CLINICO-HAEMATO-BIOCHEMICAL CHANGES IN HORSES AFTER TROT EXERCISE

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ABSTRACT

Since ancient times, horses were identified for their unique aerobic capacity. The purpose of the present study is to evaluate alteration in clinical, haematological, and biochemical parameters after exercise in horses. Twenty apparently healthy non-pregnant females of approximately equal age group (8-10 years) were divided into group I and II. Group I was given 30 kilometres of trot exercise for two hours and Group II was taken as the control without giving exercise. Recording of clinical parameters and collection of blood samples were done both at rest and after exercise in the case of group I but without exercise in group II. Clinical parameters viz. rectal temperature, pulse rate, and respiration rate; haematological parameters viz. haemoglobin, PCV, TEC, TLC, DLC, and platelet count; and biochemical parameters viz ALT, AST, GGT, total protein, albumin, creatinine, BUN, total bilirubin, and direct bilirubin were investigated in the present research. All the clinical parameters viz. rectal temperature, respiration rate, and pulse rate were increased significantly after the exercise. Among haematological parameters, haemoglobin and PCV were significantly higher after exercise in the treatment group while no effect of exercise was observed on TEC, TLC, DLC, and platelet count. There was no effect of exercise found on any of the biochemical parameters in the present study. Alteration in clinico-haemato-biochemical parameters in horses affirms physiological adjustment to maintain desired performance throughout, and also most of the parameters were close to normal range.

Keywords: Trot exercise, horse, clinical, haematological, biochemical parameters

Introduction

Horses exclusively perform physical activities, including running or jumping, at a level that surpasses most of the other animals of similar body size. The maximal aerobic capacity of horses is approximately 2.6 times that of similarly sized cattle, and on a body weight basis is approximately 2–2.5 times that of highly trained men. Several physiological adaptations in horses increase aerobic capacity and athletic capacity in horses (Hinchcliff and Geor, 2008). Exercise is performed out by using metabolic internal power, in which energy is generated by muscle cells in form of adenosine triphosphate (ATP) by utilizing aerobic and anaerobic mechanisms. Muscle tissue capillarization, blood volume, blood composition, and cardiac output are the various circulatory factors that aid in producing exercise of high intensity. Individual anatomical and physiological adaptation depends upon the physical fitness and genetic potential of an athlete and improvements did occur on basis of the type of exercise selected, its frequency, intensity, and duration (Knuttgen, 2007).

The horse appears well suited for exercise studies and also an unusual model in studying the wide range of metabolic states like heart rate and cardiac output responses together with its ability to dramatically increase haematocrit, haemoglobin, and oxygen-carrying capacity allowed VO_2, to increase some 36-fold over resting values. This is more than twice that normally seen in humans (Thomas and Fregin, 2019). Study of responses of some cardiovascular and respiratory parameters and thermoregulation following graded exercises in Lipizzaner horses in various seasons, and to a recognition of the complex physiological processes that take place during exercise, and represents a basis for further research in the field of exercise testing and sports medicine for horses (Cebulj-Kadunc *et al*., 2019). Clinical and haemato-biochemical changes are indicative of a physiological response to an acute intense exercise which can be confirmed by the optimum recovery time of some parameters (i.e., heart rate, respiratory rate, body temperature). The changes in the heart and respiration rate may be attributed to increasing the release of catecholamines which increased metabolic rate, aerobic and anaerobic glycolysis and subsequently leads to major changes in cardiovascular and respiratory function(Hassan *et al*., 2015).

