

## Changes in Blood Parameters of Endurance Horses in 30-km Training

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### Abstract

Eight endurance horses registered for competition in different categories of Sultan Cup Endurance Ride, in November 2009 were selected for an evaluation of the soundness of the horses and an examination of the changes of blood parameters in training prior to the competition. Three blood samples were taken from each horse: pre-ride, immediate post ride and 24 hours post-ride. All horses were trained at 30 km and departed at the same time and tested on the same track. Blood samples were evaluated for both haematology and biochemistry components: red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), segmented neutrophil, lymphocyte counts, electrolytes concentration, total protein (TP), aspartate aminotransferase (AST), creatine kinase (CK) and lactate. One horse was diagnosed with exertional rhabdomyolysis post-training and was removed from statistical analysis. In this study, the significant changes in most blood parameters indicated that the 30-km endurance training induced some physiological responses in horses with minimal stress and loss of water and electrolytes as reflected in the changes of blood parameters. Although elevation in serum muscle enzymes and lactate was significant, it was believed to be a normal physiological response of horses towards training without noticeable muscle injuries and/or metabolic acidosis.

**Keywords:** training, endurance horses, haematology, biochemistry, adaptive physiological response

### Introduction

Through evolution and artificial selection, horses have become extraordinary athletes compared to other species of animals of the same size. With high maximal aerobic capacity, large intramuscular storage of energy substrates especially glycogen, high mitochondrial volume in muscles, the ability to increase oxygen carrying capacity via splenic contraction, and the efficiency in thermoregulation as well as in their gaits, have produced horses with excellent athletic ability (Hinchcliff, 2005).

Training is defined as the induction of anatomical, physiological and functional adaptations in response to stresses and strains through repetitive exercise (Hinchcliff, 2005). Hence, appropriate training program induces favorable adaptation, which increases the fitness and improves the performance of horses. A fit endurance horse, on the completion of an endurance ride, must still be sound in the aspect of metabolism and gait. In terms of metabolic evaluation, it is the determination of physiological

soundness, which can be reflected in the changes of blood parameters in both components of haematology and biochemistry. This study is to determine the trend of changes of blood parameters in endurance horses trained under tropical condition and to evaluate the fitness and soundness of endurance horses by examining different blood indicators before entering the competition.

## **Materials and Methods**

Eight clinical healthy Arabian horses registered to participate in Sultan Cup Endurance Rides, in November 2009, were selected for the study. Training at 30 km was organized two weeks prior to the competition, though horses were regularly trained before this, but at a shorter distance of 10 to 20 km. The training started in the morning, with all horses starting simultaneously on the same track, and progressed at the speed of 16 km/h. Water points were provided at every 10 km so all horses had access to water. It ended within 2 hours, when all horses returned at the same time for veterinary check.

Three blood samples were taken from the jugular vein of each horse, i.e. pre-ride, immediate post-ride and 24 hours post-ride samples. Each sample of blood was collected into a plain blood tube and an EDTA-containing tube. Blood in plain tubes was centrifuged and serum obtained was kept in serum tubes for biochemistry testing. Haematology component was evaluated for differential leukocyte count, microhematocrit for PCV while others were tested using a haematology automatic analyzer. All serum samples were processed using a Hitachi 902® biochemistry automatic analyzer.

## **Results**

All horses which returned from the 30 km training were fit with only 5 minutes of recovery period for heart rate measurement, except for a horse that had an extended heart rate recovery period of 30 minutes. During veterinary check, all horses were sound and fit without noticeable dehydration, and muscle fatigue, except for the horse with a prolonged recovery period which was detected to have muscle stiffness and lameness and had persistent and exceptionally high serum aspartate aminotransferase (AST) and creatine kinase (CK) post training in the blood. Thus a final diagnosis of exertional rhabdomyolysis was made on the horse and it was excluded from the statistical analysis as the AST and CK values post training were considered to be outliers.

### ***Haematology***

Data are presented as mean  $\pm$  standard error for haematology parameters studied, as shown in Table 1.

The mean values for immediate post training were the highest for all parameters studied except for lymphocyte count, which was the lowest compared to that of pre-training and 24 hours post training. Significant ( $P < 0.05$ ) differences were observed between pre-training and immediate post-training in total WBC count, segmented neutrophil count, and lymphocyte count, while significant differences between immediate post training and 24 hours post-training were detected in all parameters except for lymphocyte

count. While expecting many of the blood parameters would return to pre-rides values, however, significant differences were noticed between pre-training and 24 hours post training in RBC count, PCV, and segmented neutrophils. Therefore, the hypothesis of no differences in the above parameters were rejected.

