

The Effects of Endurance Training on the Hemogram of the Horse

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ABSTRACT : The purpose of this study was to evaluate the changes and readjustment capacity in the hematological characteristics of the horse during and after a prolonged training program. One pony and two hot-blooded horses were used in this study. Resting or basal blood parameters were assessed by collecting blood samples of the animals for 1 to 2 months prior to start of the training program. Each animal was subjected to arbitrary exercise for 30 min by an automatic hot trotter and was bled at 0, 15, 30, 45 (15 min of recovery), 60 (30 min of recovery), and 75 min (45 min of recovery) after onset of exercise. All animals were exercised 3 times a week over a five-month period. Hematological parameters including average white blood cell counts (WBC, $\times 10^3/\mu\text{l}$), erythrocyte concentrations (RBC, $\times 10^6/\mu\text{l}$), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), number of platelets (PLT, $\times 10^4/\mu\text{l}$), hemoglobin concentration (Hb, g/dl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dl) were analyzed using an automatic cell counter. All animals showed that RBC, WBC, and HCT were significantly ($p < 0.05$) increasing from 7.09, 8.55, and 43.5 to 8.11, 9.67, and 49.5, respectively, during the 30 min of exercise and were back to or lower than the initial basis (resting and 0 min) 30 min after exercise. However, no significant differences were detected in MCV (50.3-51.3 fl), MCH (17.2-17.4 pg), and MCHC (33.7-34.4 g/dl) values ($p > 0.05$) regardless of the training periods. Similar trends were observed after 1, 3, 4, and 5 months of training when compared to the resting state. When these parameters were analyzed by the effect of training periods (month), mean WBC concentrations significantly reduced in the fourth and fifth month after onset of training compared to that in resting condition or the first month of training program ($p < 0.05$). The RBC values elevated at the second month (9.40) and reaching a significantly low level ($p < 0.001$) at the fifth month (8.62) after training compared to the first month of training (7.89). In conclusion, a mild training program enhances blood parameters gradually in both the horse and the pony. Therefore, an optimized training program is beneficial in promoting the endurance performance of the horse. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 9 : 1348-1353)

Key Words : Arbitrary Exercise, Endurance Training, Hematology, Horse

INTRODUCTION

Horses are the fastest animals among the four main athletic species including humans, camels, and greyhounds. Horse racing has been a great industry in many countries all over the world. Therefore, horses attracted great attention in the study of athletic science only next to that in humans (Derman and Noakes, 1994). The performance of a horse during competition is the result of a combination of many complex interactions. These 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.

The first factor that influences athletic performance of horses is its genetic background. It is obvious that the major focus of the horse racing industry was on the animal's past, i.e., in examining the breeding history of the horse. As a result, progress in equine exercise science research lags behind that in humans by decades. Little attention, if any, has been paid to its future, which should involve the application of scientific knowledge and techniques to the study of equine science and/or horse racing. Training would

be one of the critical factors that enable a potent animal to achieve its full performance. In previous work, we initiated the investigation of blood cell parameters and their reproductive characteristics of the horse in central Taiwan (Ju et al., 1993, 2002). In continuation of this work, we looked further into the changes of these parameters during exercise and after a prolonged period of moderate training. This information would be of value for understanding the exercise physiology and training of the mixed-breed horses in Taiwan.

MATERIALS AND METHODS

Animals, feeds and feeding

Three healthy mature horses, including one 6 years old pony and two 8-year old hot-blooded horses (Quarter and Quarter \times Thoroughbred), were used in this study. Animals were kept individually in an area of 3.5 \times 3.5 m² stable and a common peddock was available by the animals twice daily. Total feed was supplied based on 2.5% of their body weight. Animals were fed three times a day with 1-2 kg alfalfa cubes supplemented with 0.5-1.0 kg/day of oats. *Ad libitum* roughages and free choice of water were offered.

