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Effects of Race Distance on Physical, Hematological and Biochemical Parameters of Endurance Horses

[1]Lawan Adamu, [2]Noraniza Mohd Adzahan, [3]Rasedee Abdullah and [2]Bashir Ahmad
[1]Department of Veterinary Medicine, Faculty of Veterinary Medicine,
University of Maiduguri, PMB1069, Borno State, Nigeria
[2]Department of Veterinary Clinical Studies,
[3]Department of Veterinary Pathology and Microbiology,
Faculty of Veterinary Medicine, University Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia

Abstract: Problem statement: Endurance horses are generally exposed to severe stress during endurance competitions. These horses are however conditioned to cover the long distances of the competitions at moderate speeds. **Approach**: This study was conducted in Malaysia to determine the post-race hematological, blood electrolyte and biochemical and physical parameters of horses of 40, 80 and 120 km endurance race categories and eliminated and horses that completed the races with good performance. Whole blood, plasma and serum samples were collected after each race. **Results**: After physical examination 7 (9.72%) 120 km-, 48 (66.67%) 80 km- and 17 (23.61%) 40 km-race horses were eliminated. Eight horses, all from the 80 km-race category completed the race with good performance. The mean heart of the good performance horses (74.2±13.9 beats min^{-1}) was higher than that of the eliminated 40 km- (68.2±14.7 beats min^{-1}), 80 km-race (62.9±9.7 beats min^{-1}) horses. The blood lactate concentration of good performance horses was 9.2±2.2 mmol L^{-1}, which was significantly higher than in the 40 km- (6.0±2.9 mmol L^{-1}), 80 km- (6.7±3.2 mmol L^{-1}) and the 120 km-race (6.4±1.6 mmol L^{-1}) horses. The blood glucose concentration was lower in the good performance horses (1.6±0.9 mmol L^{-1}) than the eliminated 40 km- (5.7±1.9 mmol L^{-1}), 80 km- (4.3±2.4 mmol L^{-1}) and the 120 km-race (5.0±1.4 mmol L^{-1}) horses. **Conclusion**: The study showed that eliminated horses exhibited poorer glucose utilization than good performance horses, which may have resulted in poor lactate production. Thus the blood lactate and glucose concentrations of horses during training may be used to predict their performance in endurance races.

Key words: Hematological and biochemical parameters, physical parameters, glucose, endurance horses, plasma electrolyte, Lactate Dehydrogenase (LDH), Ethyl Diaminotetra-Acetic Acid (EDTA), Aspartate Transaminase (AST)

INTRODUCTION

Endurance horses undergo severe stress during the course of a competitive ride. These horses are trained and conditioned to perform over long distances at moderate speeds. When conditioning a horse for long distance competitions, the training program must be designed and monitored to match the specific exercise type and intensity of competitive endurance riding (Linder *et al*., 2006). The major physiological adaptations that can directly influence exercise capacity and stamina of endurance horses include the efficiency of gas exchange, oxygen uptake and delivery to the exercising muscles. The working muscle of endurance horses depends on aerobic metabolism of its glycogen stores, blood fatty acids and volatile fatty acids from hindgut fermentation, heart size and capacity to deliver large volumes of blood to the tissue.

Determination of the fitness or exercise tolerance of a horse is by assessment, through physical examination, of heart and respiratory rates (Cottin *et al*., 2006; Bashir and Rasedee, 2009). Hematological and biochemical changes may also be evaluated by obtaining the post-ride blood samples (Valberg, 2009). Post-exercise blood

