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Research Article

HEMATO-BIOCHEMICAL TREND IN STALL HOUSED HORSES UNDER HOT AND HUMID CLIMATIC CONDITIONS OF ODISHA, INDIA

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ABSTRACT: Blood is an important and reliable medium for assessing the health status and performance level of horses. Variations in hematological parameters in horses are associated with several factors such as exercise and training, feeding, age, sex, breed, diurnal and seasonal variation, temperature and the physiological status etc. The objective of this study was to determine the influence of exercise on hematological indices in horses maintained under hot and humid climatic conditions of Odisha, India. Blood samples were drawn from jugular veins of the animals in the morning and 30 minutes, 4 hours and 8 hours after exercise. The blood samples were assessed for Total erythrocyte (RBC), Total leucocytes (WBC), Hemoglobin, Packed cell volume (PCV), Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Erythrocyte Sedimentation rate, Serum total glucose, Whole blood glucose, Serum Sodium, Serum Potassium, Serum Calcium, Serum magnesium and Serum chloride before and after exercise.

Key words: Hematological parameters, Exercises, Horses.

INTRODUCTION

During recent years there has been an increase in concern over the welfare of horses and as a consequence the equine industry has come under scrutiny over its management and training practices (McGreevy 2007). It has been observed that housing horses in single stalls limits expression of natural behaviour, especially exercise and social behavior to a great extent (Werhahn *et al.* 2012), while free exercise on pasture or dry lots can improve the degree of animal welfare in the system (Houpt 2005). For a long time, the measurement of many biochemical and other types of variable has been suggested to allow for an objective assessment of the competitive capability of

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sport horses (Rico *et al*. 1983; Linder 2000).

The objectives of this study were to determine how well hematological and biochemical variables correlated with performance in the Jumper horses before and after exercise, a gauge of athletic potential in horses thus addressing a paucity of scientific data in this area. Further, it was hoped that this work would provide a foundation to develop a regime that can be used for screening the potential of competition horses. A fair idea of hematological changes in these animals is necessary to show as to how they behave in the Odisha state climate, after work stress, under different climatic conditions.

MATERIALS AND METHODS

Four gelds and two mares within the age group of five to fourteen years and having good health were used in the present study. The horses were maintained by N.C.C Organization at College of Veterinary Science and Animal Husbandry, Bhubaneswar on balanced diet. They were regularly put to exercise six days per week. However, before the animals were used for experiments, routine checkup of fecal samples were done. The horses were vaccinated against Anthrax and Mallein test was conducted as a regular routine.

Group-I: The six animals were used as the control group in order to record the normal hematological values. The haematological parameters like total erythrocyte (RBC), Total leucocytes (WBC), Hemoglobin, Packed cell volume (PCV), Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, Erythrocyte Sedimentation rate as well as biochemical parameters like Serum total glucose, Whole blood glucose, Serum Sodium, Potassium, Calcium, magnesium and chloride parameters were estimated in the early morning at 06.30AM.

Group-II: Six animals used in Group I, were put to exercise for exactly 30 minutes. The exercise was limited to trot in each horse immediately after recording the normal values. The parameters examined in Group I, were again recorded immediately after the exercise was completed. The data were taken as those of Group II horses.

Group-III: The same six animals were allowed to rest in the stable. The hundred forty minutes (4 hours) after exercise, estimation of all the parameters of the horses were again repeated and noted as in the Group-I (control) and Group II. These data were taken as the data of Group III horses.

Group-IV: The same six horses were used for recording all the parameters as in the control group 480 minutes (8 hours) after the exercise. The data formed the data of Group IV horses.

Collection and preservation of blood and serum

Fifteen milliliters of blood were collected at 05.40 hrs by jugular vein puncture into evacuated collection tubes. For hematological study, 5 ml blood was collected in a sterile vial using EDTA as anticoagulant @ 1 mg/ml of blood. For serum biochemical analysis, the

tubes containing 10 ml of blood and without having any anticoagulant were kept in slanting position. After the blood got clotted, the tubes were transferred to refrigerator at 4^0 C for 12 hours to allow maximum secretion of serum from the clot. The clear serum was collected carefully into sterilized vials and stored in the frozen chamber without any preservatives. Before using the frozen serum for experiment, it was allowed to defrost at room temperature.