Training program and sample collection

Animals were raised normally without additional loading of exercise 1-2 months prior to onset of the training

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program. In this pre-training period, blood samples of the horses were collected once in a week and analyzed as the resting level. A diameter of 8 m lounging area was provided for the training purpose. Several trial lounging were applied to the animals during this period. In the training program, horses were led by an automatic hot trotter for 30 min (Figure 1), during which the direction of movement was reversed at 15 min after trotting. Blood was withdrawn from the jugular vein prior to (0 min) and after (15, 30, 45, 60 and 75 min) exercise with 15 min intervals using heparin-rinsed plastic syringes attached to a gauge #16 epidermic needle (Rose and Hodgson, 1994). All horses were subjected to a 30 min training for 3 times weekly over a 5 month period, but only one blood sample was collected each week. After collection, blood samples were stood in an icebox (4°C) till accomplishment of all collections and were then carried back to the lab for analysis in 2 h.

Evaluation criteria

Hematological parameters including white blood cell counts (WBC, $\times 10^3/\mu\text{l}$), red cells concentrations (RBC, $\times 10^6/\mu\text{l}$), platelet concentrations (PLT, $\times 10^4/\mu\text{l}$), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), hemoglobin concentration (Hb, g/dl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dl), and mean platelet volume (MPV, fl) were analyzed using an automatic cell counter (Sysmex F-800).

Statistical analysis

The means of hematologic parameters were analyzed using General Linear Model (GLM) in SAS software (1988). The effects of time of exercise and training durations on all variables observed among horses were compared using the LSMEANS procedure.



Figure 1. A speed-adjustable hot-trotter was used for the endurance training. The horse was arbitrary trotted for 30 min three times a week in an 8 m lounging area. During the training period, blood samples were collected prior to and after exercise in a 15 min interval.

RESULTS

Resting levels of hematologic parameters

Prior to onset of the training program, resting levels of the horses' hematologic parameters or indices were assessed for 8 weeks.

All hematologic indices including WBC ($8.45 \times 10^3/\mu\text{l}$), RBC ($7.99 \times 10^6/\mu\text{l}$), HCT (40%), MCV (50.1 fl), PLT ($177 \times 10^3/\mu\text{l}$), Hb (13.7 g/dl), and MCHC (34.4 g/dl) values were relatively constant and no significant differences in these parameters were observed among animals ($p > 0.05$, Table 1). However, MCH and MPV differed significantly among animals ($p < 0.001$, Table 1). Although not significant, RBC counts appeared slightly greater in the pony (Horse #3) than in other horses (8.62 vs 7.87 and 7.85, $p > 0.05$). This information served as the background for comparison with their subsequent exercise and training effects.

Changes of hematologic parameters after exercise and training

Average RBC counts of the horses increased immediately to a plateau in 15 and 30 min of exercise (Figure 2). When animals were removed from the trotter, RBC counts also dropped immediately to a level even significantly lower than that prior to exercise (8.55 vs $7.47 \times 10^6/\mu\text{l}$, $P < 0.001$). A similar trend was observed in the WBC concentrations (7.09 vs $6.51 \times 10^3/\mu\text{l}$, $p < 0.001$), HCT values (43.5 vs 38.0%, $p < 0.001$), and Hb concentrations (14.8 vs 13.0 g/dl, $p < 0.001$) after exercise (Figures 2, 3 and 4). However, no significant changes were observed in MCV, MCH, MCHC, and MPV values ($p > 0.05$) prior to and after exercise (Figures 3, 4 and 5).

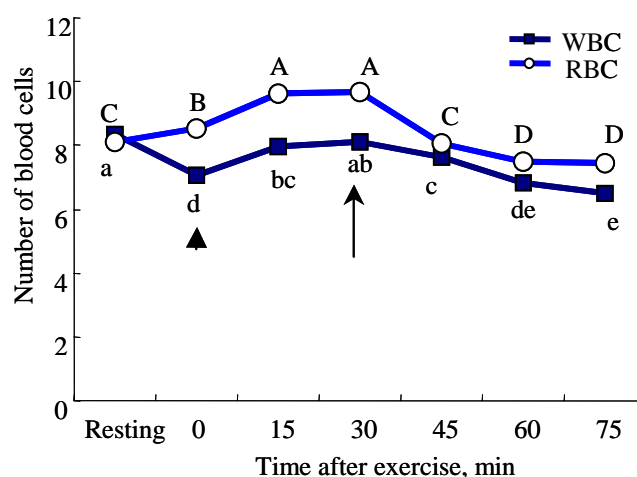


Figure 2. The effects of arbitrary exercises on the blood cell counts in horses. Means without the same alphabetic characters at different time points within the same parameter represent statistical differences ($p < 0.01$), i.e., ABCD for RBC and abcde for WBC counts (WBC: White blood cells, $\times 10^3/\mu\text{l}$; RBC: Red blood cells, $\times 10^6/\mu\text{l}$; arrow head: beginning of exercise; arrow: the end of exercise).

