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Diurnal rhythm of blood biochemical profile of sun exposed *Martina Franca* jacks in semitropical desert climate

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Abstract
Diurnal rhythm of blood biochemical profile was studied in adult and apparently healthy, male *Martina Franca* (Poitou) jacks (n=6). The jacks weighing between 270 to 350 kg were fed with standard ration for maintenance as per NRC (2007) from 10:00h to 14:00hr. The donkeys remained exposed to solar radiation. Blood was collected for biochemical parameters at 07:00h, 10:00h, 14:00h and 16:00h 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). Plasma albumin and globulin (g/dl) increased significantly on day 1 of HS from 07.00h to 14.00h & 16.00h. Plasma Lactate (mM/l) increased significantly at 14.00h & 16.00h as compared to 07:00h in both ‘HS’ and ‘C’. The lactate levels were significantly lower at the respective diurnal time points in period ‘C’. Urea (mg/dl) increased significantly ($p < 0.05$) at 14.00h & 16.00h when compared to its values at 07.00h & 10.00h on in ‘HS’. Maximum urea level was observed at 16.00h on day 1 of ‘HS’. During winter, although urea levels increased significantly at 10.00h, 14.00h & 16.00h from the values observed at 07.00h, the levels remained lower when compared to ‘HS’ at 14:00h and 16:00h. Similar trends were observed for plasma creatinine (mg/dl) which remained significantly higher in ‘HS’ than in ‘C’. The cholesterol decreased at 10.00h & 14.00h and again increased significantly ($p<0.05$) at 16.00h on all the days. Heat stress in October caused overall higher cholesterol in jacks as compared to winter month. Triglyceride levels increased significantly ($p<0.05$) at 14.00h when compared to that at 07.00h & 10.00h on day 7 of ‘HS’ in October.

Keywords: *Martina franca* (Poitou), diurnal rhythm, biochemical profile, heat stress, plasma lactate, urea, creatinine, cholesterol, TG, cortisol

1. Introduction

Determination of baseline biochemical indices helps veterinarians to confirm clinical diagnoses, estimate the severity of cases and administer appropriate treatment in domestic animals (Babeker and Elmansoury, 2013) [6]. Biochemical tests evaluate the function of different organs. Cholesterol (CT) and triglyceride (TG) are considered an indicator of energy metabolism. The indicators of protein metabolism are urea, total protein (TP) and albumins. Urea and creatinine are indicators of the basic parameter reflecting kidney function, nutritional status and stress responses (Ghada, 2014) [12]. The season is known to affect these indices in several species like buffalo (Verma *et al.*, 2000) [20], Holstein heifer (Rasooli *et al*., 2004) [19] and camel (Al-Harbi, 2012) [2]. In Rajasthan, heat stress is one of the important factors affecting the working capacity of all the animals, including equines. Many of the researches on blood biochemical parameters in *Martina Franca* (Poitou) are incomplete or lack references to fluctuations in the parameters studied caused by environmental conditions or time of sampling during the day, and these researches had not been conducted in relation to the hormonal profile of this species. The present study evaluate heat tolerance in *Martina Franca* (Poitou) Donkeys in the subtropical climate, where even a solar exposure for short period during October is going to induce a heat stress as these animals have black coat and dense hairs being native of cold and temperate region. Effect of heat tolerance on the serum concentrations of some hormone (COR) and blood biochemical indices (CT, TG, Glu, TP, urea and creatinine, the correlations between these parameters in *Martina Franca* (Poitou) was evaluated.

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. All blood metabolites were estimated spectrophotometrically as per the protocols given in the respective kits manufactured by Spinreact. The various parameters estimated, the wavelength for measurement of absorbance, and units of measurement are given in the table below:

Table 1: Various biochemical parameters, the wavelength for measurement of absorbance, and units of measurement

S. No.	Biochemical Parameter	Wavelength (nm)	Units of measurement
Plasma metabolites			
	Lactate	505	mg/dl Converted to mM/l by multiplication with factor 0.1123.
	Creatinine	492	mg/dl
	Urea	340	mg/dl
	Total Protein	540	g/dl
	Albumin	630	g/dl
	Globulin	(Total Protein-Albumin)	g/dl
Plasma Hormone	Cortisol (Calbiotech Human specific and Bluegene Biotech, China horse specific Kit)	EIA in 450nm in a multimode microplate reader (Tecan MPro 200) Calbiotech kits	ng/ml

3. 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.

