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Diurnal rhythm of blood biochemical plasma enzyme profile and minerals of sun exposed *Martina franca* jacks in semitropical desert climate

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Abstract
Diurnal rhythm of blood biochemical profile and plasma minerals 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 14:00h. 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 enzyme activities (in U/l for ALP, γGT, AST, ALT and LDH) increased at 14:00h & 16:00h when compared to 07:00h and 10:00h during HS and remained higher than in C. The Mg values increased with diurnal progression of the day and were maximum at 14.00h than at 07.00 & 10.00h. Mg levels were higher in HS than in C. The *Martina Franca* jacks exhibited specific diurnal rhythm of several plasma biochemical constituents specifically related to heat stress. The impact was only transient and reduced when the climatic THI reduced, both with the diurnal time point in day as well as season.

Keywords: *Martina franca* (Poitou), diurnal rhythm, biochemical profile, heat stress, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyl-transferase

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) [1]. Liver condition is reflected in the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyl-transferase while as urea and creatinine are indicators of the basic parameter reflecting kidney function, nutritional status and stress responses (Ghada, 2014) [3]. The season also affects these indices in several species like buffalo (Verma *et al.*, 2000) [8], Holstein heifer (Rasooli *et al*., 2004) [5] and camel (Babeker and Elmansoury, 2013) [1]. 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 biochemical indices in animals, including goats, lamb and equines (Yousef., 1990) [10]. Therefore, this study was planned to study the 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.

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 staying exposed to sun during the period 10:00h to 14:00h in the open sun in their sheds. All serum enzymes 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 plasma enzymes and minerals, the wavelength for measurement of absorbance, and units of measurement.

S. No.	Plasma Enzymes	Wavelength (nm)	Units of measurement
	Plasma enzymes		
1.	Lactate Dehydrogenase	340	IU/l
2.	ALT	340	IU/l
3.	AST	340	IU/l
4.	Alkaline phosphatase	405	IU/l
5.	Gamma amino transferase	405	IU/l
	Plasma Minerals		
6.	Phosphorus	340	mg/dl
7.	Magnesium	340	mg/dl

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 plasma enzymes and minerals 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 2: Effect of heat stress on alkaline phosphatase in *Martina Franca* donkeys (Poitou Jacks)

	ALP(U/L)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	239.07 ±25.82^{aAB}	345.95 ±42.59^{B}	205.33 ±2.72^{A}	323.40 ±51.6^{B}
10:00 h	317.72 ±28.78^{abB}	294.62 ±35.82^{AB}	226.05 ±15.5^{A}	314.42 ±33.5^{B}
14:00 h	409.75 ±54.27^{B}	430.10 ±57.27^{B}	219.63±13.8^{A}	480.33 ±53.3^{B}
16:00 h	404.43 ±23.69^{AB}	461.63 ±99.87^{B}	205.33 ±16.3^{A}	464.20 ±99.3^{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 thermoneutral climate in winter

Table 3: Effect of heat stress on gamma-glutamyl transferase in *Martina Franca* donkeys (Poitou Jacks)

	GGT (U/l)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	21.09 ±1.27^{a}	20.63±0.85^{a}	20.74±1.27^{a}	20.37±0.84^{a}
10:00 h	20.56±0.47^{aA}	29.09±1.63^{bC}	21.15±0.54^{aA}	25.26±1.37^{bB}
14:00 h	32.79±1.77^{bB}	42.77±0.75^{cA}	33.39±1.50^{bB}	30.81±1.61^{cB}
16:00 h	34.18±1.57^{bA}	42.11±0.85^{cB}	31.36±2.80^{bA}	31.67±1.65^{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 4: Effect of heat stress on aspartate aminotransferase in *Martina Franca* donkeys (Poitou Jacks)

	AST(U/L)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	203.10 ±11.83^{a}	191.33 ±6.05^{a}	199.49 ±6.97^{ab}	223.13 ±24.33^{a}
10:00 h	214.47 ±8.06^{a}	209.32 ±5.75^{a}	222.83 ±11.74^{a}	200.38 ±18.19^{a}
14:00 h	251.22 ±7.63^{b}	241.50 ±8.17^{b}	241.31 ±6.37^{a}	243.44 ±28.06^{ab}
16:00 h	259.00 ±3.39^{bA}	247.43 ±5.04^{bA}	251.71 ±9.27^{bA}	296.72 ±14.88^{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 thermoneutral climate in winter

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

	ALT(U/L)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	19.25 ±1.37a	18.28 ±0.90a	17.02 ±0.70a	19.83 ±1.29
10:00 h	19.25 ±1.02a	19.25 ±1.36a	17.79 ±0.60a	20.81 ±1.33
14:00 h	25.66 ±2.08bB	23.43 ±1.23bAB	23.43 ±1.05bAB	19.45 ±1.13A
16:00 h	26.66 ±1.01bB	25.76 ±0.74bB	21.78 ±0.79bA	19.55 ±1.93A