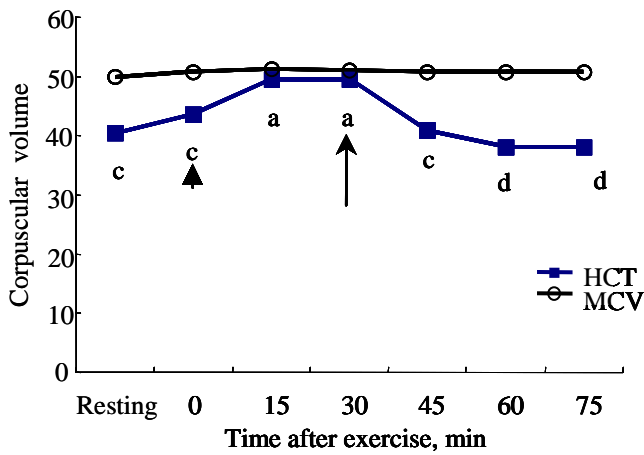


Figure 3. The effects of arbitrary exercises on the hematocrit and mean corpuscular volume of horse blood. Means without the same alphabetic characters at different time points within the same parameter represent statistical differences ($p < 0.01$; HCT: Hematocrit, %; MCV: Mean corpuscular volume, fl; arrow head: beginning of exercise; arrow: the end of exercise).

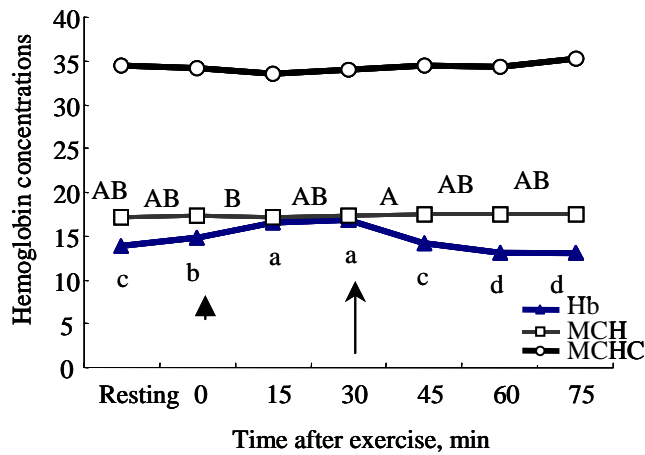


Figure 4. The effects of arbitrary exercises on the hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentrations of horse blood. Means without the same alphabetic characters at different time points within the same parameter represent statistical differences ($p < 0.01$, i.e., AB for MCH and abcd for Hb concentrations (Hb: Hemoglobin, g/dl; MCH: Mean corpuscular hemoglobin, pg/dl; MCHC: Mean corpuscular hemoglobin concentration, g/dl; arrow head: beginning of exercise; arrow: the end of exercise).

The effects of the 5 month endurance training on the hematology of the horse are presented in Table 2. The WBC counts decreased from $8.41 \times 10^3/\mu\text{l}$ in the first month to $7.31 \times 10^3/\mu\text{l}$ in the fifth month of exercise. A different pattern of RBC counts was observed. It increased to a plateau during the second and the third month and then decreased slightly during the fourth and the fifth month of the training period, which were significantly greater than

