
Comparison of Some Hematological Parameters Between Horses in an Endurance Competition

Fereydon Rezazadeh^{1,*}, Neda Hosseinzadeh², Raziollah Jafari Jozani¹, Nima Dianati¹

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

²Department of Basic Sciences, Faculty of Veterinary Medicine, University of Shiraz, Shiraz, Iran

Email address:

f_rezazadeh@tabrizu.ac.ir (F. Rezazadeh)

*Corresponding author

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Abstract: This study conducted to comparison of some blood constituents between horses participated an endurance racing. Jugular vein puncture was done to collect blood from 15 horses (1 gelding, 2 mares, and 12 male). These horses had been classified to three breeds include 12Kurd, 1 pure Arab, 2cross-breed and participated in the endurance racing of 0 (before competition), 15 (After first loop) and 30 km (final loop). Horses were classified in three age-groups include below 5(n=1), 5-10 (n=10) and above 10years of age (n=4). Hematocrit and Hemoglobin percentage, Total protein concentration, Red blood cell and White blood cell counting, glucose and cortisol level were measured at before starting competition (0), 15 and 30 km. Data was analyzed by SPSS (version 22). Results showed significant difference in glucose level after 30 km ($P<0.05$) but not after 15km ($P>0.05$). No significant difference was observed between cortisol concentration and age groups ($P>0.05$) but, there was significant difference between cortisol concentration and gender ($P<0.05$). Breed had influenced on HT, Hb, TP and WBC ($P<0.05$). Otherwise breed had no effect on RBC, glucose level and cortisol concentration ($P>0.05$). HT and WBC counting were higher in Arab, cross breed and Kurd respectively. Hemoglobin percentage was found higher in Arab, Kurd and Cross breed respectively. Total protein concentration in Arab was higher than Cross breed and Kurd. In conclusion, in short distance endurance competition of horses, stress is not effect on metabolism of animal, furthermore, gender and breed could important in blood parameters alterations.

Keywords: Horse, Blood, Endurance

1. Introduction

The horse is an extraordinary athlete with characteristics of speed and endurance that were subsequently modified or enhanced by selective breeding by human [1]. The performance of a horse during competition is the result of a combination of many complex interactions; include the horse age, gender, breed, genetic potential, gait, diet, psychology, strength, and neuromuscular coordination, as well as the capacity for work, or fitness [2]. Endurance riding is a competition in which the speed and endurance of a horse is tested, and riders are also challenged with regards to the effective use of pace and thorough knowledge of their horses through cross-country. Horses can suffer from dehydration and heat stress leading to a lot of problems

such as metabolic disorders and if does not compensated for, lead to cardiovascular and thermoregulatory instability [3, 4]. Endurance riding is an internationally recognized sport in which horse and rider team cover a designated course within a set of time. Endurance rides fall into two categories based on distance: (1) short up to 90; (2) long up 120-160 km. both two rides occur in a 24-hour period [5, 6]. The exercising horse produces a tremendous amount of metabolic heat and, like humans, cools primarily by the evaporation of sweat leading to fluids and electrolytes losses that, if uncompensated for, can lead to cardiovascular and thermoregulatory instability [7]. Given the substantial fluid losses incurred during prolonged exercise, it is likely

that reductions in plasma volume play a greater role in changes in hematocrit [8]. Arabian and Arabian-crosses are the predominant breeds used for endurance riding. This distinction is probably based on muscle fiber composition and preferential ability to utilize lipid during sub-maximal exercise [5]. Arab-based horses possess a flexible gene pool and the selection of genes favorable for a given set of environmental circumstances under a given set of environmental pressures will produce the necessary physiological adaptations for successful performance [9]. Indoubtedly, hematology and plasma or serum biochemistry are important tools for assessing the health of athletic horses [10]. This field study monitored three groups of horses that successfully completed short distances in semi-tropical conditions, determining hematologic and hormonal alterations after rides. This study was conducted on three different breeds of horses participating in 30km races to determine the effect of distance/breed in some blood profile.