Haematological parameters

Sahli's acid hematin method was employed for estimation of hemoglobin by using N/10 Hydrochloric acid (HCl) and expressed as g/dl. Wintrob's hematocrit method was used for PCV estimation and the result was expressed as percentage (%). The estimation of TEC ($\times 10^6$/µl of blood) and TLC ($\times 10^3$/µl of blood) was done by hemocytometer method. The ESR was and the values were expressed as mm//hr fall. All the estimations were done by using the procedure described by Coles (1986).

Serum biochemical parameters

The whole blood glucose, serum calcium, phosphorous, magnesium, sodium, potassium levels were estimated by Modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method as per the procedure described by Burtis and Ashwood (1999), using the diagnostic kits supplied by M/s Crest Biosystem™, a division of Coral clinical systems, Goa and were expressed in milligrams per decilitre (mg/dL). Serum total protein was estimated by Biuret method, as per the standard procedure described by Johnson *et al*. (1999). using the diagnostic kits supplied by M/s Crest Biosystem and was expressed in grams per deciliter (g/dL).

Statistical Analysis

All behavior and weather parameter data were averaged to obtain values for statistical analysis. Rectal temperature, RR, and PR were measured and analyzed. Different behavior of horse was recorded for last 15 days in each month for analysis. The results of the study were recorded and statistically analyzed as per the methods suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Total Erythrocyte

The mean total erythrocyte concentration in the horses of Group I was estimated to be 5.28 ± 0.44 x10^6 per cmm. Immediately after exercise the value was 6.73 ± 0.43 x 10^6 cmm. Analysis of variance showed significant ($P \leq 0.05$) increase in total erythrocyte concentration immediately after exercise (Table1). Critical difference also indicated a significant ($P \leq 0.05$) increase. Subsequent estimations of total erythrocyte values at 240 minutes and 480 minutes after exercise were not found to be significant (Table 1).

Total Leucocyte

The mean total leucocyte value of the experimental animals at rest in Group I was evaluated to be 9708.33 ± 352.40 per cmm. There was a rise in the leucocyte level in Group

II and III. However, this was not statistically significant (Table1). The level of total leucocytes at 480 minutes after exercise was though less than the control group and was not significant (Table 1).

Hemoglobin

The mean hemoglobin level of the experimental horses in control group was recorded to be 10.33 ± 0.63 g/dl. Analysis of variance showed significant ($P \leq 0.01$) increase just after exercise (Table1). Immediately after exercise the horses (Group II) showed an increase in hemoglobin level to 11.83 ± 0.54 g percent and this was statistically signification of hemoglobin level were found to be not significant (Table 1).

Packed Cell Volume (PCV)

The mean packed cell volume of the control group of horses (Group I) was found to be 29.83 ± 1.19 per cent. Analysis of variance indicated significant ($P \leq 0.01$) increase immediately after exercise (Table1). The mean packed cell volume immediately after exercise was estimated to be 32.83 ± 1.35 per cent. This was significantly ($P \leq 0.05$) high in comparison to group I. The mean values of this parameter at 240 and 480 minutes after exercise were not significant (Table 1).

Mean Corpuscular Volume

The mean corpuscular volume of the horses in group I was calculated to be 57.84 ± 3.61 fL. Following exercise estimation of the mean corpuscular volume at different intervals was found to be not significant with regard to the control group (Table 1).

Mean Corpuscular Hemoglobin

The mean corpuscular hemoglobin level of the horses in the control group was assessed to be 20.13 ± 1.66 pg. Estimation of this parameter at different intervals after exercise was found to be not significant (Table 1).

Mean Corpuscular Hemoglobin Concentration

The mean corpuscular hemoglobin concentration of the horses in Group I was calculated to be 34.63 ± 1.54 g/dl. The mean value of this parameter following exercise at different interval of time was found to be not significant (Table 1).