Exercise generally results in the mobilization of splenic erythrocytes and, thus, increases the oxygen transport capacity. Substantial fluid losses incurred during prolonged endurance exercise, likely produce reductions in plasma volume play a greater role in changes in haematocrit value. In contrast to high-intensity exercise, endurance exercise is associated with leukocytosis, resulting from neutrophilia and lymphopenia which is probably due to an increase in circulating corticosteroids. Furthermore, an increase in albumin and plasma protein concentrations during high-intensity exercise is mainly due to fluid losses and it takes longer for plasma protein concentration to return to normal following prolonged endurance exercise (Kingston, 2008). Physiological, haematological, and biochemical parameters change with the performance of horses and are used to assess the status of an athlete and the degree of its training adaptability provides an opportunity to modify the training schedule to achieve the desired performance (Allaam *et al*., 2014).

Materials and Methods

The proposed study was undertaken at a Private equine farm, Jaipur, and all laboratory work was performed in the Department of Veterinary Medicine, PGIVER, Jaipur and National Research Centre on Equine (NRCE), Bikaner, Rajasthan. After initial deworming and clinical examination of all animals, twenty apparently healthy non-pregnant females of almost the same age group (8-10 years) and body weight

were selected from a private equine farm at Jaipur (Rajasthan). These horses were divided into two groups (Group I and Group II) comprising 10 animals in each. All the animals included in the study were kept under the same type of feeding, housing, and environmental conditions by the farm owner.

To understand the complex physiological and biochemical processes taking place inside the body during exercise Group I was given 30 kilometres of trot exercise for two hours and Group II was taken as the control without giving any exercise. All the animals were examined thoroughly and clinical parameters (rectal temperature, respiratory rate, and pulse rate) were recorded before and after exercise from Group I and before and after without exercise from Group II according to the standard procedures described by Radostits *et al.* (2009). To analyse modifications in haemato-biochemical parameters blood samples were collected before and after two hours from Group I and before and after without exercise from Group II following all necessary aseptic procedures and ethical measures. Blood samples collected in the heparinized tubes were used for the determination of haematological parameters. Blood in heparinized tubes was centrifuged (30 minutes, 3000 rpm) to separate the plasma from whole blood. Separated plasma was aliquoted into different microcentrifuge tubes, which were then stored in a deep freezer at -20°C until the time of biochemical parameter analysis.

The blood samples were analysed for determination of Haemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocyte count (TLC), Differential leukocyte count (DLC), and Platelet count as per the standard methods described by Jain (1986). Biochemical analysis of plasma samples was done to estimate Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Protein, Albumin, Blood Urea Nitrogen (BUN), Creatinine, and Bilirubin (total and direct). Estimation of ALT, AST, Total Protein, Albumin, and Bilirubin was done by using kits manufactured by SPINREACT, S.A./S.A.U. Ctra. Santa Coloma, 7-E-17176 SANT ESTEVE DE BAS-(GI) SPAIN. Estimation of Blood Urea Nitrogen (BUN) and Creatinine was done using kits manufactured by TRANSASIA BIO-MEDICALS LTD., Nalagarh Road, Village Malpur, Baddi, Dist. Salon, HP.

The statistical analysis of collected observations will be done as per methods described by Snedecor and Cochran (1994).

Results and Discussion

The study includes pre and post-exercise evaluation of the picture of clinico-haemato-biochemical parameters. Table 1 lists the mean ± SE values of rectal temperature, respiratory rate, and pulse rate in the group I and II. Mean values of clinical parameters recorded in the present study were more or less similar to the values recorded by Onmaz *et al.* (2011) and Dedar (2012). In the present study, statistical analysis of pre and post-exercise values in group I revealed a significant increase in rectal temperature ($p<0.05$), respiration rate ($p<0.01$), and

Table 1: Mean ± SE values of clinical parameters in group I before and after exercise and in group II before and after two hours of without exercise.

Clinical parameters	Group I (n=10)		Group II (n=10)	
	Before exercise	After 2 hrs of exercise	Before exercise	After 2 hrs of without exercise
Rectal Temperature (°F)	99.37±0.33	99.89±0.26*	99.09± 0.30	99.12±0.38
Respiration rate (breath/min)	19.3±0.90	21.9±0.91***	18.00±0.78	18.3±0.82
Pulse rate (beats/min)	37.90±1.11	43.90±1.62**	38.20±1.06	38.10±1.35

*significant difference after exercise at <0.05; ** significant difference after exercise at <0.01; *** significant difference after exercise at <0.001

Table 2: Mean ± SE values of haematological parameters in group I before and after exercise and in group II before and after two hours of without exercise.