2. Material and Methods

Equine venous blood samples collected by Ethylenediaminetetraacetic acid (EDTA) venojects from 15 horses (1 gelding, 2 mare, and 12 male) categorized in three breeds include 12Kurd, 1Arab, 2 cross-breed during distances 0; before starting the competition, 15 km (after the end of the first loop for veterinary examination in vet gate) and 30 km (the final loop that finished the competition). We have only one pure Arab horse in the competition and most of cross-breed was from Arab-Kurd, Arab-Turkmen or other native breeds in Iran. Blood serum separated and were preserved at -20°C . Horses were classified in three age groups include below 5 years (1), 5-10 (10) and above 10years (4). Hematocrit and hemoglobin percentage, concentration of plasma total protein, red blood cell and white blood cell counting, glucose level and cortisol concentration were assessed in 0, 15 and 30 km. Hematocrit percentage was determined by micro hematocrit tubes. Red blood cell and White blood cell counting were calculated by hemacytometer method. Total protein concentration of plasma and glucose level was determined by Buiret, Trucky's method by using commercial Biochemical kits (Pars Azmoon, Iran). Data was analyzed by SPSS (version 22).

3. Results

The blood Hematocrit and Hemoglobin percentage, total protein concentration, Red Blood Cell and White Blood Cell counts, glucose levels and cortisol concentration of endurance horses at three different distances were presented in Table-1. No significant difference detected in hemoglobin percentage, total protein concentration, RBC and WBC

counting and cortisol concentration in our study ($P>0.05$). Significant difference was detected in glucose level after 30 km ($P<0.05$) but not after 15km ($P>0.05$). No significant difference was observed between cortisol concentration and age groups ($P>0.05$). Table-2 represents that there were significant positive correlation between breed and hematocrit ($P=0.001$) and hemoglobin percentage ($P=0.000$), plasma total protein concentration and WBC counting ($P=0.000$). In other hand, breed had no effect on RBC counting, glucose and cortisol concentrations ($P>0.05$) in this study. Figure 1 shows that hematocrit percentage in Arab was more than cross-breed and Kurd respectively. Hemoglobin concentration in Arab was more than Kurd and cross-breed (Figure 2). Total protein concentration in Arab was more than cross-breed and Kurd (Figure 3). WBC counting in Arab was more than cross-breed and Kurd respectively (Figure 4). No significant difference was observed ($P>0.05$) between gender and other hematologic parameters such as hematocrit and hemoglobin percentage, plasma total protein concentration, WBC and RBC counting and glucose level.

Table 1. The hematologic, biochemical and cortisol values at three distance points.

Parameter	Endurance race distance (km)		
	0	15km	30km
HT (%)	48.46±1.40	50.66 ±1.40	52.60±1.13
Hb (g L ⁻¹)	15.40±10.35	15.88 ±0.28	16.04 ±0.23
TP (g L ⁻¹)	7.06±0.27	7.10±0.28	7.20±0.27
RBC(*10 ⁶ /μl)	5.53 ±0.12	5.76±0.09	5.84±0.11
WBC(*10 ³ /μl)	8.20±0.19	8.42±0.2	8.58±0.23
Glu (mmol L ⁻¹)	98±2.59	106.86 ±3.17	110.73±3.08*
CORT (mmol L ⁻¹)	3.22±0.13	3.45 ±0.13	3.33±0.17

All values are expressed as Mean±SD. Value with* indicates significant parameter at $P<0.05$ HT=Hematocrit; Hb=Hemoglobin; TP= Total protein; RBC= Red blood cell; WBC= White blood cell; Glu=glucose; CORT=cortisol.

Table 2. The hematologic, biochemical and cortisol values in three breeds of horses.

Parameter	Horse breeds		
	Arab	Cross - breed	Kurd
HT(%)	60.77 ^a	50.50 ^a	49.75 ^a
Hb(g L ⁻¹)	18.53 ^b	15.38 ^b	15.60 ^b
TP(g L ⁻¹)	9.33 ^b	8.05 ^b	6.73 ^b
WBC(*10 ³ /μl)	9.96 ^b	9.03 ^b	8.16 ^b
RBC(*10 ⁶ /μl)	5.46	6.01	5.68
Glu (mmol L ⁻¹)	117.66	109	103.52
CORT(mmol L ⁻¹)	2.96	3.42	3.35

All values are expressed as Mean ± SD. ^{a, b}: within each row means with different superscripts are significantly different at $P < 0.05$ (^a $P=0.001$, ^b $P=0.000$). HT=Hematocrit; Hb=Hemoglobin; TP= Total protein; RBC= Red blood cell; WBC= White blood cell; Glu=glucose; CORT=cortisol.