Erythrocyte Sedimentation Rate

The mean erythrocyte sedimentation rate of the experimental horses in control group was found to be 34.17 ± 0.75 mm/hr. The analysis of variance showed that following exercise there was a significant ($P \leq 0.01$) decrease after exercise. The mean level of this parameter immediately (Group II) after exercise was recorded to be 10.33 ±1.2 and 25.67 ± 1.14 millimeter respectively. The mean values of erythrocyte sedimentation rate as stated above after exercise were not significant within themselves and also in comparison with the control group (Table 1). However, the mean level of the parameter 480 minutes after exercise was not significant with regard to the control group (Table 1).

Serum Total Protein

The mean level of serum total protein in the horses at rest was estimate to be 7.45 ± 0.14 gram%. Subsequent estimation after exercise showed significant ($P \leq 0.01$) increase. Immediately following exercise the mean level of serum total protein was recorded to be 8.45 ± 0.26 g. percent and was significantly ($P \leq 0.05$) higher than the control group (Table 1). Two hundred and forty minutes (Group-III) after exercise the mean level dropped to 7.87 ± 0.27 gram% and was not significant in comparison to the levels in the horses of group I and Group II (Table 1). However, the mean value of this parameter in the horses, 480 minutes after exercise (Group IV) was found to be less than the values of Group II and Group III (Table 1).

Whole Blood Glucose

The mean level of whole blood glucose in group I (Control) was estimated to be 80.00 ±3.87 mg/dl. percent. Subsequent estimations after exercise was found to be significantly ($P \leq 0.01$) low (Table 1). The mean level in group II was 71.67 ± 4.62 mg percent and was significantly ($P \leq 0.05$) low (Table 1). The glucose level was found to be 77.00 ± 4.09 mg percent in the horse of Group III and this was not significant with regard to the values of Group I and Group II. The mean glucose level of the horses in Group II. The mean glucose level of the horses in Group IV increased to 86.50 ± 2.55 mg percent and this was significantly ($P \leq 0.05$) higher than Group II and III (Table 1).

Serum Sodium

The mean values of serum sodium in Group I was found to be 144.17 ± 2.95 mEq/L. Analysis of variance Indicated that the values after exercise at different intervals were not statistically significant in comparison with the control (Table 1) .

Serum Potassium

The mean serum potassium concentration in horses at rest was recorded to be 4.54 ± 0.08 mEq/L. The potassium levels after exercise at different intervals were not significant with regard to normal value (Table 1).

Serum Calcium

The mean serum calcium level of hours of Group I was estimated to be 12.10 ± 0.24 mg/dl. The mean values after exercise at different intervals of time ware not significantly different (Table 1).

Serum Magnesium

The mean serum magnesium level in Group I was recorded to be 2.78 ± 0.03 mg/dl. There was no significant change in the mean value after exercise (Table1).

Serum Chloride

The mean serum chloride level of the experimental animals in Group I was found to be 105.33 ± 6.12 mEq/L. There was a significant ($P \leq 0.05$) degrease of chloride level at 240 minutes after exercise (Table 1).

The results obtained after estimation of hematological parameters in horses after exercise showed change in some of the

observations. Analysis of variance of the results was first done to find out significance of each parameter after exercise in comparison to the values before exercise. Critical differences of the parameters were thereafter recorded to specifically the significance of parameters at different time intervals. This helped to compare the values within the groups and at different time intervals.

The mean level of total erythrocyte in experimental horses during rest was comparatively less than those reported by Gupta *et al*. (2002). The low mean value was probably due to inclusion of a few old animals. Immediately following exercise there was significant ($P \leq 0.05$) increase in the mean level. The finding agreed with the observation of Andriichuk and Tkachenko (2015). The effect of exercise at 4 and 8 hours was not statistically significant, although it was higher. The rise in total erythrocyte soon after exercise might be due to more of cells in circulation following splenic contraction. Stimulation of hameopcetic system can lead to an increase in total erythrocyte but this may happen due to exercise.

