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Haematological Response Associated with Repeted Show Jumping Competition in Horse

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ABSTRACT

***Background*:** Several studies have demonstrated the beneficial effect of exercise; even in human that in horse, but exercise could also represent a physical stress that challenging body homeostasis. Exercise stress pushes the body to find a dynamic equilibrium through adaptive changes to ensure proper management of stress caused by maximal exercise. These changes affect various composition of body fluids, in particular changes in blood composition including an increase in Red Blood Cells (RBC), Hematocrit (Hct) and Hemoglobin (Hgb) values and a change in Platelets (Plt) count. The objective of the present study was to evaluate the effect of repeated jumping exercise on haematological parameters in horse.

***Material, Methods & Results*:** For this purpose we evaluated the modifications occurring in cell blood count (CBC) in horses during two international three stars *** show jumping competition performed in two consecutive weekend. After routine clinical examination, twelve well trained Italian Saddle horses were enrolled for this study. For every subject, Red blood cell (RBC), haematocrit (HCT), haemoglobin (HGB) and platelets (PLT) were assessed. Blood samples were collected: in the morning after the meal (7:30-8:00 AM), before the beginning of the competition (R_1) and within 10 min after competition ending (C_1S_1, C_1S_2) and on the day after competition (R_2). The same procedure was followed on the second weekend (C_2S_1, C_2S_2, R_3). Whole blood samples were placed on ice pending analysis that were performed within 2 h after collection and immediately taken to the laboratory for analysis. The obtained data were analyzed with Stats package of R: R Core Team (2013). One-way repeated measures analysis of variance (ANOVA) showed a significant effect on sampling time ($P < 0.05$) concerning every studied parameter. In particular a significant increase of RBC in C_1S_1, C_1S_2, C_2S_1 and C_2S_2 versus R_1, R_2 and R_3. Same results were found for Hgb and Hct that have showed a significant increase in C_1S_1, C_1S_2, C_2S_1 and C_2S_2 versus R_1, R_2 and R_3. A significant increment in Plt was found in R_3 and R_2 versus R_1 and in R_3 versus C_1S_1 and C_1S_2.

***Discussion*:** In this study it was possible to observe an increasing of RBC, Hgb and Hct levels immediately after the exercise and returning to basal levels during resting time. Instead Plt values increase during resting time and the proximity of the competition induce a thrombocytosis during the second weekend. These increases may be related to the mechanisms of physiological adaptation required in course of exercise, increased oxygen demand by the muscles, release of circulating catecholamine resulting in spleen contraction, and to negative effect of the exercise like fluid loss resulting in dehydration. The return to normal levels during the resting time and the similar trend during the second weekend denotes as a horse, that has been well trained, is able to counterbalance the stress of two sessions of competition so close together. The trend of Plt denotes an increase during the resting time. This demonstrates the slowness in the reuptake of these, so during haematological evaluation in athlete particular attention must be put to Plt evaluation, because exercise stress induces not only the increase of their number, but also the increase of their reactivity and of their capacity to form aggregates that can be implicated in thrombosis and some other cardiovascular diseases.

Keywords: haematological parameters, horse, show jumping competition, physical exercise.

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INTRODUCTION

Exercise could represents a physical stress challenging body homeostasis [29]. The body reacts to stress with adaptive changes that can influence the composition of circulating blood [1,9,11,25,27] causing an increase in Red Blood Cells (RBC) number, Hematocrit (Hct) and Hemoglobin (Hgb) values and a change in Platelets (Plt) count [5,6,14]. Hematological adaptations guarantee a proper muscle function and can influence athletic performance. They are strictly related to catecholamines concentration. Catecholamines, in fact, showed several effects in athletic horse [7] such as spleen contraction. Horse spleen stores 6-12 L of red-cell-rich blood [12] and its contraction can increase some hematological values and can change blood composition and pressure. Variations in blood rheology can be also induced by post-exercise fluid shift increasing blood viscosity and several haematological values. Moreover during maximal exercise intravascular haemolysis could increase HGB value [21]. In fact, the mean erythrocyte osmotic fragility curve shifts leftward during exercise toward a position of increased fragility [8].

The aim of the study was to investigate the changes of RBC, HCT, HGB and PLT in horses during two sessions of jumping show in order to enhance the knowledge of rheological changes in blood occurring during exercise and to ensure a proper line of management minimizing the negative effects of exercise.

MATERIALS AND METHODS

Animals

Twelve regularly trained Italian Saddle horse (7 geldings and 5 females, 10-12 years old, mean body weight 490 ± 30 kg) were enrolled in this study with the informed owner consents. Before starting the study, horses were subjected to clinical examination, routine haematology and biochemistry at rest conditions, and only healthy subjects were used. Animal were feed four times a day (at 7:00, 11:00, 15:00, 19:00).

