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Alterations in Biochemical, Hematological and Physical Parameters in Endurance Horses with Metabolic Crisis

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Abstract: Metabolic crisis is the complex and detrimental physiological changes observed in eliminated endurance horses during endurance events. Therefore, this study aims to appraise the alterations in biochemical, hematological and physical parameters of endurance horses with metabolic crisis. About 174 endurance horses between the ages of 6-20 years were examined to be clinically healthy pre-ride and 115 were eliminated post-race as a result of metabolic crisis. Blood sample was collected and physical examinations were conducted at pre and post ride. The blood sample was analyzed for biochemical and hematological parameters. The parameters were assessed using one-way analysis of variance. Metabolic crisis leads to significant increases in heart rate ($p<0.0001$), capillary refill time ($p<0.005$) and significant decrease in gut motility (0.006). There were significant increases in RBC, Hb and PCV at ($p<0.0001$). There were also significant increases in WBC ($p<0.0002$), band neutrophil ($p<0.012$), segmented neutrophil ($p<0.0001$) and monocytes ($p<0.011$). Significant decreases were seen in sodium ($p<0.0196$) and chloride ($p<0.0001$). Significant increases were also assessed in plasma protein ($p<0.0001$), albumin ($p<0.0001$), lactate ($p<0.0001$), creatine kinase ($p<0.0033$), uric acid ($p<0.0017$), gamma glutamyl transferase ($p<0.0472$), urea ($p<0.0014$), triglyceride ($p<0.0041$), total protein ($p<0.0001$), icterus index and bilirubin ($p<0.0024$). Thus, these changes could be indicative of metabolic crisis and poor performance in endurance horses.

Key words: Endurance horses, metabolic crisis, biochemical, hematological, physical

INTRODUCTION

Metabolic crisis is the excessive and or inadequate supply of the physiological requirements of tissues and organs during low-intensity and prolong aerobic and anaerobic race. The biochemical, hematological and physical parameters are required by the ailing tissues and organs in order to sustain the body in a homeostatic state (Foreman, 1998; Castejon *et al*., 2006). Endurance is the ability to hold up exhaustion and fatigue psychogenically and physically (Piccione *et al*., 2010). Endurance race is a function of both maximal aerobic and anaerobic ability (McMiken, 1983; Piccione *et al*., 2010).

The ability of a horse to withstand rigors of race is gauge through measurements of its heart rate and respiratory rate (Asheim *et al*., 1970; Cottin *et al*., 2006; Bashir and Rasedee, 2009; Zobba *et al*., 2011). An excessive sweat loss of water and electrolytes during maximal aerobic and anaerobic trials at elevated rates progressively leads to metabolic alkalosis (Rose *et al*., 1979; Poso-Reeta *et al*., 2004; McKeever, 2004; Piccione *et al*., 2010) and leads to fatigue and muscle weakness and reduces the thirst response to dehydration (Francesca *et al*., 2007). Increased capillary refill time and decreased skin elasticity is indicative of dehydration (Zobba *et al*., 2011).

Elevated levels of RBC, Hb and PCV are due to splenic contraction releasing large number of erythrocytes into the circulation. This homeostatic adaptation induces an increase in the oxygen carrying capacity of blood to the ailing muscle tissues an elevated aerobic ability and a decrease in lactate production (Munoz *et al*., 1999; Kearns *et al*., 2002; McKeever *et al*., 1993; Kim *et al*., 2005; Adamu *et al*., 2010; Zobba *et al*., 2011).

During endurance events, leucocytosis is due to neutrophilia and lymphopenia as a result of redistribution

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of neutrophils from the peripherial pool in to the circulation and splenic contraction this depends on the aerobic or anaerobic nature of the exercise and the associated stress factors (Snow *et al*., 1983; Rose and Hodgson, 1994; Kastner *et al*., 1999; Piccione *et al*., 2010; Zobba *et al*., 2011).