Corresponding Author: A. Rasedee, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel: 603 89468295 Fax: 603-89468333

lactate concentration are sometimes used to indicate fitness of the horse. As fitness increases, post-exercise blood lactate concentrations of the horse should decrease. In fact, maximal blood lactate steady state concentration and anaerobic threshold have been shown to predict training and long distance race performances (Gondim *et al*., 2007). In endurance races, stress and fatigue are clearly expressed by changes in circulating erythrocyte and leucocyte numbers and in creatinine concentration of the horses. Tissue remodeling can also occur in endurance races and this is seen as changes in plasma fibrinogen, urea, proteins and creatine kinase (Benamou-Smith *et al*., 2006). Endurance horses require calcium for muscle contractions and inadequate plasma levels of calcium during strenuous exercise can cause metabolic problems and failures, including synchronous diaphragmatic flutter. However, high blood calcium concentration is undesirable because it may increase the frequency of thumps during endurance competitions (Lewis, 1995). In tying-up, horses exhibit elevated muscle enzymes, Creatine Kinase (CK), aspartate aminotransferase (AST) and Lactate Dehydrogenase (LDH) (Hodgson and Rose, 1994).

The objective of this study was to determine the effect of endurance riding distances on the hematological, biochemical and physical parameters and the differences in level of these parameters between eliminated horses those that completed races with good performance.

MATERIALS AND METHODS

Subjects: Eighty-one endurance horses that participated in three endurance competitions each consisting of 120, 80 and 40 km-race distances were sampled. Among these, 72 horses were eliminated and they comprise of 17, 57 and 7 horses that participated in 40, 80 and 120 km-races, respectively.

Veterinary inspection: Veterinary inspection was conducted after each leg of the race on all 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 limp, 3 = severe limp). The horses were also observed for soreness or injuries on the back, withers, girth area, body or distal extremities.

Sampling: Blood samples were obtained from all horses, anticoagulated with Ethyl Diaminotetra-Acetic Acid (EDTA) for hematological analysis and lithium heparin for biochemical analysis. The hematological parameters determined were erythrocyte, leucocyte thrombocyte counts and hemoglobin concentration (Cell DYN 3700, Abbot) and hematocrit (PCV) (Hettich-Hematocrit 210 and Hawksley microhematocrit reader) and differential leucocyte count, 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 by chemistry analyser (Hitachi 920) using standard diagnostic kits (Roche).

RESULTS

Seventy-two horses were eliminated from the endurance competition. Only nine horses managed to complete the race without abnormal signs and all were from the 80 km distance category. All horses from the 40 and 120 km distance categories were eliminated because of metabolic or physical ailments. Horses that completed the races with good performance showed a higher mean heart rate and more congested mucous membrane than those that were eliminated (Table 1).

The hematology, plasma/serum electrolyte and biochemical parameters are presented in Table 2-5. The most significant change in hematology and plasma biochemistry parameters were lactate concentrations which were significantly higher in good performance horses that those eliminated from the endurance race. On the other hand the plasma glucose concentrations were lower in the good performance than eliminated horses. There was no remarkable change in other parameters.

DISCUSSION

Equine sports, particularly endurances, are gaining popularity in Malaysia, with races being conducted almost every month of the year. However, the population of horses that are eligible to participate in these races is still small, resulting in the same horses being used in most of the races. This may have resulted in these horse being inappropriately conditioned, consequently causing a large percentage of these horses eventually being eliminated because of various metabolic and physical disorders.

Table 1: Post-endurance race physical parameters of horses

	Endurance race distance (km)			
	Eliminated			Good Performance
Parameter	40 (n = 17)	80 (n = 48)	120 (n = 7)	80 (n = 9)
Heart rate (beats min^{-1})	68.2^{a}±14.7	65.2^{a}±13.8	62.9^{a}±9.7	74.2^{b}±13.9
Mucous membrane	1.5^{a}±0.5	1.5^{a}±0.6	1.7^{a}±0.8	2.0^{b}±0.7
Capillary refill time (sec)	1.9±0.8	1.8±0.7	1.6±0.8	1.8±0.8
Skin recoil	1.6^{a}±0.6	1.6^{a}±0.7	2.0^{b}±0.8	2.1^{b}±0.9
Gut motility	1.6^{a}±0.6	1.6^{a}±0.8	1.0^{b}±0.0	$1.3^{a,b}$±0.5
Gait	1.6±0.7	1.6±0.7	1.6±0.7	1.3±0.7

