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Effect of Training on Diverse Hematologic Parameters in Andalusian Horses

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A study has been made of the variations experienced by the hemogram and blood volume indices in 16 Andalusian horses after carrying out an exercise test of increasing intensity consisting of 3 sequences of 1000 m each, before and after being subjected to a daily exercise programme for two months. Samples were taken by external jugular vein puncture: while at rest, within the first minute of finalizing each exercise stage and at 10 and 15 minutes of recovery. Both the red blood cells and the hematocrit value showed a significant decrease in their mean values after two months training; conversely, the hemoglobin reached higher post-training figures. Similarly, the volume indices increased their mean values with training but no significant changes for total plasma proteins were observed.

Key words: Blood, Horses, Training.

The hemogram is undoubtedly one of the most useful laboratory tests as an aid to diagnosis for its speedy execution and the large amount of information derived from it (4).

For that reason, the study of blood has become a great ally for the purpose of detecting hematologic alterations induced by exercise, always taking into account the type of effort or physical work to be undertaken as well as its intensity, duration and degree of physical condition or of training. Nevertheless, in spite of the numerous investigations carried out to determine the influence of physical exercise on blood (6-9, 13-15, 17), contradictory data are frequently being obtained making it difficult to clearly establish the hematologic alterations which may be directly acquired with training.

This led to the idea of studying the alterations in the blood caused by programmed training for which an exceptional animal, the horse, was chosen, both for

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its economic importance and the part it plays in present-day society, and in a rarely studied breed, the Andalusian horses.

Materials and Methods

The investigation was made with a total of 16 Andalusian horses, all four year-old males, belonging to the Centre of Selection and Training of the Military Stud at Jerez de la Frontera (Cádiz). All the available horses were chosen without applying any selection method.

The first part of the experiment began when the animals were duly broken in to permit them to carry out the test. The foals did the exercise on an oval-shaped sandy track 1000 m long by 6 m wide, signposted in red at every 100 m (5).

A test of increasing intensity was carried out consisting of 3 sequences of 1000 m each: 1st sequence was of 15 Km/h; the 2nd, 25 Km/h and the 3rd, 32 Km/h. Every 1000 m and before increasing the speed, they were given a five-minute rest.

On the days after this test, from Monday to Friday the animals took regular exercise (training). The exercise consisted of 15-30 minutes of walking; 25-30 minutes' trotting; 12-18 minutes' galloping: the work sequence was the following: walking → 5-7 minutes of trotting to each side → walking-trotting (transition to galloping) → 5-9 minutes galloping → trotting → walking (1).

After 2 months of receiving this training, the test mentioned previously was repeated.

In both tests, blood samples were taken by external jugular vein puncture as follows: while resting, within the first minute of finishing each exercise stage and at 10 and 15 minutes' recovery. The blood was then placed in two tubes with lithium-heparin, one for its immediate centrifugation and the other for refrigeration and subsequent analysis in the laboratory.

The parameters analysed in the blood were: the hematocrit (Hc) value by the microhematocrit method; the hemoglobin (Hb) by spectrophotometry and the red blood cell count (RBC) with an "Ibercell" cell counter. From the RBC count, the hematocrit value and the hemoglobin, the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were determined.

Finally, in the plasma, the total plasma proteins (TPP) were evaluated with an inversion refractometer.

The statistical study consisted of finding out the mean and the standard deviation, as well as making a Kruskal-Wallis non-parametric analysis (since there was a heterogeneity in the variances between stages within the same group – Bartlette test), in order to compare the first group (untrained) with the second group (trained). Finally, a Student's *t* was carried out for an observation of the significant differences between the same stages in the two groups and a correlation between the different parameters analyzed in two groups of variables studied (untrained and trained) when resting.

Results

It was seen from the Kruskal-Wallis analysis that all the parameters analyzed, except total plasma proteins, showed significant differences ($p \leq 0.001$) when comparing the results obtained before training with those observed at post-training.

