THE ROLE OF DIFFERENT IRON PREPARATIONS IN THE PREVENTION OF ANEMIA IN RACING HORSES

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The hypothesis that a high hematocrit value and high hemoglobin concentration are the most important conditions which have to be fulfilled in order to achieve top racing results has resulted in a massive use of iron preparations in healthy horses. This specially implies in racing horses during intensive training aiming at the prevention of the so-called racing anemia.

By studying the effects of the most commonly used iron preparations in Serbia and Montenegro (Fedex®, as the representative of dextrane iron preparations; Hippirion® as a well known representative of iron complexed to saccharate and Hemo® 15 which is a representative of complex iron preparations which contain other hemantics) the expected changes in erythrocyte count, hemoglobin concentration and hematocrit value were recorded. However, the recorded changes in hematological values did differ between used iron preparations. The best effects were exhibited by Hemo® 15, the application of which resulted in a prompt and significant increase in erythrocyte count, MCH, MCHC and hemoglobin concentration.

Key words: horses, racing anemia, ferrous dextran, ferrous saccharate, ferric ammonium citrate

INTRODUCTION

The hypothesis that a high hematocrit value and high hemoglobin concentration are the most important conditions which have to be fulfilled in order to achieve top racing results has resulted in a massive use of iron preparations in healthy horses. This specially implies in racing horses during intensive training aiming at the prevention of anemia, which is often considered to be the major cause of poor results during the racing season (Stewart et al., 1977; Trailović et al., 1988; Jovanović et al., 2003).

The increased physical activity in racing horses is associated to their increased iron requirements. Horses, which endure for long periods of time strenuous exercise, can suffer from iron deficiency, especially in cases of inadequate dietary intake.
Iron deficiency develops slowly due to the fact that iron losses are gradual. The magnitude of the consequences of iron deficiency depends on the duration and degree of the negative iron balance, as well as on the available iron pool present in the body depot. Severe iron deficiency with clinical manifestations can occur in cases of massive hemorrhage.

Efficient energy metabolism is possible only in the presence of oxygen, hence the need for a high hemoglobin concentration as a prerequisite for efficient oxygen binding and transport. Today on the market can be found per os preparations containing iron in the form of phosphate, gluconate, fumarate and succinate. Iron preparations for parenteral application in horses are commonly available as iron complexes bound to dextrin, sorbitol and ammonium citrate (Kamerling, 1984; Putan, 1986; McLean et al., 1987; Morris, 1989; Lawrence, 1994; Harris et al., 1995).

Preparations containing iron sulphate are most commonly used for per os application. Iron supplements for per os use are cheap and do not cause allergic reactions like the ones which are often seen after the use of parenteral iron preparations. The major setbacks of peroral iron preparations are: slow absorption, subsequent irritation of the digestive tract, as well as an unpleasant metallic taste. Their slow absorption results in low bioavailability, and high doses can cause acute intoxication and liver damage (Divers et al., 1983; Hershko C, 1989; Edens et al., 1993). To avoid all the possible side-effects caused by peroral iron supplements, the use of parenteral iron preparations is becoming more popular. In racing horses the most commonly used parenteral iron preparations are in the form of dextran, saccharate and ammonium citrate.

Parenteral application, especially intramuscular, of iron dextrane preparations can bring up an anaphylactic reaction with a lethal outcome. Beside a generalized reaction to the preparation a local painful swelling sometimes accompanied by tissue necrosis can develop on the injection site. After an intravenous application of such iron supplements, trombophlebitis or symptoms of generalized poisoning may develop (Wagenaar, 1975; Arnbjerg J, 1981; Mullaney and Brown, 1988; Edena et al., 1993).

In a follow-up study on the effect of iron preparations on racing horses stationed at Belgrade Hippodrome (Jovanović et al., 1997), 11.18% (n=17) of tested animals showed a painful reaction at the injection site, 2.63 % (n=4) developed skin rash, 0.66% (n=1) anaphylaxis and 0.66 % (n=1) liver disorders.

Fe-saccharate is a complex of Fe^{3+} and saccharate. These iron preparations are usually administered to racing horse by intravenous route. They have shown good results in racing horses as they have a fast effect due to efficient iron incorporation into hemoglobin. Such iron supplementation is almost entirely withheld within the body, and only to a small extent is excreted through the kidneys.