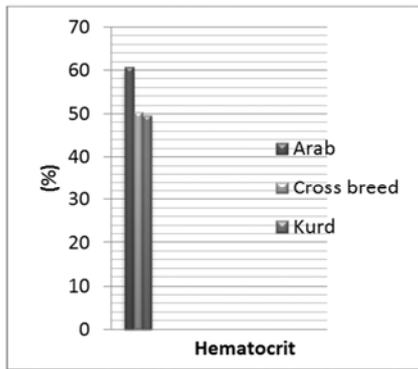


Figure 1. The percentage of hematocrit in Arab was more than cross- breed and Kurd respectively.

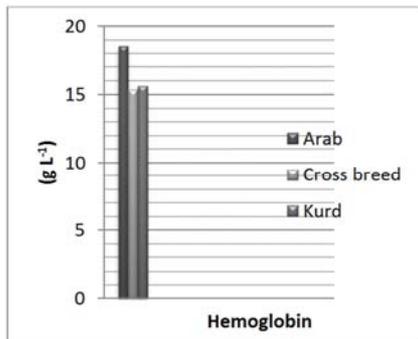


Figure 2. The hemoglobin concentration in Arab was more than Kurd and cross- breed respectively.

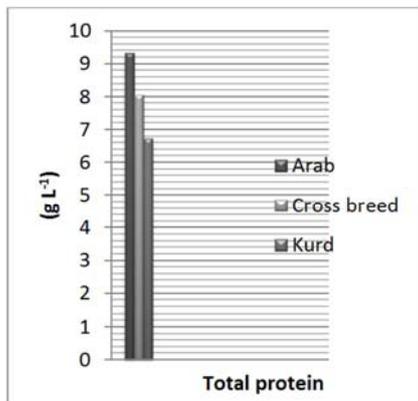


Figure 3. Total protein in Arab was more than cross-breed and Kurd respectively.

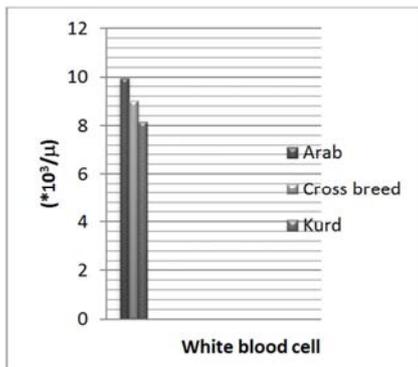


Figure 4. WBC counting in Arab was more than cross-breed and Kurd respectively.

4. Discussion

Endurance horses undergo severe stress during the course of a competitive ride. Endurance 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 [11]. The working muscle of endurance horses depends on aerobic metabolism of its glycogen stores [12-14]. Determination of the fitness or exercise tolerance of a horse is by assessment, of hematological and biochemical changes obtaining the post-ride blood samples [15].

In present study, there were no significant differences in the post-ride Hb percentage, TP concentration, RBC and WBC concentration and CORT level studied after distance of 15 and 30 km. These findings were in agreement with the results of Teixeira-Neto *et al.* [3] In endurance efforts, two physiological events can contribute to HT percentage elevation. In more prolonged exercises, splenic contraction due to an adrenergic stimulus and sweating leading to extensive body fluid losses. As thermoregulation leads to intense sweating with evident body fluids losses and haemoconcentration [16, 17]. More than 5% body weight loss, occur in after 30 km of the ride [3], and this could change some blood parameter, so there were no significant differences in the post-race HT and Hb concentration RBC counting and TP concentration [18-20]. Kingston suggested that during prolonged, low-intensity exercise, the albumin and total plasma protein concentrations increases [10]. Naylor *et al.*, believe it is likely that plasma volume decreases in response to the substantial fluid losses incurred during prolonged endurance exercise [20]. Furthermore, those fluid losses result in increases in albumin and plasma protein concentrations that are much greater than those observed in horses performing short-duration exercise [10]. Prolonged physical exercise (more than 30 km), affects plasma proteins involved in pathways related to inflammation, coagulation, immune modulation, oxidant/antioxidant activity and cellular and vascular damage, with consequent effects on whole horse metabolism [21]. Present study revealed that there is significant correlation between breed and HT and Hb percentage, TP concentration and WBC count. Fan *et al.* affirmed that significant differences in HT percentage and TP concentration could be indicative of dehydration status may be due to the action of xanthine oxidase in free radical production as a result of muscle cell membrane permeability [2]. Dehydration, to varying degrees, will inevitably occur in horses participating in endurance trail rides. This water loss is directly related to the amount of body water lost through evaporative cooling, that in turn being related to the amount and rate of work performed, and to the environmental temperature and humidity [22]. It is generally agreed by Jones and other authors that about one third up to 50% of the blood cells are stored in the spleen at rest [23]. Exercise