A high level of plasma proteins is indicative of dehydration status in endurance horses especially of those eliminated from the race due to metabolic crisis (Castejon *et al*., 2006). Creatine kinase is obtained in high concentration in excitable muscle tissues requiring high fluctuating energy (Rose and Hodgson, 1994) and is associated with muscular damage (Irving *et al*., 1990). Gamma Glutamyl Transferase (GGT) in its normal range may be an enzyme related to oxidative stress (Lee *et al*., 2004; Yang *et al*., 2007). Oxidative stress is accountable for many metabolic crises (Lee and Jacobs, 2005; Fernando *et al*., 2009).

The tendency for the occurrence of heat stress increases when endurance event is conducted under unpleasant ambient conditions, especially if the temperature and humidity are high and as a result of insufficient conditioning protocol of horses (Hodgson *et al*., 1994; Marlin *et al*., 2001; Jeffcott *et al*., 2009).

Increases in uric acid due to prolonged disproportionate energy dispensation could lead to metabolic crises during endurance events in horses (Castejon *et al*., 2006). Metabolic crisis is the excessive and or insufficient provision of the physiological needs of tissues and organs during prolong aerobic and anaerobic trials and these manifest in the forms of exhaustion, fatigue, severe dehydration, oxidative and heat stress. Thus, this study aims to appraise the alterations in biochemical, hematological and physical parameters of endurance horses with metabolic crises.

MATERIALS AND METHODS

One hundred and seventy four Arabian horses participated in an endurance competition of 40, 80 and 120 km and one hundred and fifteen of them were eliminated due to metabolic crises and these were used in this study. The age and body weight of the horses ranged between 6-20 years and 350-450 kg, respectively. Veterinary inspection was conducted at pre and post-ride of all the competing horses and physical parameters recorded.

The physical parameters evaluated were the resting heart rate (44-64 = normal, 65-70 = high, 71-90 = very high); mucous membrane (1 = normal, 2 = moderately congested, 3 = severe congestion) capillary refill time (1 sec = normal, 2 sec = moderate, 3 sec = severe) skin recoil (1 = normal skin, 2 = moderate dehydration, 3 = severely dehydrated) gut motility or sound (1 = normal, 2 = moderate, 3 = no motility or sound) and gait (1 = normal, 2 = moderate lame, 3 = severely lame). The horses were also observed for soreness or injuries on the back, withers, girth area, body or distal extremities.

The ambient temperature (°C) and relative humidity (%) were also recorded throughout the period of the endurance events at an interval of 30 min using Casella® thermo-hygrometer.

Blood samples were obtained from all the horses via jugular veinipucture into Ethyl Diaminotetra-Acetic Acid (EDTA) vacutainer tubes for hematological and into heparinized vacutainer tubes for biochemical analysis. The erythrocyte, leucocyte, thrombocyte, differential leucocyte count, hemoglobin concentration, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) were analyzed using Cell DYN 3700, Abbot® while the Packed Cell Volume (PCV) was analyzed using Hettich-Hematocrit 210 and Hawksley microhematocrit reader®. The plasma electrolyte and biochemical, sodium, potassium, chloride, calcium, urea, creatinine, bilirubin, Aspartate Transaminase (AST), Creatine Kinase (CK), glucose, lactate, total protein, albumin and globulin concentrations were determined with chemistry analyzer (Hitachi 920®) using standard diagnostic kits (Roche®). The data was analyzed using one way analysis of variance using the Statistical Software Package JMP-9. Analyses were considered as significant at $p<0.05$.

RESULTS AND DISCUSSION

One hundred and seventy four Arabian horses participated in an endurance competition of 40, 80 and 120 km and one hundred and fifteen of them were eliminated due to metabolic crises. The age and body weight of the horses ranged between 6-20 years and 350- 450 kg, respectively.

Table 1 shows the pre and post race physical parameters of endurance horses that completed the race successfully and those that had metabolic crises showing significant increases in heart rate ($p<0.0001$), capillary refill time ($p<0.005$) and a significant decrease in gut motility ($p<0.006$).