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

	LDH(U/L)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	345.07 ±55.44A	638.46 ±41.85aB	581.40 ±63.22aB	414.52 ±44.60A
10:00 h	374.14 ±47.08A	607.24 ±29.79aB	566.33 ±60.62aB	413.44 ±42.61A
14:00 h	362.84 ±47.75A	867.79 ±114.27bB	784.35 ±73.06bB	379.52 ±57.76A
16:00 h	369.84 ±58.42A	794.58 ±35.97abC	651.38 ±32.12abB	388.68 ±32.40A

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

	P (mg/dl)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	4.65 ±0.21aA	4.50 ±0.09aA	5.87 ±0.57B	4.70 ±0.12A
10:00 h	6.56 ±0.35bA	5.25 ±0.29aB	5.32 ±0.48B	4.62 ±0.34B
14:00 h	9.46 ±0.64cC	5.32 ±0.27aAB	6.29 ±0.55B	4.21 ±0.47A
16:00 h	5.26 ±0.51abAB	6.22 ±0.42bB	5.85 ±0.56AB	4.73 ±0.19A

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

	Mg (mg/dl)			
	Heat Stress Period			Thermo-neutral Period
Diurnal Time Points	1st day	4th day	7th day	TN
07:00 h	2.15±0.22AB	2.61±0.26B	2.25±0.19aB	1.50±0.22A
10:00 h	2.38±0.20B	2.87±0.22B	2.59±0.22abB	1.69±0.25A
14:00 h	2.60±0.20B	3.02±0.21B	2.89±0.12bB	1.96±0.30A
16:00 h	2.21±0.24A	2.56±0.16A	2.48±0.15abA	1.58±0.28B

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 increased alkaline phosphatase activity seen in the blood plasma of donkeys especially during the heat stress period in afternoons (14:00h to 16:00h) than in winter is possibly attributed to its role in metabolism (Table 2). The alkaline phosphatase enters the blood stream during stressful conditions from the skeletal tissues. Additionally, it can also enter from liver (Karaca 1999). Higher ALP activity might also be due to its involvement in regulating cell division, growth, homeostasis and energy generation in the animal body during heat stress (Kamal *et al.,* 2016) [4]. Bagha (2007) also presented similar increase in ALP during heat stress in dairy cows. However, contrasting results were reported by Sejian *et al.* (2010) [6] in heat stress in malpura ewes. Concurrent rise of ALT, ALP and γGT at 14:00h as compared to 07:00h in the 'heat stress period was observed (Table 4, 5). It may be their primary source as liver is the prime organ that is most affected during heat stress in the afternoons. However, the intensity of this affect was not very high and the moderate increase showed that the donkeys suffered a moderate heat stress. Vrankovic *et al.* (2015) [9] observed a significantly increased GGT level in the summers than in the winters in Holstein Stallions in Croatia. They associated this increased GGT with the variety of hepatic disorders, such as toxic hepatic factors, subclinical hepatopathy and hyperlipemia as reported earlier by Aoki and Ishil (2011). AST showed increasing during the heat stress period from 07:0h to maximum at 16:00h (Table 3). Diurnally the same increase was observed during the thermoneutral season. Correspondingly, the ALT activity did not increase during the winter from 07:00h to 16:00h. This indicates that the heat stress affected ALT activity rather than AST. Therefore, the primary organ affected is the liver rather than musculoskeletal system during the heat stress. Vrankovic *et al.* (2015) [9] also showed that AST was significantly higher in summer than in winter while ALT activity was higher in winter than in summer in Holstein Stallions. No other study has reported any effect of heat stress on the ALT and AST activity. The significantly increased LDH activity from 07:00h to 14:00h and 16:00h in the heat stress period in comparison to the thermoneutral winter is due to increased stress imposed on both the liver and musculoskeltal system (Table 6). As increased GGT and ALT indicated a stressed liver of the donkeys during this period, it is most probable that LDH activity increased in liver along with that of red blood cells and musculoskeltal system. Such a change is the most probable reason for its release from these tissues into the blood stream as has been reported previously in humans (Patel *et al*., 1991) [4] and buffaloes (Singh *et al*., 2011) [7]. The increased LDH also plays an important role in myostasis (Vrankovic *et al*., 2015) [9]. The significantly increased phosphorus and magnesium during heat stress period in comparison to the thermoneutral period in donkeys in the present study is possibly attributed to their increased requirement for several energy requiring metabolic processes and enzymatic reactions during the heat stress. The heat stress increased from 07:00h to 14:00h due to solar radiation and additionally the stress was also caused due to feed and water restriction from 10:00 h to 14:00h (Table 7, 8). This has probably led to increased mobilization of P and Mg from skeletal system bones into the blood stream. (Vrankovic *et al*., 2015) [9] reported that Mg and P level was significantly higher in summer than in winter which depends on the P availability in feed and fodder in a particular season and its absorption in the gastro-intestinal tract.

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