**Table 1.** Haematology parameters of horses in training

Blood Parameter	Pre-Training	Immediate Post	24 Hours Post
RBC ( $\times 10^{12}/L$ )	8.07 <sup>a</sup> $\pm$ 0.35	8.35 <sup>a</sup> $\pm$ 0.31	7.17 <sup>b</sup> $\pm$ 0.27
PCV (L/L)	0.41 <sup>a</sup> $\pm$ 0.01	0.42 <sup>a</sup> $\pm$ 0.01	0.36 <sup>b</sup> $\pm$ 0.01
WBC ( $\times 10^9/L$ )	7.74 <sup>a</sup> $\pm$ 0.37	10.08 <sup>b</sup> $\pm$ 0.67	8.03 <sup>a</sup> $\pm$ 0.18
Segmented Neutrophil ( $\times 10^9/L$ )	4.36 <sup>a</sup> $\pm$ 0.21	7.33 <sup>b</sup> $\pm$ 0.71	5.22 <sup>c</sup> $\pm$ 0.22
Lymphocyte ( $\times 10^9/L$ )	2.53 <sup>a</sup> $\pm$ 0.16	1.93 <sup>b</sup> $\pm$ 0.09	2.10 <sup>ab</sup> $\pm$ 0.19

All values are expressed as mean  $\pm$  se.

<sup>a,b,c</sup>Means with different superscripts, within the same row are significantly different at  $P < 0.05$ .

**Table 2.** Biochemistry parameters of horses in training

Blood Parameter	Pre-Training	Immediate Post	24 Hours Post
Na <sup>+</sup> (mmol/L)	136.87 $\pm$ 0.39	136.77 $\pm$ 1.34	137.74 $\pm$ 0.41
K <sup>+</sup> (mmol/L)	3.94 <sup>a</sup> $\pm$ 0.08	2.69 <sup>b</sup> $\pm$ 0.08	3.96 <sup>a</sup> $\pm$ 0.09
Cl <sup>-</sup> (mmol/L)	98.93 <sup>a</sup> $\pm$ 0.38	94.09 <sup>b</sup> $\pm$ 0.92	100.46 <sup>c</sup> $\pm$ 0.48
TP (g/L)	71.27 <sup>a</sup> $\pm$ 1.15	78.51 <sup>b</sup> $\pm$ 0.92	72.46 <sup>a</sup> $\pm$ 1.54
AST (U/L)	336.09 <sup>a</sup> $\pm$ 25.40	391.94 <sup>b</sup> $\pm$ 30.30	368.46 <sup>c</sup> $\pm$ 25.89
CK (U/L)	176.00 <sup>a</sup> $\pm$ 13.42	316.86 <sup>ab</sup> $\pm$ 62.64	241.86 <sup>b</sup> $\pm$ 30.76
Lactate (mmol/L)	0.97 <sup>a</sup> $\pm$ 0.05	2.33 <sup>b</sup> $\pm$ 0.23	0.76 <sup>c</sup> $\pm$ 0.07

All values are expressed as mean  $\pm$  se.

<sup>a,b,c</sup>Means with different superscripts, within the same row are significantly different at

### **Biochemistry**

Table 2 presents the data expressed in mean  $\pm$  standard error. No significant changes ( $P > 0.05$ ) were observed for serum Na concentration of all samples. Apart from that, it was obvious that the samples taken instantly post training demonstrated the lowest means for serum K<sup>+</sup> and Cl<sup>-</sup> concentration, while it showed the highest means for serum total Protein (TP), AST, CK and lactate compared to the other two samples. Significant difference was found in serum K<sup>+</sup> concentration between the means of pre-ride and immediate post ride and also between immediate post ride and 24 hours post ride, but there is no significant change between pre-ride and 24 hours post-ride samples, indicating the recovery to pre-ride values. Similar finding was observed for TP. On the other hand, serum Cl<sup>-</sup>, AST, and lactate had significant changes in all levels, i.e. pre-training, immediate post and 24 hours post training. While changes occurred in serum

CK level, significant changes of mean was only observed between pre-training and 24 hours post training.