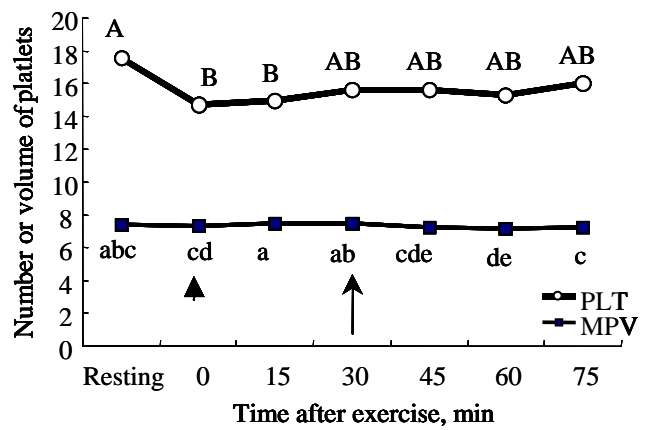


Figure 5. The effects of arbitrary exercises on the platelet concentrations and mean platelet volumes of horse blood. Different alphabetic characters at different time points within the same parameter represent statistical differences ($p < 0.01$), i.e., AB for PLT and abcde for MPV (PLT: Platelet, $\times 10^4/\mu\text{l}$; MPV: Mean platelet volume, fl; arrow head: beginning of exercise; arrow: the end of exercise).

that in the first month (8.41 and 8.62 vs $7.89 \times 10^6/\mu\text{l}$, $p < 0.05$; Table 2). The effect of training on HCT was closely similar to that on RBC counts. The pattern of PLT counts varying with the length of training appeared to be different from those of RBC and HCT values. It decreased during the 2-3 months after training and then returned to the initial value (the first month). However, MPV was the only parameter that reduced from the first month (7.38 fl) to a value (7.22 fl) lower than that at the beginning of training (Table 2).

Hb concentrations significantly increased from 13.7 g/dl for the first month to 16.3 g/dl during the second and the third months, and then compromised slightly (14.6 - 15.0 g/dl) during the last 2 months of training. No significant changes were observed in the MCV, MCH and MCHC during the 5 month training of the horse (Table 2).

DISCUSSION

The resting hemogram

Hematologic analysis of the racehorse at rest (resting values) is a possible mean of assessing animals' fitness. The average hematocrit (HCT) or packed cell volume (PCV) was 40.5% in this study (Figure 3) and 32.7 - 50.7% in Thoroughbred (Garcia and Beech, 1986; Steel and Stewart, 1960). It ranged from 30 to 56% with an average of $41.6 \pm 6.5\%$ for the horses in Taiwan and was significantly lower in the mare than that in the stallion (46 vs 40%) based on our study (Ju et al., 1993). However, poor reproducibility of RBC in the resting racehorse has been reported, which may be due to its large capacity of sequestering blood cells in the spleen (Garcia and Beech,

Table 1. The distribution of resting hematological parameters of individual horses

Criteria	Horse #1	Horse #2	Horse #3*	Average
WBC, $\times 10^3/\mu\text{l}$	8.22 \pm 0.45	8.80 \pm 0.39	8.00 \pm 0.63	8.45 \pm 1.09
RBC, $\times 10^6/\mu\text{l}$	7.87 \pm 0.32	7.85 \pm 0.27	8.62 \pm 0.45	7.99 \pm 0.78
HCT, %	39.2 \pm 1.83	39.4 \pm 1.58	43.0 \pm 2.59	40.0 \pm 4.48
MCV, fl	49.8 \pm 1.83	50.4 \pm 1.59	49.9 \pm 2.59	50.1 \pm 4.48
PLT, $\times 10^4/\mu\text{l}$	17.1 \pm 1.30	18.5 \pm 1.13	17.2 \pm 1.85	17.7 \pm 3.20
Hb, g/dl	14.0 \pm 0.53	13.2 \pm 0.46	14.7 \pm 0.75	13.7 \pm 1.30
MCH, pg	17.7 \pm 0.10 ^a	16.8 \pm 0.08 ^{bc}	17.0 \pm 0.14 ^b	17.2 \pm 0.23
MCHC, g/dl	35.6 \pm 1.0	33.7 \pm 0.87	34.2 \pm 1.41	34.4 \pm 2.45
MPV, fl	7.50 \pm 0.09 ^a	7.05 \pm 0.08 ^b	7.65 \pm 0.16 ^a	7.29 \pm 0.23

* Horse #3 is a pony.

^{abc} Means without the same superscripts in the same row differ significantly ($p < 0.01$).