The diet consisted of hay (first cut meadow hay, sun cured, and late cut; 8 ± 1 kg/d; 6.9% crude protein on average) and mixed cereals (oats and barley, 50% each; 3.5 ± 0.5 kg/d), three times a day (at 7 AM, 12 AM, and 6 PM). Cereal mixture composition (dry matter basis) was 13.0% crude protein, 20.7% crude fiber, and 3.4% other extracts; the estimated net energy content was 0.8 UFC (Unitè Fouragire Cheval).Water was available *ad libitum*. Horses took part to International *** jumping competition "Sicilia jumping tour 2011"(Sicily latitude 37.46 N; longitude 14.93 E). Each session was preceded by 20 min warm-up consisting in: walk, trot and canter with six jumps (height: from 100 to 140 cm). All shows jumping were performed at about the same time every day.

Competition type, course length, obstacle height and environment recording are shown in Table 1.

Blood sampling

Blood samples were collected by jugular venipuncture in vacutainer tubes containing EDTA[1]. The sampling was performed before the first competition day (R_1), within 10 min from the end of each exercise (C_1S_1, C_1S_2) and day after the competition (R_2), same plan was followed during second weekend (C_2S_1, C_2S_2, R_3). Whole blood samples were placed on ice pending analysis that were performed within 2 h after collection and immediately taken to the laboratory for analysis and analysed by means of an automated analyzer ultraviolet-visible spectrophotometer[2].

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). One-way repeated measures analysis of variance (ANOVA) was applied to determine statistically significant effects of exercise on Rbc, Hgb, Hct and Plt. in horses involved in this study. P values < 0.05 were considered statistically significant. Bonferroni's multiple comparison test was applied for post hoc comparison. Statistical analysis was performed using Stats package of R (R Core Team (2013) / (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, 2013, URL: http://www.R-project.org/)[3].

RESULTS

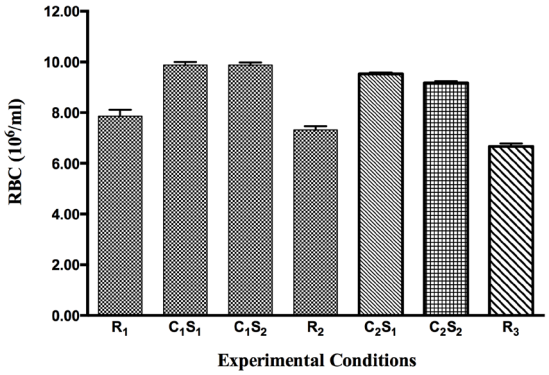
ANOVA showed significant effect of the exercise on RBC ($P < 0.05$), Hgb ($P < 0.05$), Hct ($P < 0.05$) and Plt ($P < 0.05$). Specifically, has given $P < 0.005$ for RBC, Hct, Hgb and Plt.

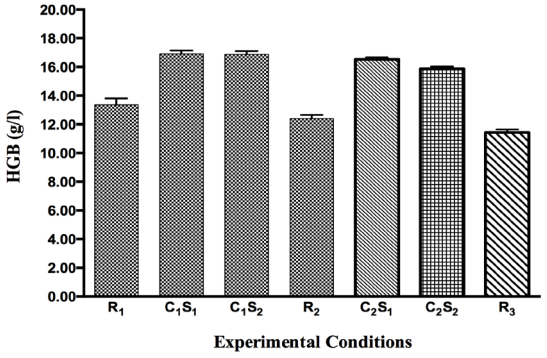
Bonferroni multiple comparison test showed, in particular, a significant increase of RBC in C_1S_1, C_1S_2, C_2S_1 and C_2S_2 versus R_1, R_2 and R_3. Same results were found for Hgb and Hct that have showed a significant increase in C_1S_1, C_1S_2, C_2S_1 and C_2S_2 versus R_1, R_2 and R_3.

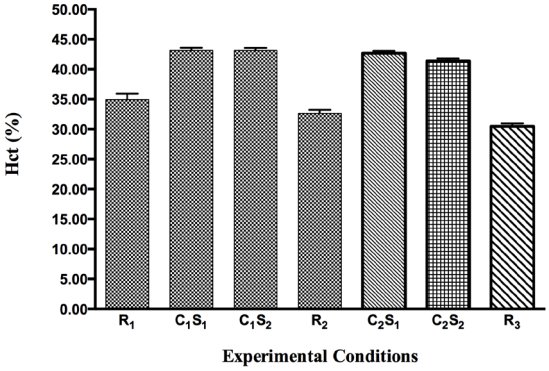
A significant increment in Plt was found in R_3 and R_2 versus R_1 and in R_3 versus C_1S_1 and C_1S_2.

Table 1. Competition type, course length, obstacle height and environmental conditions recording during the two weekends of competition.