All values are expressed as mean±Std Dev. ^{a,b}: Within each row, means with different superscripts 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 limp, 3 = severe limp)

Table 2: Post-endurance race erythrocyte parameters of horses

	Endurance race distance (km)			
	Eliminated			Good Performance
Parameter	40 (n =17)	80 (n = 48)	120 (n = 7)	80 (n = 9)
Erythrocytes (x10^{12} L^{-1})	9.7±1.1	9.9 ±1.7	9.6±1.1	9.9±0.70
Hb (g L^{-1})	162.8±15.4	157.4 ±16.6	162.6±12.4	169.3±11.4
PCV (L L^{-1})	0.5±0.05	0.5±0.06	0.5±0.03	0.5±0.04
MCV (fL)	47.8±2.9	53.1±43	47.6±2.5	47.2±1.20
MCHC (g L^{-1})	333.6±76.5	346.5±51.3	349.0±9.9	360.6±9.40

All values are expressed as mean±Std Dev. ^{a,b}: Within each row, means with different superscripts are significantly different at p = 0.05.

Table 3: Post-endurance race leucocyte and thrombocyte counts of horses

	Endurance race distance (km)			
	Eliminated			Good Performance
Parameter (x10^{9} L^{-1})	40 (n=17)	80 (n=48)	120 (n=7)	80 (n = 9)
Leucocytes	9.4±2.5	9.7±3.5	7.8± 4.1	8.9±2.8
Band Neutrophils	0.3±0.2	0.3±0.2	0.3±0.04	0.3±0.2
Segmented Neutrophils	6.9±2.0	6.8±2.1	5.8±3.5	6.8±2.7
Lymphocytes	1.4±0.5	1.3± 0.5	1.3± 0.5	1.1±0.3
Monocytes	0.4±0.1	0.4±0.1	0.3±0.1	0.4±0.1
Eosinophils	0.2±0.1	0.1±0.09	0.05±0.07	0.1±0.06
Basophils	0.15±0.1	0.14±0.1	0.09±0.06	0.15±0.06
Thrombocytes	115.1^{a}±37.4	97.1^{a}±34.0	102.7^{b}±75.6	114.9^{a}±40.6

All values are expressed as mean±Std Dev. ^{a,b,c}: Within each row, means with different superscripts are significantly different at p = 0.05

Table 4: Post-endurance race plasma electrolyte concentrations of horses

	Endurance race distance (km)			
	Eliminated			Good Performance
Electrolyte (mmol/L)	40 (n = 17)	80 (n = 48)	120 (n = 7)	80 (n = 9)
Sodium	137.0^{a}±7.2	131.3^{b}±4.2	130.9^{b}±3.3	134.8±4.3
Potassium	3.6^{a}±1.7	3.6^{a}±1.12	4.4^{b}±2.8	4.6^{b}±0.4
Chloride	92.5^{a}±7.4	87.5^{b} ±7.0	95.2^{a}±6.1	85.0±5.1
Calcium	3.3±0.6	3.2±0.4	3.4±0.5	3.4±0.3

All values are expressed as mean±Std Dev. ^{a,b}: Within each row, means with different superscripts are significantly different at p = 0.05