causes production of epinephrine, which in turn expels the spleen to eject the stored RBC into peripheral blood circulations [2]. Thus it may be concluded that elevation of HT and Hb percentage may be due to splenic contraction not related to dehydration and body water loss. Prolonged exercise, such as endurance riding, is associated with leukocytosis resulting from a neutrophilia and lymphopenia [24]. This is probably due to an increase in circulating corticosteroids [25]. Jensen et al revealed the effects of an endurance ride on neutrophil functions in endurance-trained horses and related to metabolic changes and changes in cortisol concentrations. The race resulted in elevated serum cortisol levels (<465 nmol/l) and an increased neutrophil: lymphocyte ratio [26]. Although other investigators [27] observed that older horses (>8 years) had higher leukocyte values than younger horses, in the present study no difference was observed between the analyzed age ranges. Data from the present study did not reveal an increase in WBC counts during rides. Physical exercise induces a reduction of immune defenses and an imbalance of red-ox status. Moreover, a reduction of lymphocyte responsiveness to the proliferative stimulus was observed. Homocystein is an amino acid that can reduce the proliferative capacity of resting lymphocytes as well as their responsiveness to mitogen. Similarly there were no significant differences in the post-race leukocyte counts, within the mentioned distances [18, 28].

Gurgoze et al. and Vincze et al. found that age did not influence the glucose levels in all the age-groups, which is similar to the findings of the present study [29, 30]. In previous studies, it was reported that heightened exercise increases glucose transporters, which mediate insulin responsive use of glucose in the skeletal muscle. The endurance horses that were eliminated in some studies 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 [18, 31-35] thus glucose concentration will be decreased after 30 km¹⁴ which is in parallel to our finding. The influence of age was seen in the poor performance category in relation to lactate, but age did not influence other physical and hematological parameters of the endurance horses [18].

During competition, stress may affect riders and horses. This stress can affect health, welfare, and/or performance [36]. During exercise, both the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis are activated, which increases the circulating levels of adrenocorticotropin (ACTH), cortisol, adrenaline and noradrenaline concentration. Furthermore, adrenaline inhibits the release of insulin from the pancreas. Catecholamines, adrenaline, and noradrenaline increase

glycogen breakdown in the muscles. Increasing the glucose level after 30km may be due to aforementioned hormones released in the blood circulation. In the liver, catecholamines, together with cortisol, increase blood glucose level by activating glycogen breakdown and gluconeogenesis. Cortisol and catecholamines also enhance the mobilization of free fatty acids from fat stores [37]. Data from this study demonstrated that there is no correlation between age groups and cortisol amounts. Similarly many reports showed no significant difference of cortisol amounts age-related, probably because of the variability of plasma cortisol, which is known to be the result of many influences [38]. The plasma cortisol level (CORT) is reported to be influenced by the duration of exercise [39]. Our work showed no significant difference in cortisol amount after 15 and 30 km. It was reported that prolonged exercise induced leukocytosis, which was caused by release of cortisol [2]. Thus if the distances in our study were longer, it may be seen cortisol elevation and leukocytosis successively. In a study conducted by Lacerda et al. [40] no influence of sex was observed in the hematological or biochemical parameters but Schmidta et al. [41] suggested cortisol release varied between training units and occasionally was more pronounced in mares than in male horse, which agrees with our finding.

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