4. 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 $p < 0.05$.

5. Results and Discussion

The various blood biochemical profile 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 thermo neutral period in winter are presented in the following tables.

Table 2: Effect of heat stress on albumin in *Martina Franca* donkeys (Poitou Jacks)

	A(g/dl)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	1.69 ±0.08^{aA}	2.63 ±0.33^{bAB}	3.09 ±0.46^{bB}	3.09 ±0.46^{bB}
10:00 h	1.78 ±0.15^{abA}	2.68 ±0.09^{bC}	2.09 ±0.06^{aB}	2.09 ±0.06^{aB}
14:00 h	2.08 ±0.11^{bA}	2.15 ±0.25$^{ab A}$	2.97 ±0.16^{bB}	3.71 ±0.41^{bC}
16:00 h	2.05 ±0.08^{bA}	2.00 ±0.04^{aA}	3.81 ±0.05^{cB}	3.16 ±0.26^{bC}

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 thermo neutral climate in winter

Table 3: Effect of heat stress on globulins in *Martina Franca* donkeys (Poitou Jacks)

	G (g/dl)			
	Heat Stress Period			**Thermo- neutral Period**
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	3.25 ±0.92	2.20 ±0.26	2.22 ±0.58	3.27 ±0.73
10:00 h	3.83 ±0.69	2.50 ±0.29	3.02 ±0.78	3.49 ±0.68
14:00 h	4.86 ±1.02^{B}	3.15 ±0.48^{A}	3.76 ±0.63^{AB}	2.28 ±0.58^{A}
16:00 h	4.03 ±0.98	3.76 ±0.96	2.43 ±0.22	2.80 ±0.45

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 thermo neutral climate in winter

Table 4: Effect of heat stress on plasma lactate in *Martina Franca* donkeys (Poitou Jacks)

	LA mM/l			
	Heat Stress Period			Thermo- neutral Period
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	1.15 ±0.15^{aBC}	0.96 ±0.14^{aAB}	1.33 ±0.07^{aC}	0.77 ±0.05^{aA}
10:00 h	1.29 ±0.14^{abB}	1.32 ±0.14^{aB}	1.49 ±0.07^{abB}	0.86 ±0.04^{abA}
14:00 h	1.44 ±0.12^{abB}	1.40 ±0.07^{aB}	1.60 ±0.06^{bcB}	0.98 ±0.04^{bA}
16:00 h	1.57 ±0.10^{bA}	1.89 ±0.23^{bA}	1.74 ±0.09^{cA}	1.00 ±0.05^{bB}

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 thermo neutral climate in winter

Table 5: Effect of heat stress on plasma urea in *Martina Franca* donkeys (Poitou Jacks)

	UREA (mg/dl)			
	Heat Stress Period			Thermo- neutral Period
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	47.15 ±5.30^{aB}	42.16 ±1.56^{aAB}	34.97 ±2.02^{aA}	35.96 ±1.87^{aA}
10:00 h	58.12 ±6.12^{aB}	48.04 ±2.78^{aAB}	51.63 ±5.26^{aAB}	44.91 ±1.53^{bA}
14:00 h	92.05 ±8.43^{bB}	88.89 ±7.00^{bB}	88.40 ±7.57^{bB}	48.92 ±2.48^{bA}
16:00 h	102.27 ±8.49^{bA}	84.48 ±5.06^{bA}	85.30 ±6.10^{bA}	49.23 ±1.42^{bB}

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 thermo neutral climate in winter