Haematological Parameters	Group I (n=10)		Group II (n=10)	
	Before exercise	After 2 hrs of exercise	Before exercise	After 2 hrs of without exercise
Hb (g/dl)	10.63±0.36	11.37± 0.32*	10.88±0.24	10.90± 0.22
PCV (%)	35.7±1.38	37.1±1.57*	35.60±1.43	36.00±1.36
TEC (10^6/µl)	6.77±0.19	7.02±0.26	6.692±0.35	6.68±0.35
TLC (10^3 /µl)	9.524±0.42	9.884±0.65	9.755±0.49	9.76±0.53
Neutrophils (%)	51.40±1.20	52.10±1.49	51.00±0.98	51.20±1.01
Lymphocytes (%)	42.90±0.95	42.00±1.05	41.70±1.55	41.30±1.54
Monocytes (%)	3.10±0.57	2.90±0.48	3.50±0.45	3.30±0.47
Eosinophils (%)	2.30±0.40	2.80±0.59	3.50±0.45	3.70±0.45
Basophils (%)	0.30±0.15	0.20±0.13	0.30±0.15	0.40±0.27
Platelet count (10^3 /µl)	144.1±13.49	146.5±13.42	138.8±13.15	138.4±13.19

*significant difference after exercise at <0.05

pulse rate ($p<0.001$) while values in group II did not varied.

The present study was found relevant to other studies recorded by McConaghy (1994); Snow and Mackenzie (1997); Allaam *et al.* (2014) and Hassan *et al.* (2015) attributing that change in clinical parameters reflect physiological adaptation and results due to compensatory mechanism during exercise. Increase in muscular contractions require a constant, supply of energy generated from fat metabolism and the energy derived from the breaking down of ATP molecule is generally used to maintain body temperature (Fritzsche and Coyle, 2000). Increase demand for oxygen and energy by muscles for compensatory mechanisms during exercise leads to an increase in the respiration rate. This may help to increase the uptake of oxygen by blood from the lungs (Lamba, 2013). The muscles demand increased blood flow during exercise, which is achieved by both a rise in heart rate and stroke volume (Wallsten, 2012).

Table 3: Mean ± SE values of biochemical parameters in group I before and after exercise and in group II before and after two hours of without exercise.

Biochemical Parameters	Group I (n=10)		Group II (n=10)	
	Before exercise	After 2 hrs exercise	Before exercise	After 2 hrs of without exercise
	Mean±SE (Range)	Mean±SE (Range)	Mean±SE (Range)	Mean±SE (Range)
ALT(U/l)	16.70±0.989 (11.58-24.18)	17.60±0.98 (12.12-25.44)	16.35±1.44 (11.65-22.05)	16.23±1.53 (11.22-26.34)
AST(U/l)	276.05±15.90 (231.45-367.27)	282.70±17.08 (212.87-390.93)	283.51±15.91 (214.18-354.28)	282.92±16.18 (212.61-376.61)
GGT(U/l)	22.89±1.47 (16.61-29.76)	24.97±1.40 (15.45-30.37)	20.50±1.29 (15.12-28.49)	20.43±1.20 (16.21-27.71)
Total Protein (g/dl)	5.97±0.288 (4.88-7.43)	5.97±0.29 (4.85-7.27)	5.82±0.29 (4.62-7.45)	5.82±0.29 (4.62-7.42)
Albumin (g/dl)	3.19±0.18 (2.18-4.07)	3.22±0.14 (2.29-3.96)	3.33±0.17 (2.41-4.21)	3.32±0.17 (2.41-4.19)
BUN (mg/dl)	23.14±0.94 (18.57-27.14)	22.93±1.05 (17.14-26.43)	23.43±0.84 (18.57-26.43)	23.36±0.78 (19.29-27.14)
Creatinine (mg/dl)	1.67±0.17 (0.91-2.23)	1.72±0.17 (0.96-2.23)	1.65±0.21 (0.41-2.36)	1.66±0.22 (0.40-2.48)
Total Bilirubin (mg/dl)	1.16±0.09 (0.78-1.66)	1.16±0.09 (0.76-1.60)	1.12±0.09 (0.61-1.49)	1.12±0.093 (0.61-1.45)
Direct Bilirubin (mg/dl)	0.53±0.05 (0.32-0.77)	0.54±0.04 (0.35-0.74)	0.49±0.04 (0.36-0.74)	0.50±0.39 (0.36-0.74)