Significantly medium correlation was found between gut motility and capillary refill time ($r = 0.3617$; $p<0.0001$). While there were significantly weak correlations between capillary refill time and heart rate ($r = 0.2700$; $p<0.0006$), gut motility and heart rate ($r = 0.2321$; $p<0.0031$).

Table 2 shows the pre and post race erythrocytes of endurance horses that completed the race successfully

Table 1: Physical parameters of endurance horses

	Completed race		Metabolic crisis	
Parameters	Pre-ride	Post-ride	Pre-ride	Post-ride
Heart rate (bpm)	39.39±4.9^C	57.50±7.7^B	43.14±7.8^C	67.18±12.8^A
Mucus membrane (sec)	1.06±0.2^B	1.41±0.5^A	1.14±0.4^{A,B}	1.43±0.600^A
Capillary refill time (sec)	1.03±0.1^B	1.29±0.4^B	1.07±0.2^B	1.82±0.800^A
Gut motility (sec)	1.17±0.4^B	1.48±0.7^B	1.00±0.0^B	1.86±0.900^A
Gait	1.00±0.0^B	1.09±0.3^B	1.00±0.0^B	1.50±0.700^A

All values are expressed as mean±SD. $^{A-C}$Within each row; means with different superscript are significantly different at p<0.05. Heart rate (44-64 = normal, 65-70 = high, 71-90 = very high); mucous membrane (1 = normal, 2 = moderately congested, 3 = severe congestion); capillary refill time (1 = normal, 2 = moderate, 3 = severe); skin recoil (1 = normal, 2 = moderate dehydration, 3= severely dehydrated); gut motility and sound (1 = normal, 2 = moderate, 3 = no motility or sound); gait (1 = normal, 2 = moderate lame, 3 = severe lame)

Table 2: Erythrocytes of endurance horses

	Completed race		Metabolic crisis	
Parameters	Pre-ride	Post-ride	Pre-ride	Post-ride
Erythrocytes ($\times10^{12}$ L^{-1})	7.88±1.00^B	10.67±1.500^A	7.38±0.70^B	10.34±1.700^A
Hb (g L^{-1})	124.31±13.3^B	168.19±20.30^A	121.14±11.5^B	162.19±22.70^A
PCV (L L^{-1})	0.32±0.10^B	0.47±0.100^A	0.33±0.03^B	0.47±0.100^A
MCV (f L^{-1})	42.77±3.40^B	45.31±4.700^A	44.43±2.10^{A,B}	45.81±4.020^A
MCHC (g L^{-1})	370.42±20.4^A	362.90±39.70^{A,B}	369.00±6.60^{A,B}	346.10±58.70^B

All values are expressed as mean±SD. ^{A,B}Within each row; means with different superscript are significantly different at p<0.05

and those that had metabolic crises showing significant increases in RBC, Hb and PCV at ($p<0.0001$). Significantly strong correlation were found between Hb and RBC ($r = 0.6274$; $p<0.0001$), PCV and RBC ($r = 0.7465$; $p<0.0001$), PCV and Hb ($r = 0.5580$; $p<0.0001$).

Table 3 shows the pre and post race leucocytes and thrombocytes counts of endurance horses that completed the race successfully and those that had metabolic crises showing significant increases in leucocytes (WBC) ($p<0.0002$), band neutrophil ($p<0.012$), segmented neutrophil ($p<0.0001$) and monocytes ($p<0.011$).

Significantly strong correlations was found between segmented neutrophils and White Blood Cells (WBC) ($r = 0.9058$; $p<0.0001$), monocytes and WBC ($r = 0.7759$; $p<0.0001$), monocytes and segmented neutrophils ($r = 0.7562$; $p<0.0001$) while significantly medium correlation was found between monocytes and band neutrophils ($r = 0.3977$; $p<0.0001$) and a significantly weak correlation was observed between band neutrophils and WBC ($r = 0.4702$; $p<0.0001$) segmented and band neutrophils ($r = 0.4887$; $p<0.0001$).