Table 2. The monthly effects of arbitrary exercise on hematological parameters of the horse

Criteria	Month 1	Month 2	Month 3	Month 4	Month 5	Overall average
WBC, $\times 10^3/\mu\text{l}$	8.41 \pm 0.18 ^a	7.83 \pm 0.17 ^b	7.44 \pm 0.14 ^{bc}	7.51 \pm 0.11 ^{bc}	7.31 \pm 0.13 ^c	7.58 \pm 0.73
RBC, $\times 10^6/\mu\text{l}$	7.89 \pm 0.22 ^c	9.40 \pm 0.21 ^a	9.42 \pm 0.18 ^a	8.41 \pm 0.14 ^{bc}	8.62 \pm 0.17 ^b	8.70 \pm 0.91
HCT, %	40.0 \pm 1.19 ^c	48.1 \pm 1.14 ^a	48.3 \pm 0.95 ^a	42.6 \pm 0.75 ^{bc}	44.3 \pm 0.88 ^b	44.5 \pm 4.85
MCV, fl	50.3 \pm 0.36	51.1 \pm 0.35	51.3 \pm 0.29	50.7 \pm 0.23	51.4 \pm 0.27	51.1 \pm 1.47
PLT, $\times 10^4/\mu\text{l}$	18.2 \pm 0.59 ^a	10.3 \pm 0.56 ^d	14.1 \pm 0.47 ^c	16.5 \pm 0.37 ^b	17.5 \pm 0.44 ^{ab}	15.9 \pm 2.40
Hb, g/dl	13.7 \pm 0.38 ^c	16.3 \pm 0.36 ^a	16.3 \pm 0.34 ^a	14.6 \pm 0.24 ^{bc}	15.0 \pm 0.28 ^b	15.0 \pm 1.53
MCH, pg	17.2 \pm 0.09	17.4 \pm 0.08	17.3 \pm 0.08	17.3 \pm 0.06	17.4 \pm 0.07	17.4 \pm 0.36
MCHC, g/dl	34.4 \pm 0.26	33.9 \pm 0.25	33.7 \pm 0.23	34.2 \pm 0.16	34.0 \pm 0.19	34.0 \pm 1.05
MPV, fl	7.38 \pm 0.04 ^b	7.45 \pm 0.07 ^{ab}	7.61 \pm 0.04 ^a	7.26 \pm 0.03 ^c	7.22 \pm 0.03 ^c	7.37 \pm 0.17

^{abc} Means without the same superscripts in the same row differs significantly ($p < 0.01$).

1986; Rose, 1982; Schalm et al., 1982). The RBC ranged between 1.8-2.8% of the body weight at rest and up to 8% during exercise, which is workload-dependent and is mediated by sympathetically splenic mobilization (Evans et al., 1990), i.e., up to a 50% increase in the population of equine RBC in circulation during exercise (Persson et al., 1973). Therefore, HCT values in resting horses may not be a good indicator representing the whole blood profile. Intravenous injection of epinephrine (2.5 $\mu\text{g}/\text{kg}\text{-BW}$) might be required to empty the spleen reserve of RBC into the general circulation (Hanzawa et al., 1998). Negative correlation between the HCT and erythrocyte sedimentation rate (ESR) was observed in previous study (Ju et al., 1993). Although a poor correlation was reported between resting RBC and racetrack development (Evans et al., 1990), this study is valuable in determining the fitness of the training horses. It also provides illustrations of athletic horses' responding to an endurance training program under the environment of Taiwan.

The exercise hemogram

The effects of exercise : Among the blood parameters detected in this study, no obvious differences in WBC counts were observed in resting animals, although the pony mare showed a slightly greater WBC counts (Ju et al., 1993). Persson et al. (1973) reported that prolonged

exercise induced leukocytosis, which was caused by release of cortisol. The leukocyte counts usually returned to normal levels within 24 h after exercise. However, we found that the WBC levels decreased to a level below their resting or initial level in 45 min after exercise (Figure 2). The WBC counts responded clearly in a time-dependent manner after exercise as well as other parameters examined in this study including RBC, HCT, and Hb values. They largely followed a pattern of increase-with-time of exercise and then returned to their initial values at the end of recovery stage or 45 min after exercise (Figures 2-4).

It is generally agreed that about one third upto 50% of the blood cells are stored in the spleen at rest (Jones, 1989). Exercise causes production of epinephrine, which in turn expels the spleen to eject the stored RBC into peripheral circulations (Garcia and Beech, 1986; Schalm et al., 1982). However, endurance horses working at slow speeds for long periods experienced a different hematological response. Due to the incomplete splenic mobilization, the RBC, WBC or HCT would not increase as much as that in intensive exercise such as competitive racing (Evans et al., 1990).

