Laus *et al.*, 2015) [10, 16, 13]. The heat stress induces inflammatory response in the vasculature of the arteries and veins resulting in increased number of white blood cells primarily due to increased neutrophils and lymphocytes amongst the WBCs to counter the inflammation. The cardiovascular system increases the cardiac output during heat stress leading to increased blood flow in the vasodilated blood vessels in order to supply extra oxygen and metabolites for work and heat dissipation in respective tissues. Extreme stress has also been reported to cause an increased influx of erythrocytes in the circulation from spleen which acts as a reservoir of erythrocytes in horses during stressful conditions such as exercise (Piccione *et al*., 2005, 2007; Escribano *et al.*, 2013) [3]. The hematological indices studied during thermoneutral climate and during the morning hours were in agreement with the normal range observed in horses and donkeys (Jordana *et al*., 1998 Mori *et al*., 2003; Garba *et al*., 2015; Laus *et al*., 2015; Burden *et al*., 2016) [10, 16, 13, 2]. Meteorological factors control the dynamics of hematological parameters in equines (Satue *et al*., 2013) [21]. Higher PCV, RBC and Hb were observed during summer in the Carthusian mares (Satue *et al*., 2013) [21], while cold climate is found to reduce the erythrocyte count in horses (Ruiz *et al.*, 2004) [20]. Gill *et al*. (1979) [6] observed lower Hb and RBC during summer in Arabian horses. In the tropics, where most of the animals are kept in open sheds, there is substantial heat load on these animals due to direct solar radiation (Zakari *et al*., 2014) [23]. These animals therefore have to alter their behavior and physiology in order to adapt to the climatic changes in order to dissipate heat and remain comfortable. Oxygen consumption and average heart rate is increased in the equines under work or under exercise (Art and Lekeux 1993; Kumar and kumar 2013) [14, 12]. Circadian variations in Hb, PCV, and RBC reported in adult horses, mares and foals (Gill and Kompanowska 1986; Komasa *et al*., 1990; Paglieroni *et al*., 1994; Ruiz *et al*., 2004) [5, 11, 18, 20] are more or less in agreement with our findings. A too high or low ambient temperature would lead to higher metabolic activity stimulating erythropoiesis. Lower morning temperature in the winters could have led to high values of RBC at 07:00 h. Additionally enhanced sympathetic activity in winter could lead to increased splenic mobilization with the release of RBCs in the bloodstream (Hata *et al.,* 1982; Satue *et al*., 2013) [8, 21]. Contrarily a previous report has indicated that intense cold could reduce RBC by reducing their half life (Lurie *et al.,* 1993) [15]. Satue *et al*. (2013) [21] also found higher RBCs and Hct in the summer months than in winter months which is due to heat, physical activity and change in the feeding grass which has different composition. High temperature and associated decrease in the body fluids in association with the thermoregulatory mechanisms could influence RBC and Hct, limiting the importance of hemoconcentration, because of evaporative sweating and /or breathing (Satue *et al*., 2013) [21]. Sexual activity is also higher in horses during summer and spring causing hemoconcentration and decreases during winter, ceasing completely in winter (Ginther *et al*., 2008) [7].

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