Table 4 shows the pre and post race electrolytes concentrations of endurance horses that completed the race successfully and those that had metabolic crises showing significant decreases in sodium ($p<0.0196$) and chloride ($p<0.0001$). There was a significantly strong correlation between chloride and sodium ($r = 0.5038$; $p<0.0001$).

Table 3: Leucocytes and thrombocytes counts of endurance horses

	Completed race		Metabolic crisis	
Parameters	Pre-ride	Post-ride	Pre-ride	Post-ride
Leucocytes	5.69±1.40^B	9.93±3.30^A	5.53±1.600^B	10.19±3.50^A
Band neutrophils	0.09±0.04^B	0.31±0.20^A	0.07±0.040^B	0.34±0.30^A
Segmented neutrophils	3.40±0.90^B	7.74±2.90^A	3.59±1.900^B	7.45±2.60^A
Lymphocytes	1.66±0.50^A	1.39±0.80^A	2.10±0.500^A	1.59±1.90^A
Monocytes	0.29±0.09^B	0.45±0.20^A	0.29±0.080^B	0.45±0.20^A
Eosinophils	0.18±0.10^A	0.12±0.10^A	0.09±0.100^A	0.16±0.20^A
Basophils	0.06±0.05^A	0.05±0.06^A	0.08±0.030^A	0.05±0.10^A
Thrombocytes	63.58±44.3^C	122.75±46.2^A	76.87±55.80^{B,C}	95.96±52.4^B

All values are expressed as mean±SD. $^{A-C}$Within each row; means with different superscript are significantly different at p<0.05

Table 4: Electrolytes concentrations of endurance horses

Parameters	Completed race		Metabolic crisis	
(mmol L^{-1})	Pre-ride	Post-ride	Pre-ride	Post-ride
Sodium	139.77±2.7^A	136.52±3.9^B	138.13±1.8^{A,B}	133.62±5.6^C
Potassium	4.13±1.1^{A,B}	4.52±0.8^A	4.09±0.4^{A,B}	3.79±1.5^B
Chloride	101.43±2.6^A	86.64±5.4^C	101.10±1.9^A	89.62±6.7^B
Calcium	3.07±0.2^A	3.26±0.4^A	3.14±0.3^A	3.20±0.5^A

All values are expressed as mean±SD. $^{A-C}$ within each row; means with different superscript are significantly different at p<0.05

Table 5 shows the pre and post race biochemical parameters of endurance horses that completed the race successfully and those that had metabolic crises showing significant increases in plasma protein ($p<0.0001$), albumin ($p<0.0001$), lactate ($p<0.0001$), creatine kinase ($p<0.0033$), uric acid ($p<0.0017$), gamma glutamyl transferase ($p<0.0472$), urea ($p<0.0014$), triglyceride ($p<0.0041$), total protein ($p<0.0001$), icterus index and bilirubin ($p<0.0024$).

Significantly strong correlations were observed between albumin and plasma protein ($r = 0.5780$; $p<0.0001$), total protein and plasma protein ($r = 0.7755$; $p<0.0001$), total protein and albumin ($r = 0.5938$; $p<0.0001$), lactate and plasma protein ($r = 0.5110$; $p<0.0001$), lactate and triglyceride ($r = 0.6742$; $p<0.0001$), uric acid and plasma protein ($r = 0.5140$; $p<0.0001$), uric acid and triglyceride ($r = 0.7510$; $p<0.0001$), uric acid and lactate ($r = 0.7283$; $p<0.0001$).

Table 6 shows the ambient temperature (°C) and relative humidity (%) recorded at an interval of 30 min throughout the period of the endurance events mean±standard deviation was used.