Gutschow et al., (1975) have studied the effect of Fe-saccharate (Hippiron®) on the number of erythrocytes, hemoglobin concentration, hematocrit and hematological indices. After treatment the authors registered an increased erythrocyte count by 17.78%, hemoglobin concentration and hematocrit value increased by 10.07% and 10.20% respectively, compared to the initial value. They
have registered as well a decrease in CMV and MCH by 6.67% and MCHC by 0.92%. Gutschow et al., (1975) consider the increased values of erythrocyte count, hemoglobin concentration and decreased MCV, MCH and MCHC values to be one of the crucial factors in the improvement of the performance of racing horses.

Cornelia Detlef (1985) studied the effects of Fe-saccharate (Hippiron®) on hematological parameters and serum iron concentration in pregnant mares and their progeny. In mares to which Hippiron® was administered during pregnancy, there was not a fall in erythrocyte count, common throughout pregnancy due to the increasing needs of the growing fetus. Foals, whose mares have been treated with Fe-saccharate had a significantly (p<0.05) higher erythrocyte count and hemoglobin concentration when compared to their mothers and foals of mares not treated with Fe-saccharate. The author noted as well a significant difference (p<0.01) in the increase of serum iron in foals of mares treated with Fe-saccharate compared to foals of untreated mares.

Jovanović et al., (1997) studied the effects of Fe-saccharate (Hippiron®) in the English Thoroughbred and Yugoslav Trotter, with a special insight into the negative side-effects of this preparation. In 60% of the studied horses there was a sharp increase in erythrocyte count and hemoglobin concentration. This showed a positive effect on the racing performances of the studied horses. About 10% of the studied animals did not show any significant changes, in 31.5% after an initial rise the hemoglobin concentration and erythrocyte count decreased by 30% compared to the initial values, accompanying marked hyperbilirubinemia was recorded.

MATERIAL AND METHODS

In the study a total of 18 racing horses (English Thoroughbred) were included in the trial. The animals between three and four years of age, of both sexes, were randomly assigned into three groups (n=6).

The first group was treated with Fe-dextrane Fedex-100® (Zdravlje, Leskovac). One milliliter of the preparation contains 100 mg Fe³⁺, 200 mg dextrane and 0.50 mg phenol. A dose of 8 mL was applied i.m., three times, every second day.

The second group was treated with Hippiron® (Janssen-Cilag Sa Vifor Inc., St Gallen). A 50 mL vial (used as a single application) contains a Fe(OH)₃ - saccharose complex, i.e. 20 mg Fe³⁺/mL. The preparation was applied via intravenous route, combined to 500 mL saline every second day, three times.

The third group was treated with Hemo-15®(Rhone Meriux). One milliliter of this preparation contains ferric ammonium citrate 15mg, vitamin B₁₂ 150 μg, riboflavine (Na-5-phosphate) 10 mg, pyridoxine hydrochloride 10 mg, niacin amide 100 mg, d-panthenol 15 mg, cobalt gluconate 0.7 mg, copper gluconate 0.2 mg, holin chloride 10 mg and biotin 10 μg every second day.

Blood samples were taken five days before the first treatment, as well as on the day respective treatments were carried out, and two days after the last application. In the obtained blood samples the values for erythrocyte count,
hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined. For serum iron analysis, the samples were taken five days before the treatment and two days after the last iron preparation application.

Blood samples were taken from the jugular vein in test tubes containing EDTA. Serum samples were taken by withdrawing blood from the same site using sterile vacuum tubes containing SST gel and coagulation activators. All hematological analyses were performed on the automatic analyzer "Tehnicon" H-1 (USA).

The procedure used for the measurement of total serum iron concentration is based on the automatized method by Giovanella et al. (1968) and the manual method by Stookey (1970) using a Tehnicom SMAK – System (USA).

Statistical analysis was done after determining the mean values and standard deviations of the studied parameters. The significance of differences between groups was calculated using Analysis of Variance (F-test) and individual LSD-test.

RESULTS AND DISCUSSION

The results obtained in this study are shown on Figure 1-7.

The effects of the three most commonly used iron preparations: Fedex® (Zdravlje Leskovac) as a representative of ferrous dextrane preparations for intramuscular application, Hippiron® (Jessen – CilagSA Vifor Inc., St. Galen) the most popular iron preparation in the chemical form of ferrous saccharate and Hemo® (Rhone Meriux) containing iron in the form of ferrous ammonium citrate were tested on 18 healthy racing horses.

At the start of the racing season the studied horses, after hematological tests, were diagnosed as preanemic. The erythrocyte count ranged from 8.2 x 10^{12}/L in the third group (Hemo® 15) to 8.76 x 10^{12}/L and 8.84 x 10^{12}/L in the second and first group respectively (Figure 1). These values can be considered to be within the physiological level for racing horses, however they are considered to be insufficient for top racing results (Stewart et al., 1977; Carlson, 1996; Christian, 2000; Martin and Nankervis, 2002).