$P < 0.05$ .

## **Discussion**

In comparison to previous studies that involved longer distances in temperate condition, endurance horses training at 30 km under tropical condition had consistent findings, with significant changes in blood parameters immediate post-training and reversion of these changes 24 hours later, except for RBC count, PCV and CK parameters.

Significant increase of segmented neutrophil count was also reflected in a significant increase of leukocyte count immediately post training, and with a significant reduction of lymphocyte count immediate post training, which indicated that the training at 30 km had caused stress to the endurance horses. Stress leukogram of neutrophilia and lymphopenia was typically observed in two horses immediately post training, though the other 5 horses only showed mild to modest changes in segmented neutrophil and lymphocyte count as was also reported by Carlson (1987) and Snow (1983).

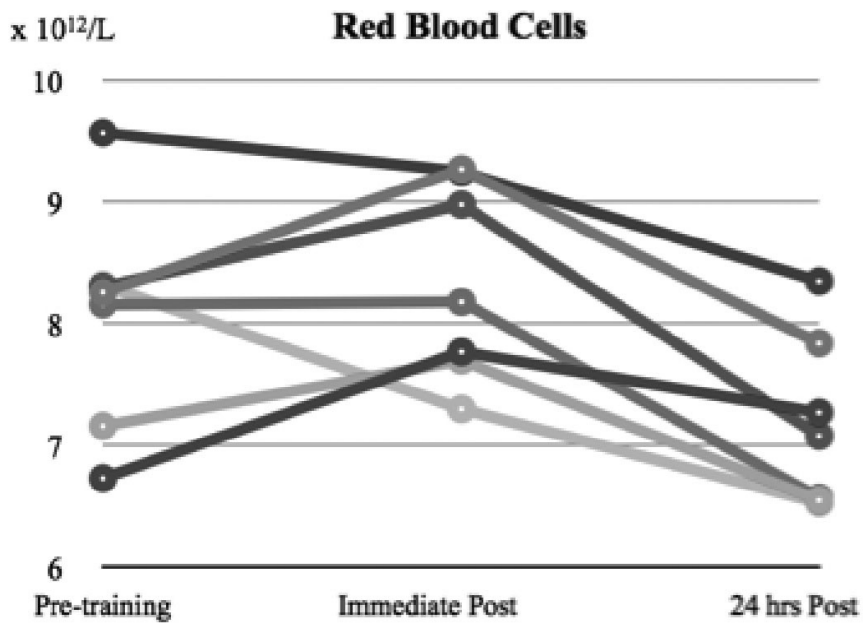
As the  $\text{Na}^+$  concentration was closely associated with the volume of water intake during and after the training, 3 out of 7 horses showed reduction immediately post training while 4 horses showed elevation. Therefore, there was no significant changes in  $\text{Na}^+$  concentration immediately post training, consistent with earlier findings but a net loss of  $\text{Na}^+$  through sweating was expected (Topliff, 2006; Grosskopf, 1983). The loss of  $\text{K}^+$  and  $\text{Cl}^-$  ions through sweating was significant immediately post training, but the magnitude of loss was estimated to be lesser than that of  $\text{Na}^+$  loss. (Topliff, 2006; Carlson, 1987). At the same time, the loss of water through sweating was high immediately post training, which was reflected in the significant elevation of TP.

All AST values exceeded normal range (120 – 160 U/L) for all three samples, which was most likely caused by regular training, causing minor muscle injuries or muscular leakage. With prolonged removal half life of AST, there was not much decrease 24 hours post training (Valberg, 2006). Increase energy demand had caused a significant elevation of lactate values immediately post training, that there was an increase in glycolysis over the threshold of oxygen-dependent citric acid cycle and electron transport chain leading to the increase of conversion of pyruvate to lactate. However, with all values of serum lactate less than 4 mmol/L, it is estimated that all horses worked aerobically (Islas, 2006; Topliff, 2006).

Blood parameters that were not congruent with earlier findings were RBC count, PCV and CK. As PCV is closely related to RBC count, similar statistical results were obtained. There was no significant change between pre ride and immediate post ride for both RBC count and PCV. Through individual interpretation, there were two horses with elevated pre-training values as indicated in Figures 1 and 2, which was highly suggestive of the cause of excitement during blood sampling as they were unfamiliar with the procedures, or due to the transportation of these horses to the track for the preparation for training

(Carlson, 1987; Persson, 1983). Besides, it could be due to higher water intake during and after the training. As for CK, though the value of immediately post training was the highest among all, it was not a significant increase from its pre-training sample. From individual interpretation, there were two horses with exceptionally elevated CK readings immediate post training, that caused noticeable individual variation (Figure 3).