Alterations in the values of biochemical, hematological and physical parameters in endurance horses with metabolic crises were associated to low-intensity and prolonged racing trial under aerobic conditions and complex interactions between environmental conditions, speed, nature of the tracts, horse and riders attitude, training protocols and adaptation (Lekeux *et al*., 1991; Foreman, 1998; Barton *et al*., 2003) which manifested in the forms of exhaustion, fatigue, severe dehydration, muscular

Table 5: Biochemical parameters of endurance horses

	Completed race		Metabolic crisis	
Parameters	Pre-ride	Post-ride	Pre-ride	Post-ride
Urea (mmol L^{-1})	5.39 ± 1.100^{C}	8.77 ± 1.9000^{A}	5.31 ± 1.300^{C}	7.61 ± 1.9000^{B}
Bilirubin (µmol L^{-1})	29.88 ± 8.400^{B}	50.86 ± 17.070^{A}	29.43 ± 20.40^{B}	49.38 ± 17.700^{A}
AST (U)	328.62 ± 110.8^{B}	$562.09\pm413.70^{A, B}$	$349.04\pm97.00^{A, B}$	634.81 ± 518.80^{A}
CK (U L^{-1})	245.88 ± 159.7^{B}	1405.27 ± 763.10^{A}	348.86 ± 111.4^{B}	1510.79 ± 1165.9^{A}
Glucose (mmol L^{-1})	6.47 ± 0.700^{A}	4.26 ± 2.7000^{C}	$6.73\pm0.800^{A, B}$	5.48 ± 2.2000^{B}
Lactate (mmol L^{-1})	1.07 ± 0.200^{C}	8.19 ± 2.9000^{A}	1.00 ± 0.100^{C}	6.09 ± 2.8000^{B}
Total protein (gL^{-1})	68.15 ± 8.500^{C}	78.42 ± 8.8000^{B}	68.43 ± 4.700^{C}	82.25 ± 8.9000^{A}
Albumin (gL^{-1})	26.92 ± 3.800^{B}	35.48 ± 5.4000^{A}	27.73 ± 4.500^{B}	36.20 ± 5.0000^{A}
Plasma protein (gL^{-1})	63.92 ± 4.200^{B}	79.58 ± 8.3000^{A}	65.14 ± 5.400^{B}	78.23 ± 8.9000^{A}
Icterus index (Unit)	18.27 ± 4.700^{C}	55.77 ± 22.700^{A}	17.86 ± 4.900^{C}	40.40 ± 20.200^{B}
Fibrinogen (sec)	2.46 ± 0.600^{A}	3.18 ± 3.0000^{A}	2.14 ± 0.400^{A}	2.60 ± 0.9000^{A}
GGT (U L^{-1})	14.69 ± 4.700^{C}	23.35 ± 6.1000^{A}	14.43 ± 2.900^{C}	18.76 ± 5.7000^{B}
Triglyceride (mmol L^{-1})	0.26 ± 0.100^{B}	0.67 ± 0.400^{A}	0.25 ± 0.100^{B}	0.59 ± 0.3000^{A}
Uric acid (µmol L^{-1})	6.96 ± 5.600^{B}	74.85 ± 50.500^{A}	10.1 ± 5.3000^{B}	66.21 ± 52.000^{A}

All values are expressed as mean±SD. $^{A-C}$Within each row; means with different superscript are significantly different at $p<0.05$. AST = Aspartate Transaminase; CK = Creatine Kinase

Table 6: Ambient temperature (°C) and relative humidity (%)

Ambient temperature (°C)	Relative humidity (%)
29.06±1.10	71.73±4.05

All values are expressed as mean±SD

weaknesses, muscle enzymes leakage, heat stress, oxidative stress and drastically decreased performance.

In the present study, statistical increases were seen in the values of heart rate and capillary refill time and a significant decrease in gut motility. The increased in heart rate and delayed recovery time post race was indicative of metabolic crises and subsequent elimination from the race thus, the heart rate was perceived as indicator of fitness level in equine endurance events as reported by Asheim *et al.* (1970), Cottin *et al.* (2006), Bashir and Rasedee (2009) and Zobba *et al.* (2011). Increased capillary refill time and decreased skin elasticity and gut motility are indicative of severe dehydration, heat stress, fatigue, exhaustion, muscle enzyme leakage and onset of oxidative stress (Hodgson *et al.*, 1994; Marlin *et al.*, 2001; Lee *et al.*, 2004; Lee and Jacobs, 2005; Yang *et al.*, 2007; Fernando *et al.*, 2009; Jeffcott *et al.*, 2009; Harold, 2010; Zobba *et al.*, 2011) which is in consonant with the findings of this study.