On carrying out the Student's *t* between the same stages in both groups (trained and untrained), a significant diminution in the red blood cell values (fig. 1) during resting and in the three

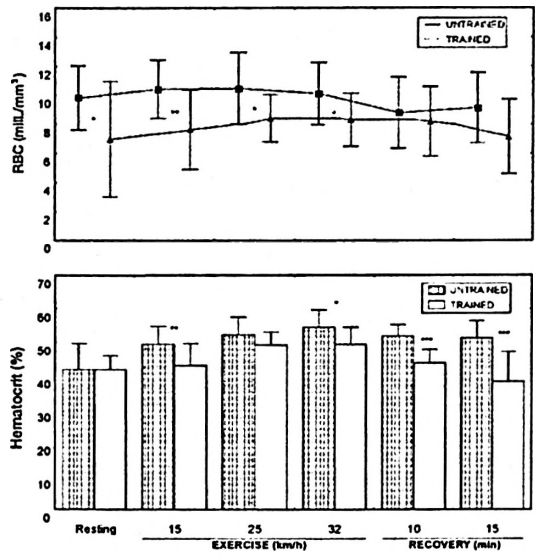

Fig. 1. *Resting red blood cells, and hematocrit in each of the exercise levels, and during recovery in trained and untrained Andalusian horses.*
Values are means ± SD. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ for difference between the two groups (*t*-test).

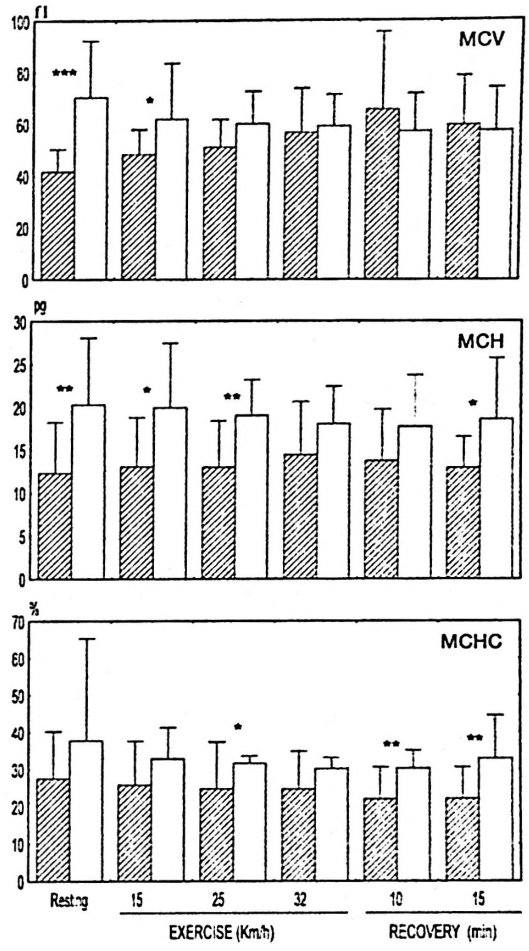

Fig. 2. *Resting MCV, MCH and MCHC in each of the exercise levels and in recovery period in Andalusian horses.*
Values are means ± SD. Trained, white square; and untrained, striped square. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$ for difference between the two groups (t-test).

exercise levels (15, 25 and 32 Km/h) was observed in the test corresponding to post-training with respect to the one made two months previously. The greatest differences ($p \leq 0.01$) were noted in the 15 Km/h stage (10.41 ± 2.00 mill./mm^3 to 7.62 ± 2.73 mill./mm^3).

The hematocrit value (fig. 1), which started at similar resting values in untrained and trained horses, also significantly decreased after training, and maximum differences ($p \leq 0.001$) were obtained in the 10 and 15 min recovery stages. However, neither the hemoglobin nor the total plasma proteins underwent any changes when comparing the same stages before and after training (over 13 g/dl).

An increase in mean corpuscle volume (fig. 2) with training was revealed in the rest period and in the 15 Km/h stage, as well as an increase in mean corpuscle hemoglobin at rest and in the 15 and 25 Km/h and 15 min recovery stages.

The mean corpuscle hemoglobin concentration reached significantly higher values in the 25 Km/h levels (31.6 ± 2 %) and at the two recovery stages (10 and 15 min) when they were compared with the figures obtained before training.