Table 5: Post-endurance race blood biochemical parameters of horses

	Endurance race distance (km)			
	Eliminated			Good Performance
Parameter	40 (n = 17)	80 (n = 48)	120 (n = 7)	80 (n = 9)
Urea (mmol L^{-1})	7.4^a±1.7	7.4^a±1.2	6.6^b±1.2	7.7±1.4
Creatinine (µmol L^{-1})	165.7^a±44.2	190.4^b±46.4	201.1^b ±87.0	187.9±32.7
Bilirubin (µmol L^{-1})	44.5±13.0	44.1±13.3	45.4±10.9	40.3±10.2
AST (U L^{-1})	875.0^a±745.9	563.5^b±467.2	818.6^a±562.0	700.1±668.0
CK(U L^{-1})	1346.4±762.2	1250.5±808.7	1004.3±225.2	1689.0±937
Glucose (mmol L^{-1})	5.7^a±1.9	4.3^b±2.4	5.0^a±1.4	1.6^c±0.9
Lactate (mmol L^{-1})	6.0^a±2.9	6.7^b±3.2	6.4^a±1.6	9.2^c±2.2
Total Protein (g L^{-1})	81.5±11.6	83.4±8.05	77.8±6.3	82.6±6.4
Albumin (g L^{-1})	37.1^a±4.6	35.8^b±3.4	34.0^b±4.1	37.5±1.9
Globulin (g L^{-1})	44.4^a±9.3	47.6^b±6.0	43.8^a±3.6	45.1±5.6

All values are expressed as mean±Std Dev. ^{a,b,c}: Within each row, means with different superscripts are significantly different at p = 0.05 AST = aspartate transaminase; CK = creatine kinase

This study was conducted on horses participating in sanctioned 40, 80 and 120 km races, to determine the effect of the race distances on the physical, hematology and blood biochemistry parameters. The result showed that only approximately 10% of these horses managed to complete the races in good condition. The rest of the horses were eliminated.

Apart from the inherent characteristics, performances of the horses in races are closely associated with cardiac function and tissue oxygenation. These are dependent on the oxygen-carriage capacity of blood, which is dependent on erythrocyte number and hemoglobin concentrations. Endurances horses that showed metabolic disorders in races could not produce optimal muscle performance either due to inadequate oxidation or poor glucose utilization to produce sufficient energy for muscular actions during endurance ride. In exercise the blood lactate concentrations are expected to increase in these horses. The level of blood lactate in the horse is used as a measure of performance and fatigue (Pösö, 2002; Letafatkar *et al*., 2009). It has been shown, in exercise blood lactate concentrations increase, but the levels seldom exceed 4 mmol L^{-1} even in sick horses. In fact the speed of the horse when the blood lactate concentration attains 4 mmol L^{-1} (V_{LA4}) is used as an indicator of fitness of horse during conditioning (Fielding *et al*., 2009; Lindner *et al*., 2009; 2010). In our study, however, the mean blood lactate concentration for all horses were higher than 6 mmol L^{-1}. This value was attained after endurance races irrespective of distance. Horses that completed the races with good performance had even higher blood lactate concentrations reaching mean values of higher than 9 mmol L^{-1}, which were significantly higher than those in the eliminated horses.

The endurance horses that were eliminated in our study had lower circulating blood lactate and higher blood glucose concentrations than good performance horses. It is uncertain why the eliminated horses produced less lactate from tissue metabolism during exercise. From the increased blood glucose concentration there is a possibility that decrease in lactate production could be associated with poor glucose utilization by the metabolizing tissue. In acute exercise, the glucose transporters in the skeletal muscle, which mediate insulin-responsive uptake of glucose, increases (Hayashi *et al*., 1997; Tomás *et al*., 2002; Hirshman *et al*., 1988; Prenen *et al*, 2005; Alhusseini *et al*., 2010). Consequently, the exercising muscles produce lactate from glucose through the anaerobic process. Thus from the result of this study, perhaps it is the inherent lack of the glucose transporters in the skeletal muscle that causes the poor performance of susceptible horses (Hayashi *et al*., 1997), thus resulting in high blood glucose concentrations and low blood lactate in the eliminated horses.

CONCLUSION

In conclusion, the study showed that eliminated horses had low blood lactate and high blood glucose concentrations than the good performance horses. This blood biochemical manifestation in eliminated horse may be associated with inefficient muscle tissue metabolism. These parameters may be used as indicators of performance potential of horses during the conditioning regime.

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