All three tested iron preparations resulted in an increase of hematological parameters such are erythrocyte count, hemoglobin concentration and hematocrit value. Hence, the group of animals treated with Fe-saccharate had an increase of the mean erythrocyte count by 5.7% (9.25 x 10^{12}/L). However, Gustschow et al., (1975) in a similar experiment reported an increase in erythrocyte count up to 17.78%.

Racing horses treated with Fe-dextrane had an increase in erythrocyte count by 4.80% (9.27 x 10^{12}/L). This change is lower than the one reported by Detlef (1985). However, the effect of such supplementation in our trial was followed up for a shorter period of time and a delayed effect of Fedex® after the last application cannot be excluded.
The highest increase in erythrocyte count was recorded in horses treated with ferric ammonium citrate. After applying three times Hemo-15 the erythrocyte count increased by 12.8%, i.e. from $8.2 \times 10^{12}/L$ to $9.25 \times 10^{12}/L$ by the end of the experiment. We believe that the highest increase in erythrocyte count in this group can be explained by the fact that Hemo® 15 has a complex composition, containing other hemantics like vitamin B12 and folic acid. Hemo® 15 is applied via an intravenous route, hence has a higher bioavailability when compared to preparations which are applied intramuscularly.

If the effects of these three iron preparations are compared it is evident that there are differences in the resulting erythrocyte count. The highest increase (+12.8%) was recorded in horses treated with ferric ammonium citrate; an intermediate increase (+5.7%) was achieved by ferric saccharate and the lowest (+4.8%) by ferric dextran. However, these differences were not statistically significant ($p>0.05$).

By increasing the erythrocyte count a respective increase in hemoglobin concentration is to be expected. However, this is not equal between groups, as shown by our results. At the start of the trial the initial hemoglobin concentration ranged from 118.33 g/L in horses treated with ferric dextran to 126.5 g/L in horses treated with ferric ammonium citrate. This can be considered as within the physiological limits for the English Thoroughbred. (Ralston et al., 1988; Lassen and Swardson, 1995; Cuddeford, 2001), however these values are not adequate for the workload a horse endures during a race (Figure 2).

In the group treated with ferric saccharate the increase in hemoglobin concentration, when compared to the initial value, was statistically significant ($p<0.01$). Compared to the initial values this increase in hemoglobin concentration was 13.63%, which is higher than the value of 10.07% reported by Gutschow et al. (1975). In the experimental group of animals supplemented with Fe- saccharate the percental increase in hemoglobin was higher than the percental increase in erythrocyte count. The increase in hemoglobin
concentration in this group was statistically significant ($p<0.01$) when compared to the group treated with ferrous dextran.

Horses treated with ferric ammonium citrate had a significant ($p<0.05$) increase by 10.93% compared to initial values in hemoglobin concentration. Only in this group, when the percent values were compared, the increase in erythrocyte count was higher than the increase in hemoglobin concentration.

On testing the effects on hemoglobin concentration, between the three different iron preparations, there were no significant differences ($p>0.05$), however by comparing the values when expressed as percentage, the best results were obtained by ferric saccharate (+13.63%).

The third parameter relevant for the estimation of the effects of an iron preparation on hematopoiesis is the hematocrit (packed cell volume, PCV). The hematocrit value increases as the erythrocyte count rises, however it does not do so at the same rate. Before treatment the hematocrit value ranged from 0.35 L/L to 0.37 L/L. The same comment as for hemoglobin and erythrocyte count applies for the hematocrit, i.e. they are within the physiological limits of the tested animals, but these values are far from sufficient to enable optimal racing results, hence these horses are often described as pre-anemic (Ralston et al., 1988; Smith et al., 1989; Lassen and Swardson, 1995; Harris and Krenfeld, 2003). Results for hematocrit values are given in Figure 3.

By statistical analysis of the values obtained for the hematocrit no significant differences ($p>0.05$) between Fe-saccharate and Fe-ammonium citrate were recorded. However, when the results were expressed as percentage a growing trend in hematocrit value was evident. Horses treated with ferric saccharate and ferric ammonium citrate showed an increase in hematocrit value by 11.04% and 6.62% respectively. The increase in the hematocrit is due to the increase in erythrocyte count and volume. The increase in the experimental group supplemented with ferric saccharate coincides with the value (10.20%) recorded by Gustschow et al. (1975).