Table 2 lists the Mean ± SE values of Hb, PCV, TEC, TLC, DLC, and platelet in the group I and II. Mean values of haematological parameters observed in the present study were found more or less similar to Piccione *et al.* (2008); Meliani *et al.* (2014); Vazzana *et al.* (2014); Burlikowska *et al.* (2015) and Andriichuk and Tkachenko (2017). The statistical increase in post-exercise values of Hband PCV ($p<0.05$) in group I agreed with results obtained by Piccione *et al.* (2008); Piccione *et al.* (2010); Vazzana *et al.* (2014) stated it as a physiological response for better athletic in horses. Other hematological parameters did not varied significantly in the present study was supported by various authors Snow *et al.* (1983); Zobba *et al.* (2011); Adamu *et al.* (2012) Burlikowska *et al.* (2015) and Andriichuk and Tkachenko (2017).

Oxygen transport from the lungs to exercising muscle is achieved by circulation. Horses achieve rapid increases in the oxygen-carrying capacity of blood by increasing haemoglobin concentration in blood through the splenic contraction. With the increase in erythrocyte count

Table 2 lists the Mean ± SE values of ALT, AST, total protein, albumin, bun, creatinine, and bilirubin (total and direct) in the group I and II. Mean values of biochemical parameters obtained in the present study were more or less similar to the findings of Arslan *et al.* (2002); Piccione *et al.* (2010); Simenew *et al.* (2011); Adamu *et al.* (2012a); Adamu *et al.* (2014); Allaam *et al.* (2014) and Burlikowska *et al.* (2015). Post-exercise values of AST, ALT, GGT, albumin, and creatinine in the group I increased moderately while BUN appeared slightly decreased. Other values of biochemical parameters like total protein and bilirubin did not change ingroup I after exercise, but all these biochemical parameters did not vary significantly. Non-significant change in the values of AST, ALT, and GGT corresponds to the findings of Arslan *et al.* (2002) reported that physiological adaptation tends to decrease secretion of the enzyme from muscle under physical training and an increase in enzymatic activity occurs only in liver and muscle diseases. In the present study, values of biochemical parameters varied without any significance, and post-exercise values appeared much close to values recorded before exercise and this study was found in agreement with Judson *et al.* (1983); Jagric-Munih *et al.* (2012); Krumrych (2010); and Adamu *et al.* (2012a).

Conclusion

The results of this study proved beneficial in analysing the physiological response of the body to a single episode of long-distance trot exercise by determining alterations in clinical, haematological, and biochemical parameters. An increase in clinical parameters is might be due to increasing demand of oxygen and energy to muscles and heat production during exercise and additionally, haemoglobin and PCV increased after exercise is might be due to the achievement of a compensatory increase in oxygen-carrying capacity and

hemoconcentration. The study concludes that horses possess the impressive aerobic capacity and physiological adjustment of the body to maintain desired performance throughout, herein maintained most of haemato-biochemical parameters close to normal range.

Conflict of interest

The author declares that they have no conflict of interest.

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