All changes in blood parameters recovered 24 hours later, although segmented neutrophil count, lymphocyte count, and AST did not return to the pre-ride values due to their prolonged correction due to disruption of the homeostasis caused by the training (Valberg, 2006; McWilliams, 1995).



**Figure 1.** Changes of RBC count in individual horses

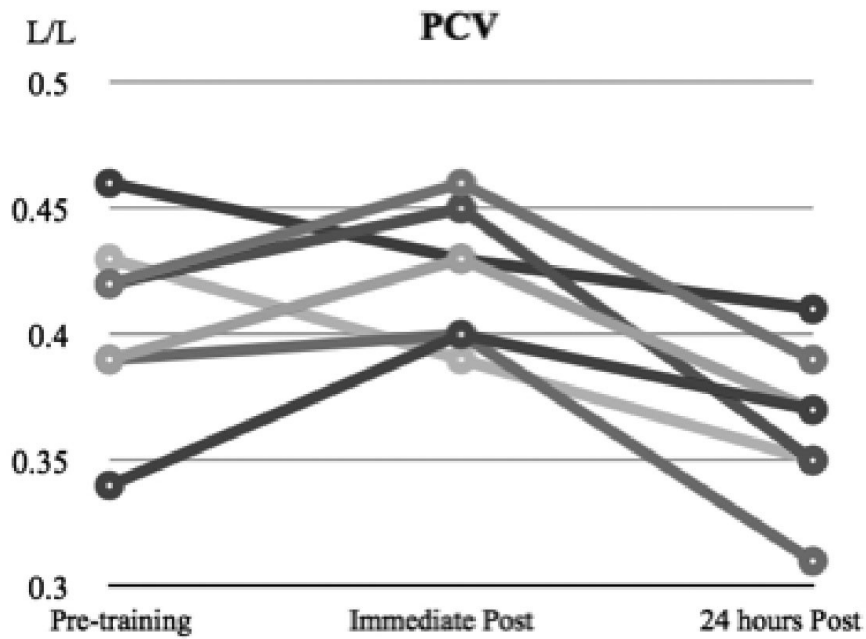


Figure 2. Changes of PCV in individual horses

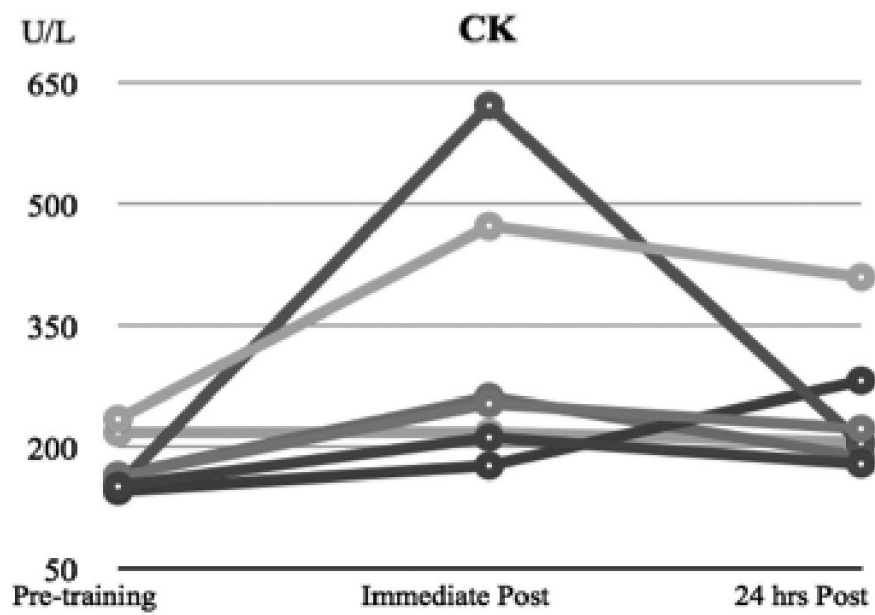


Figure 3. Changes of serum CK in individual horses

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