Figure 2. Changes in hemoglobin concentration (g/L) in the blood of racing horses treated with different iron preparations

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When the results relative to erythrocyte count, hemoglobin concentration and hematocrit value are inconclusive, a good indicator of the efficiency of the tested supplements are the erythrocyte indices, which are more reliable. The mean values of the hematological indices (MCV, MCH, MCHC) at the start of the trial were within physiological limits for racing horses. The mean MCV ranged from 40.55 fl to 42.8 fl; MCH from 12.86 pg to 14.21 pg, and MCHC from 269 g/L to 323.5 g/L (Figures 4, 5, and 6). These values coincide with the values published by (Rose and Hadgson, 1982; Radin et al., 1986; Ralston et al., 1988; Smith et al., 1989).
A significant increase \((p<0.05)\) in MCV was in the group of animals treated with Fe-dextrane. The increase, when expressed as percentage, was 9.26\%. The same preparation resulted in a significant change in MCH, which actually decreased by 1.62\%. MCHC significantly increased by 25.77\% \((p<0.01)\).

After fortification with ferric saccharate no significant changes \((p>0.05)\) in MCV, MCH and/or MCHC were recorded. However, mean MCH and MCHC increased by 7.34\% and 1.64\%, respectively. Gustschow \textit{et al.} (1975) reported a decrease in the erythrocyte indices MCV and MCH by 6.67\% and MCHC by 0.92\%.

Horses treated with ferrous ammonium citrate had an increase in MCV value by 4.19\%, however this increase was not significant \((p>0.05)\). MCH and MCHC values significantly increased \((p<0.01)\), MCH increased by 16.64\% and MCHC increased by 26.30\%.

Figure 5. Changes in MCH (pg) in the blood of racing horses treated with different iron preparations

Figure 6. Changes in MCHC (g/L) in the blood of racing horses treated with different iron preparations
One of the most relevant biochemical indicators which was followed-up throughout the experiment is serum iron. Before the start of the treatments, serum iron values ranged from 27 µmol/L in the group treated with Hippirion®, 28 µmol/L in the group treated with Hemo-15 and 28.5 µmol/L in horses treated with Fedex® (Figure 7). These values for serum iron are higher when compared to those published by Tobin (1984) and Stone and Freden (1990), but close to the values reported by Rose and Hadgson (1982) and Johnson (1990).

In the first experimental group of horses treated with a dextrane iron formulation there was no significant increase of the mean values of serum iron at levels of significance 0.01 and 0.05. In this group a decrease of 0.61% in serum iron was recorded.

Values for serum iron in the experimantal animals treated with ferri saccharate where higher by 17.25% when compared to initial values. This increase in serum iron has shown to be statistically significant (p>0.05).

The third group of animals, treated with ferric ammonium citrate the serum iron concentrations differed significantly (p<0.05) between the initial value and at day 8. from the start of fortification. The increase in serum iron concentration in horses treated with ferric ammonium citrate was 27.14%.

By comparison of the effects of different iron preparations on serum iron concentration a statistically significant difference (p<0.01) between the treatment with ferrous ammonium citrate and the treatment with ferrous dextran was reported.

Based on the obtained results it can be concluded that all three preparations have a positive effect on hematological parameters and blood serum iron concentration. The effect of Hemo®-15 is more marked because beside Fe³⁺ (whose concentration is lower compared to Fedex-100 and Hippirion) contains other hemantics. Other components of this preparation, especially vitamins B2, B6, B12, choline and microelements such as cobalt and copper have a positive
effect on hemoglobin concentration and red blood cell count in treated racing horses. We consider the application of Hemo®-15 to be more appropriate, specially bearing in mind that with this preparation the chance for iron hypermedication is lower.

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U ovom radu su studirane uticaje tri najčešće korišćene preparata gvožđa kod trkačkih konja u našoj zemlji (Fedex kao predstavnik dekstranskih preparata gvožđa, Hippiron kao najpoznatiji predstavnik gvožđa u kompleksu sa saharatom i Hemo 15 koji je predstavnik kompleksnih preparata koji uz gvožđe sadrže i druge hematinkne) utvrđene očekivane promene u broju eritrocita, koncentraciji hemoglobina i hematokritskoj vrednosti. Primena sva tri preparata gvožđa dovela je do povećanja vrednosti svih ispitivanih hematoloških parametara kod trkačkih konja. Najbolji efekat ispoljio je preparat Hemo 15 čija je primena dovela do najvećeg povećanja broja eritrocita, MCH, MCHC i koncentracije hemoglobina, a i sam efekat je nastajao najbrže.