Table 6: Effect of heat stress on plasma creatinine in *Martina Franca* donkeys (Poitou Jacks)

	Creatinine (mg/dl)			
	Heat Stress Period			Thermo- neutral Period
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	2.46 ±0.15^{B}	1.75 ±0.06^{aA}	1.76 ±0.06^{aA}	1.64 ±0.10^{A}
10:00 h	2.43 ±0.22^{B}	1.82 ±0.04^{aA}	1.80 ±0.06^{aA}	1.78 ±0.08^{A}
14:00 h	2.78 ±0.21^{B}	2.40 ±0.09^{bB}	2.46 ±0.09^{bB}	1.77 ±0.10^{A}
16:00 h	2.85 ±0.42^{A}	2.42 ±0.07^{bA}	2.43 ±0.09^{bA}	1.56 ±0.09^{B}

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 thermo neutral climate in winter

Table 7: Effect of heat stress on cholesterol in *Martina Franca* donkeys (Poitou Jacks)

	CHOL(mg/dl)			
	Heat Stress Period			Thermo- neutral Period
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	114.01 ±10.88^{Bab}	127.81 ±11.55^{abB}	111.81 ±8.55^{abA}	96.31 ±7.88^{A}
10:00 h	84.20 ±7.67^{a}	93.77 ±11.74^{a}	95.77 ±10.04^{a}	90.09 ±7.21
14:00 h	84.94 ±4.23^{a}	97.95 ±10.71^{a}	90.85 ±10.71^{a}	86.99 ±4.25
16:00 h	140.32 ±14.14^{b}	140.46 ±16.43^{b}	125.26 ±8.65^{b}	103.23 ±5.69

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 thermo neutral climate in winter

Table 8: Effect of heat stress on triglycerides in *Martina Franca* donkeys (Poitou Jacks)

	Triglycerides (mg/dl)			
	Heat Stress Period			Thermo- neutral Period
Diurnal Time Points	**1st day**	**4th day**	**7th day**	**TN**
07:00 h	116.75 ±4.64	114.74 ±5.85	106.19 ±2.59^{a}	118.33 ±4.31
10:00 h	119.58 ±4.38	123.44 ±4.58	116.42 ±3.27^{ab}	122.61 ±5.71
14:00 h	122.50 ±4.82	126.50 ±6.58	120.46 ±4.26^{b}	128.05 ±4.98
16:00 h	120.52 ±5.88	117.95 ±3.38	113.08 ±3.74^{ab}	118.93 ±4.64

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 thermo neutral climate in winter

Table 9: Effect of heat stress on cortisol in *Martina Franca* donkeys (Poitou Jacks)

	Cortisol (ng/ml)		
	Heat Stress Period		Thermo- neutral Period
Diurnal Time Points	**1st day**	**7th day**	**TN**
07:00 h	.	.	.
10:00 h	111.02 ±29.42	85.60 ±23.90	152.30 ±26.96

14:00 h	90.17 ±24.45	106.17 ±29.97	97.52 ±25.75
16:00 h	117.21 ±29.17	104.33 ±26.92	>200

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, 7d: days of exposure from 10AM-2PM; TN: animals kept in similar condition in thermo neutral climate in winter