Competition 1			
Time	Race type	Course and obstacle	Ambiental enviroment
First day	Two phases	550 ± 50 m; 1.40 cm	25 ± 6°C; 65±5% HR
Second day	Mixed competition	550 ± 50 m; 1.45 cm	27 ± 4°C; 66±3% HR
Competition 2			
Time	Race type	Course and obstacle	Ambiental enviroment
First day	Two phases	550 ± 50 m; 1.40 cm	26 ± 5; 68 ± 6% HR
Second day	Mixed competition	550 ± 50 m; 1.45 cm	28 ± 3; 70 ± 5% HR







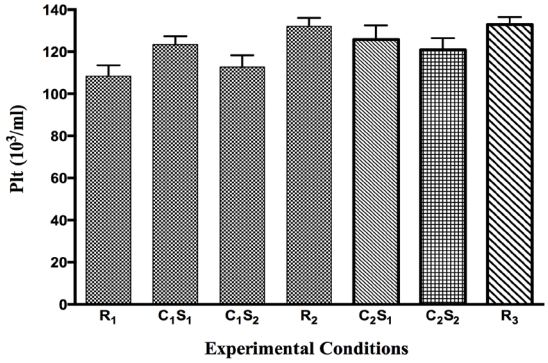


Figure 1. The pattern of mean values (± standard error) of RBC and Hct. In 12 Italian Saddle Horse, the day before competition (R1), 10 min after the competition for three days (C1S1, C1S2), the first rest day (R2), 10 min after the second session of competition (C2S1, C2S2) and the second rest day (R3).

Figure 2. The pattern of mean values (± standard error) of Hgb and Plt. In 12 Italian Saddle Horse, the day before competition (R1), 10 min after the competition for three days (C1S1, C1S2), the first rest day (R2), 10 min after the second session of competition (C2S1, C2S2) and the second rest day (R3).

DISCUSSION

The results of the present study could confirm how jumping competitions affect hematological parameters by increasing RBC, Hct, Hgb levels immediately after the exercise and returning to basal levels during resting time. Instead Plt values increase during resting time and the proximity of the competition induce a thrombocytosis during the second weekend. The obtained results could be due to physiological adjustment likely to occur during exercise [10,18,20]. These changes are necessary to guarantee correct oxygenation, provision of metabolites and removal of catabolites for the proper functioning of muscles [10]. Moreover, it is well recognized that, following high intensity exercise, some endocrine modifications take place in the organism during exercise stress and the release of catecholamine like epinephrine [17] is one of

the most important. Epinephrine causes spleen contraction and consequent release of RBC and PLT in circulating blood (auto transfusion) [16,19] causing a significant increment of these parameters [4]. Furthermore, splenic erythrocyte release, lead to a significant increase in Hct, in fact RBC numbers, and Hct percentage are strictly correlated and an increase of RBC number provokes a concomitant increase of Hct percentage [13,15,23]. This might be due to that fluid shift of the plasma into the cells likely to occur during exercise [25]. The increase of RBC number improves blood capacity to carry oxygen, and consequently enhance the aerobic capacity [28]. Furthermore, the gain of a greater ability to bind oxygen is due to the increase in Hgb caused by a greater number of circulating RBC. After exercise the endocrine stimulus decreases and erythrocytes are reuptake from spleen. RBC decreasing and concomitant rehydration, due to reverse of liquid shift and drinking, allow the expansion of plasma volume with return to normal of RBC, Hgb and Hct levels (Figures 1 & 2). The trends observed in RBC, Hct and Hgb were the same during the first and the second weekend of competition. Therefore, it is possible that within four days of rest a well-trained horse was able to establish adjustments in order to minimize the stress, related to competition. Effectively, no statistical differences were observed in RBC, Hct and Hb values during R_1, R_2 vs R_3.

Spleen contraction induces, also, an increment of Plt (Figure 2). The results showed the different trend between Plt and other parameters. This difference is due to lower spleen's reuptake speed between RBC and Plt, which is lower for Plt. Spleen is unable to re-establish a balance during resting time for Plt value. During rest days Plt value were higher than during other time points. This high value could induce a thrombocytosis and the thrombocytosis could be worsened by the proximity of the competitions. In fact in R_3 PLT value were higher than the other time points. Exercise thrombocytosis was widely studied in human athlete and horse athlete [30]. So during CBC evaluation in athlete particular attention must be put to Plt evaluation, because exercise stress induces not only the increase of their number, but also the increase of their reactivity and of their capacity to form aggregates [2,3,22,24], that can be implicated in thrombosis and some other cardiovascular diseases [26].

In conclusion, the present study showed how in well-trained subjects there may be an adaptation of the spleen that reduces the haematological changes due to the proximity of two sessions of competition. Effectively, the trends of RBC, Hct and Hgb did not change after the exercise and this could be due to a rapid return to the initial conditions of the spleen. Therefore, the interpretation of haematological parameters in athletes cannot be limited to the comparison with a static normal range, but in relation to their dynamic evolution with the progression of training.

The results suggest that the evaluation of the effects of training through the assessment of haematological profile, and consequently of spleen activity, could provide more information on horses' response to the loads, which may be useful for periodic monitoring of their welfare and health status and to understand the degree of their ability to adapt to specific training programs. These information are an important tool to provide an opportunity to modify the training schedule in order to improve physical performance in athlete horse.

MANUFACTURERS

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Ethical approval. All treatments, housing and animal care reported above were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the continent and writing of the paper.

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