Horses that are conditioned for high-intensity athletic bouts are characterized by increase of RBC counts, Hb concentrations, and blood volumes. No relationship between racing performance and any one of the hematological parameters has been found. However,

racehorses with resting HCT above 0.47 and below 0.36 are less likely to perform as expected. This was observed in over-trained Standardbred horses and several months were usually required for recovery (Evans et al., 1990). Horses with an HCT value consistently lower than 0.36 perform below their potential and are commonly referred to as being anemic. This results in a lowered oxygen-carrying capacity in the blood (Evans et al., 1990).

The morphology and membrane characteristics of RBC may be altered by exercise. RBC osmotic fragility (Boucher, 1987, 1989) and the proportion of the crenated RBC, an abnormal RBC morphology known as echinocytes, increased (Bessis, 1973; Boucher et al., 1981; Hanzawa and Watanabe, 2000).

The effects of training : Although environmental factors such as nutrition, track surfaces, shoes, and the jockey are all critical in a successful racing, adequate training is one of the most important variables determining athletic performance after genetics. The type and duration of training influenced animals' physiologic responses differently. Lower exercise intensities resulted in slower rates of responses, i.e., longer training time was required to achieve the same responses (Allen, 1987).

Performance of dynamic exercise depends on adequate alveolar ventilation and Hb saturation, coupled with adequate cardiac output and efficient delivery of oxygen to exercising muscles, by which the chemical energy is transformed into kinetic energy (Allen, 1987). It has been well known that oxygen is carried in the blood in two forms. Most oxygen is carried in combination with Hb but some in dissolved form. The amount of dissolved oxygen is proportional to the partial pressure of oxygen and is inversely proportional to body temperature. However, transportation of the dissolved oxygen is inadequate for physiological requirement of the animal. Therefore, oxygen-binding capacity of Hb becomes much more critical. The oxygen-carrying capacity of horse blood, generally reflected in an increased level of Hb, also increased with conditioning of the animals. This parameter is easily measured and could be an important indicator for the level of conditioning. Some trainers in fact spent big money on blood tonics to build up the Hb content of the horse. In this study, the Hb concentrations were elevated steeply after the second month of training (13.7 vs 16.3 g/dl) and eventually stayed at 15 g/dl, which was also significantly greater than that at the initial stage (Table 2). The Hb molecule comprises hemes and the protein globin. Each Hb molecule is able to carry four oxygen molecules. It has been known that oxygen combines reversibly with Hb concentration. Under normal circumstances, each gram of oxygen-saturated Hb binds 1.34 ml of oxygen. Therefore, a horse with 15 g/dl of Hb concentration in the blood has an oxygen-carrying capacity of 20.1 ml per 100 ml of the

blood. In other words, the oxygen carrying capacity of these horses increased about 2 g/dl after exercise or training. If the total blood volume is estimated as 12-14% of body weight (Evans et al., 1990), average blood volume of these horses (BW 360 kg) is around 50 liters. With 2 g/dl increase in Hb concentration, a total increment of oxygen binding capacity is 1,340 ml for each horse. When compared with the total oxygen-carrying capacity (9,179 ml) at the beginning of training, a 15% increment was achieved after this moderate training program, which is effective and significant.

McMiken (1983) reported that training increased the capacity of horses to use muscle glycogen in glycolytic pathway. Lactate concentration in circulation and number of mitochondria in muscle cells increased during exercise and after endurance training, respectively. In general, horses in less fitness showed a rise in blood lactate concentrations at lower exercise intensities or running speeds. An earlier lactate threshold appeared in a less fit horse than a fit one (McMiken, 1983). Therefore, blood lactate concentration after exercise signals how well the horse being trained (Persson and Ullberg, 1974). These important parameters will be incorporated into our next study.

In conclusions, a performance profile is incomplete without thorough clinical examinations on the musculoskeletal and respiratory, as well as on the hematologic parameters of the cardiovascular system. This profile helps to assess the response of training or racing and to detect abnormalities due to overtraining. However, The availability of time and labor limits the numbers of animals used and parameters examined. In this study, we clearly demonstrated that an effective training program promoted several hematological parameters, which would in turn enhance the performance of both the athletic and leisure horses.

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