Discussion

The mean red blood cell values obtained when resting in untrained Andalusian horses showed similar figures to those reported by RUBIO *et al.* (14) in stallions of the same breed, and to those proposed by MCCLAY *et al.* (6) and ROSE (10 - 12) in horses of different breeds; conversely, the resting trained horses displayed similar values to those observed by BARREY and VALETTE (2) in French breeds. Both before and after training, a rise in mean red blood cell values was noted when the animals were subjected to a progressively-increasing intensity speed test. This was as a consequence of an increase in the sympathetic activity responsible for spleen contraction and a speed of 25 Km/h was seen to cause the greatest stress in the animal which was reflected in a maximum value in the number of red blood cells.

The fact that there were lower mean red blood cell values after training possibly meant that, on one hand, the animals had become accustomed to being handled, which would result in a lesser degree of stress and, on the other, that there might be a hemodilution caused by an increase in plasma volume with training and/or a continuous hemolysis caused by intense daily exercise (16).

The mean values for the hematocrit of the resting animal, both trained and untrained, coincided with those reported in previous studies made on foals and stallions of the same breed (14).

After two months' training, the resting hematocrit value was similar to that obtained in untrained animals in spite of starting with a lower total number of red blood cells. This was probably due to the great variability shown by this parameter (see SD, fig. 1) in that group of animals, and to a possible macrocytosis.

As it occurred with the number of red blood cells, exercising signified an increase in the hematocrit value, which was more apparent in the untrained animals, but low hematocrit values were maintained in the trained animals. Therefore, it was suggested that, together with the aforementioned hemolysis, when the degree of training was higher, the possible hydric losses during exercise were smaller, with less notable reductions in plasma volume. On the other hand, the most significant differences between the two groups of animals were obtained during recovery, since in the trained animals not only was the increase in the post-effort hematocrit smaller but, also, a return to its normal figures was much sooner in time.

The mean values of blood hemoglobin were higher in trained animals in spite of the fact that the number of red blood cells was lower in that group; this increase might be a result of a hemolysis occurring during training. Likewise, the increase obtained in the total plasma proteins of trained animals might be due to an increase in plasma hemoglobin. As it occurred with the number of red blood cells and the hematocrit, an increase in both parameters with exercise was evident.

The mean corpuscle volume obtained in this work for resting trained animals was seen to be significantly higher than in the untrained animals, which corroborated the idea of a hemolysis occurring during training with a resulting decrease in the number of red blood cells and a consequent greater production from the bone marrow of new red blood cells (macrocytic) with a higher MCV than usual, since young erythrocytes are larger in comparison with older cells (3).

Besides, the diminution of the total number of red blood cells and the hematocrit and the increase in blood hemoglobin after training resulted in higher mean

values of hemoglobin indices (MCH and MCHC) in the trained animals.

Furthermore, a positive and significant correlation between the hematocrit value and the number of red blood cells was only observed in the untrained group, whilst in the trained animals the correlation was significant and positive for hemoglobin and total plasma proteins. These results reinforced the hypothesis of an intravascular hemolysis suffered as a consequence of the daily exercise to which the animals studied had been subjected.

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B. M. ESCRIBANO, F. M. CASTEJÓN, R. SANTISTEBAN, E. I. AGÜERA y M. D. RUBIO. *Efecto del entrenamiento sobre diversos parámetros hematológicos en caballos andaluces.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (4), 207-212, 1995.

Se estudian las variaciones que experimenta el hemograma y los índices volumétricos sanguíneos en 16 caballos de Pura Raza Española tras realizar una prueba de intensidad creciente, compuesta por tres series de 1000 m cada una, antes y después de ser sometidos a un programa de ejercicio diario durante dos meses. La toma de muestras se realiza por punción en la vena yugular externa en reposo, dentro del primer minuto de finalizada cada etapa de ejercicio y a los 10 y 15 minutos de recuperación. Tanto los eritrocitos como el valor hematocrito experimentan un descenso significativo de sus valores medios tras dos meses de entrenamiento; por el contrario, la hemoglobina alcanza cifras más altas en el post-entrenamiento. Asimismo, los índices volumétricos incrementan sus valores medios con el entrenamiento pero no se observan cambios significativos para las proteínas plasmáticas totales.

Palabras clave: Sangre, Caballos, Entrenamiento.

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