Furthermore, this present study indicated significant increases in RBC, Hb and PCV. The increases observed in RBC in the study were as a result of splenic contraction releasing large number of erythrocytes into the peripheral blood circulation and caused increased oxygen carrying capacity of blood to the ailing muscle tissues and which lead to an elevated aerobic capacity and subsequent decrease in lactate production (Munoz *et al.*, 1999; Kearns *et al.*, 2002; McKeever *et al.*, 1993; Kim *et al.*, 2005, Adamu *et al.*, 2010; Zobba *et al.*, 2011) due to glycolytic flux (Myburgh, 2003). In endurance races, stress and fatigue are vividly articulated by alterations in circulating erythrocytes (Benamou-Smith *et al.*, 2006).

Similarly, increases in PCV after endurance race could be due to fluid shift but the mainstay effect is as a result of the release of concentrated erythrocytes from the spleen (Kastner *et al.*, 1999).

The present study revealed a significant leucocytosis which was dependent on the intensity, duration and the stress levels of the endurance horses experiencing metabolic crises and also due to splenic contraction and these findings relate well with those of (Snow *et al.*, 1983; Kastner *et al.*, 1999; Wong *et al.*, 1992; Piccione *et al.*, 2010; Zobba *et al.*, 2011). These changes in the white blood cells are also seen during sub-maximal strenuous endurance race (Snow *et al.*, 1983) leading also to neutrophilia (Rose and Hodgson, 1994). The leucocytosis observed in this study could be due increase in neutrophils and monocytes number as a result of acute intense endurance race as reported by Benschop *et al.* (1996), Gabriel and Kidermann (1997) and Ceddia *et al.* (1999).

Moreso, there were significant increases in sodium and chloride concentrations in the present study post race which may be due to excessive loss of these electrolytes from sweat during intense endurance rides resulting in the weaknesses and metabolic crises observed and this findings was equally observed in the study conducted by Rose *et al.* (1979), Poso-Reeta *et al.* (2004), McKeever (2004) and Piccione *et al.* (2010). Hyponatremia caused swelling or edema in the brain with a resultant fatal effect in a study conducted on marathon runners in human (Davis *et al.*, 2001).

Significant increases in plasma protein, albumin, lactate, creatine kinase, uric acid, gamma glutamyl transferase, urea, triglyceride, total protein, icterus index and bilirubin were assessed in the endurance horses with metabolic crises in the present study and these alterations could be as a result of severe dehydration, exertional rhabdomyolysis, accumulation of free radicals and extended disproportionate energy levels and a similar findings were reported in the research of Tullson and Terjung (1991), Valberg *et al.* (1993), Tullson *et al.* (1995), Foreman (1998), Mastaloudis *et al.* (2004), Lee *et al.* (2004), Lee and Jacobs (2005), Yang *et al.* (2007) and Fernando *et al.* (2009). In this study, there were strong correlations between uric acid and plasma protein, lactate, heart rate, triglycerides and erythrocytes and a similar findings were reported by Castejon *et al.* (2006) and Zobba *et al.* (2011).

This present study was conducted under ambient temperature of 29.06±1.1 (°C) and relative humidity of 71.73±4.05 (%) the high relative humidity under this study could possibly contribute to the metabolic crises due to possible anhidrosis Bashir and Rasedee (2009) thereby increasing the core body temperature (Hodgson *et al.*, 1994) and redistribution of blood flow with resultant consequences on muscles, oxygen uptake and metabolism (Marlin *et al.*, 2001). This situation is harsh and exhausting on horses (Jeffcott *et al.*, 2009).

CONCLUSION

The alterations in biochemical, hematological and physical parameters of endurance horses with metabolic crises could be used as indicators of metabolic crises and poor performance in endurance horses.

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