The average value of total leucocyte concentration in the experimental horses was within the range observed by Gopala Krishnan *et al.* (1973) in the Indian race horses and was comparatively less than those reported by Gupta *et al*. (2002). The mean level of total leucocyte showed an increase immediately after exercise but was not statistically significant. Similar results were reported by Octura *et al*. (2014). The level dropped at 4 and 8 hours after exercise reaching the reaching the range of the control group. Increased blood cell number due to splenic contraction might have attributed to increase in total leucocyte level during exercise, though it was not significant.

The mean hemoglobin level of the horses during rest was less than that reported by earlier workers by Octura *et al*. (2014) and Andriichuk and Tkachenko (2015). Similar low value was also obtained in total erythrocyte level in these horses before exercise. The reason for a low value may be the inclusion of more number of old horses, which presented lot of variation in confirmation and breed character. Low hemoglobin was directly proportional to low erythrocyte level. Immediately following exercise there was an increase in hemoglobin concentration. This was related to a similar increase in erythrocyte count after exercise. Four and eight hours after exercise the hemoglobin dropped to normal range as was in control group. Identical observation was also noted in total erythrocyte level.

The average level of packed cell volume of the horses was lower than the levels reported by Gopala krishnan *et al.* (1973) Fregin (1979) and Blood and Hendersen (1981). Such low value was evident as the total erythrocyte level was also lower in the horses. However, an increase ($P \leq 0.01$) in level was recorded in the horses after exercise. Similar results were also obtained by Fregin (1979). The significant increase in the packed cell volume soon after exercise was due to significant increase in erythrocyte count and loss of fluid during exercise through sweating. The average values

reached the control level at 4 and 8 hours after exercise. A significant increase in packed cell volume in horses immediately after exercise denoted hemocontration during increased packed cell volume results from hemoncocentration following exercise and due to release of more number of erythrocyte from spleen.

These parameters were calculated using the erythrocyte and hemoglobin values. A decrease in M.C.V. and M.C.H. and an increase in M.C.H.C. have been observed immediately after exercise but none of the values were statistically significant. Reduced mean corpuscular volume is probably the result of shrinkage of red cell wall resulting from changed osmotic pressure, Thus, there were more cells in a comparatively lesser PCV. Low MCH was also related to the above factor. The corpuscular hemoglobin concentration was higher than the control level but was not significant though the whole blood hemoglobin concentration was significantly ($P \leq 0.05$) higher than the control value immediately after exercise.

The normal erythrocyte sedimentation rate in the horses was near the range reported by Octura *et al*. (2014). The sedimentation rate was significantly ($P \leq 0.05$) low after exercise. Such condition was due to increased muscular action and respiratory rate during exercise leading to quicker movement of circulating blood in the tissues. It was also observed that though the sedimentation rate increased after 4 hours of exercise, yet it was significantly ($P \leq 0.05$) lower than the control level. Further, it has also been recorded that though the mean erythrocyte level increased significantly ($P \leq 0.05$) less at 4 hours after exercise. The sedimentation rate reached the normal level after 8 hours of exercise

In the present experiment, the horses were allowed to trot for 30 minutes and at the end of exercise blood sample was collected to estimate the erythrocyte sedimentation rate. ESR during rest after exercise were 34.17 ± 0.75 and 10.33 ± 1.20 mn/30 minutes. Such a low erythrocyte sedimentation rate was not observed in this experiment even though the horses were given 30 minutes of trotting.

The normal mean serum total protein value of the experimental horses was higher than those reported by other Anderson *et al*. (1975) significant ($P \leq 0.01$) increase after exercise was observed. Critical difference further revealed that the total protein level was not significantly high at 4 hours after exercise than the control and was not significantly low than the level immediately after exercise. These changes in total protein indicated that exertion and less water intake might have caused significantly ($P \leq 0.05$) high total protein level following exercise. Two hours after exercise all the horses were supplied with their normal ration.

The normal mean whole blood glucose level in the experimental horses was 80.00 ± 3.87 mg/dl, whereas, Blood and Henderson (1981) has mentioned the normal level to be 60-100 mg/dl. Immediately after exercise a significant ($P \leq 0.05$) fall in the glucose level was observed. The finding is in agreement with the

observation of Bhatti and Shaikh (2007). The glucose level improved at 4 and 8 hours after exercise. The low value immediately following exercise was due to utilization of glucose during exercise. The stress was relieved after 4 and 8 hours of exercise and the horses had their norm al food and water. This caused a significant ($P \leq 0.05$) increase in whole blood glucose at 8 hours after exercise during which the horse had adequate rest.