The heat stress did not induce any significant change in the total protein concentration either within different diurnal time points or between the two climates. However albumin concentration showed uneven trend on different days of the heat stress period (increasing from 07:00h to 1600h on day 1 and decreasing on day 4 and 7). On the thermoneutral day, it increased from 07:00h to 14:00h. Overall protein and albumin levels ranged within the normal range as reported previously by Laus *et al*., 2015 [15]; Burden *et al*., 2016 [8] and Gupta *et al*., 2016 [14]. During heat stress period, total protein, albumin and globulin levels increased as solar radiation increased heat load on the animals at 14:00h and 16:00h except on day 4 in heat stress period in case of albumin. This effect seen in the heat stress period was absent for total protein and globulins on the thermoneutral day in winter. The reason for this increased protein and globulin and to a slight extent albumin is attributed to decreased plasma volume in blood as the water intake was restricted and heat stress increased water loss through sweating (Wenderley *et al*., 2015) [22]. The significant increase in lactate at diurnal time points in the afternoons during the 'heat stress period' is due to the increased activity of LDH enzyme in the tissues of the musculoskeletal, liver and reticuloendothelial system which favours increased lactate production from pyruvate for energy purposes. In Brazilian donkeys, the mean lactate concentration in jacks was 17.30±4.24 mg/dl (Mori *et al*., 2003) [17] which is higher than the values observed in the present study. We observed 17.05±2.05 mg/dl at 16:00h in the heat stress period after solar radiation and a minimum of 6.88±0.42 mg/dl at 07:00h in the winter. The primary cause of increased urea and creatinine levels in plasma of donkeys during heat stress at 14:00h and 16:00h in comparison to 07:00h and also in comparison to that during winter is attributed to combined effect of dehydration and increased with catabolism. Similar findings have been seen also by Alrhman (2016) in Sawari police horses in Khartoum, Sudan. Similar increase has been shown in other studies during heat stressed goats (Urwat *et al*., 2015) [21], camel (Bargaa *et al*., 2006) [7], buffaloes (Gudev *et al.*, 2007) [13]. Higher cholesterol levels were recorded at 07.00h and 16:00h on all the days of study in both thermoneutral and heat stress periods (Table 7). The cholesterol decreased at 10.00h & 14.00h and again increased at 16.00h significantly on day 1, 4 & 7 of heat stress period (Table 7). The trend was similar but non-significant on thermoneutral day. Overall, cholesterol remained higher during heat stress period than on the thermoneutral day at corresponding time points. The cholesterol decreased significantly from 07:00h to 10:00h and 14:00h for the in the heat stress period for the reason that cholesterol in plasma was transported with lipoproteins to the binding sites in the target cells of glucocorticoid synthesis in adrenals and other such glands to combat the stress. Solar radiation at the diurnal time points in the afternoon of the heat stress period was significantly effective to cause a heat load than in the corresponding time points in winter. During stress, 80% of circulating cortisol derives from plasma cholesterol, which is the main precursor of steroid biosynthesis in steroidogenic tissue (Vyroubal *et al*., 2008). The triglyceride levels did not differ significantly at the corresponding time points in the heat stress period and winter, although slightly less triglycerides were observed on day 7 in the Heat stress period. Within a day, on all the days, the triglyceride levels increased in the afternoon hours at 14:00h which was significant on day 7 (Table 8). This could have occurred due to increased synthesis of triglyceride in liver as a result of the increased free fatty acid level in the blood circulation after absorption from the intestines (Ozcan *et al*., 2002) [18]. On the other hand, Assenza *et al* (2016) [4] reported significant increase in TG level after exercise and decrease during recovery period. The cortisol was found to be higher in the morning on first day of heat stress period and Thermoneutral day in winter (Table 9). The higher cortisol observed in this study in the morning upto 10.00 h is due to the increased diurnal secretion of cortisol in the blood stream in the morning hours and lower at about 14:00h-15:00h (Lekeux *et al*., 1991) [16]. The higher cortisol observed at 14:00 and 16:00h is supposed to be due to increased heat accumulation in the donkeys due to solar exposure by activation of the hypothalamo-adrenal axis. Activation of this axis increases cortisol secretion from the adrenals to provide sufficient changes in the energy metabolism to combat heat stress (Atkinson *et al*., 2006 [5], Aggarwal and Upadhyay 2013.

6. Conclusions

The biochemical indices creatinine, urea, lactate, were significantly increased in response to heat stress. Other biochemical responses also showed increase with varying significance on different days of heat stress period. The similar exposure in the thermoneutral climate in the winter elicited a comparatively very low increase in the afternoons in the most of the hemato-biochemical responses. The increased plasma lactate, urea and creatinine, indicated induction of heat stress in these donkeys during afternoons of October in Rajasthan as compared to the winter in December.

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