The normal mean serum sodium and potassium levels in the horses were found to be within the ranges as stated by Blood and Henderson (1981). An increased sodium level immediately after exercise was recorded but the increase was not statistically significant. The levels after 4 hours and 8 hours of rest were within normal range. The serum potassium level showed a decrease after exercise at all the intervals of time but was not significant at any stage. The findings were not in agreement with Soliman and Nadim (1967). These workers reported that a slight decrease in sodium and a significant fall in potassium level occurred after a strenuous exercise. This might have a relation to the timing of blood collection after exercise.

The average serum calcium and magnesium levels in the horses were in the same range as mentioned by Blood and Henderson (1981). The levels in these electrolytes did not show any deviation after exercise.

The estimation of serum chloride before and soon after exercise did not show significant change. However, the mean level was significantly ($P \leq 0.05$) low at 4 hours after exercise. This could not be explained.

A complete study of the above said parameters indicated that significant ($P \leq 0.05$) increase in total erythrocyte, total hemoglobin, packed cell volume and total protein and significant ($P \leq 0.05$) fall in erythrocyte sedimentation rate and whole blood glucose occurred after exercise. There was no change in blood electrolytes. It may be concluded that analysis of blood for hemoglobin, erythrocyte sedimentation rate, total protein and whole blood glucose after exercise are indicative of stress. In any diseased condition the hemoglobin and protein mainly, may not be in normal range.

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Table 1. Influence of exercise on hematological indices of horses maintained under hot and humid climatic conditions of Odisha, India.

Horse Group	RBC (Cmm)	WBC (Cmm)	Hb (g%)	PCV (%)	MCV cuμ	MCH (ppg)	MCH C (%)	ESR mm/30 mins	Total protein (g%)	Glucose (mg%)	Na $m^E q/L$	K $m^E q/L$	Ca (mg%)	Mg_2 $m^E q/L$	Cl_2 $m^E q/L$
1. (before exercise)	$5.28x10^{6a}$ ±0.44x10^6	9,708.33^a ±352.40	10.33^a ±0.63	29.83^a ±1.19	57.84^a ±3.61	20.13^a ±1.66	34.63^a ±1.54	34.17^a ±0.75	7.45^ac ±0.147	80.00ac ±3.87	144.17^a ±2.95	4.547a ±0.088	12.10^a ±0.241	2.78^a ±0.033	105.33^a ±6.12
2. (after exercise)	$6.73x\ 10^{6b}$ ±0.43x10^6	11.725.0^a ±409.54	11.83^b ±0.54	32.82^b ±1.35	49.55^a ±3.04	17.94^a ±1.32	36.05^a ±0.79	10.33^b ±1.20	8.45^b ±0.265	71.67^b ±4.62	149.17^a ±2.61	4.51^a ±0.261	12.08^a ±0.32	2.80^a ±0.02	102.17^a ±7.15
3. (4 hrs after exercise)	$5.75x10^{6a}$ ±0.56x10^6	9.833.33^a ±658.10	10.58^a ±0.65	29.33^a ±1.43	52.46^a ±1.29	19.19^a ±2.18	36.16^a ±1.85	25.67^c ±1.14	7.87^ab ±0.27	77^ab ±4.09	142.67^a ±4.75	4.33^a ±0.196	12.55^a ±0.628	2.75^a ±0.02	96.83^b ±5.14
4. (8 hrs after exercise)	$5.30x10^{6a}$ ±0.40x10^6	9.216.66 a±475.76	10.42a ±0.58	29.50a ±3.55	56.66a ±3.55	20.14a ±1.67	35.35a ±1.29	55a± 0.93	7.25a ±0.241	86.50a ±2.55	140.83a ±4.69	4.34a ±0.167	12.21a ±0.20	2.75a ±0.02	105.33a ±6.29

Values with different superscripts differ significantly ($P \leq 0.05$).

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