

Variability of electrophoretic protein profile in Quarter Horses during reining trials

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ABSTRACT

The aim of this study was to assess the effect of a reining session on serum total protein and their fractions. In the present study, eight Quarter Horses aged 5-15 years were subjected to reining pattern 5 of the National Reining Horse Association (NRHA). Blood samples were collected from each animal before (T0), immediately after the pattern (T1), after 1h (T2), 2h (T3) and 24h (T4), during the recovery period. On all samples serum total proteins (TP), albumin (Alb), α_1 -globulins, α_2 -globulins, β_1 -globulins, β_2 -globulins and γ -globulins concentrations, and albumin/globulin ratio (A/G) were assessed. One way repeated measure analysis of variance (ANOVA) showed the statistical significant effect of time on TP and Alb. The application of Bonferroni's post-hoc comparison showed a statistical significant increase in TP values at T1 compared to T0. Alb concentration increased at T1 compared to T0, T2, T3 and T4. The present study shows how reining exercise affects electrophoretic parameters by increasing TP and Alb levels immediately after exercise. These results provide insight into the reining horse's physiological response to exercise, allowing better evaluation of the athletic performance of this sport horse.

Key words: serum total proteins, electrophoretic profile, reining, physical exercise, Quarter Horses

Introduction

It is known that exercise induces various changes, depending on its characteristics, duration and intensity. In recent years, interest in reining competitions has greatly increased and expanded worldwide. Specialized equine reining events are largely regulated by the National Reining Horse Association (NRHA) and represent a significant business

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venture, offering high monetary awards. The Quarter Horse is the most common breed used in reining events (CLAYTON, 1991). As specified by the NRHA, reining consists of 11 predetermined patterns, including small circles at slow lope, large circles at fast lope, straight runs to sliding stops, spins and rollbacks in both directions, and backing up. The relatively short duration of the reining pattern and the intermittent bursts of moderate to high intensity exercise suggest that reining horses have high energy needs during both training and competition sessions (RAMMERSTORFER et al., 1998; VAZZANA et al., 2014). Intense and continuous exercise can induce changes in the serum concentrations of numerous laboratory parameters (BANFI et al., 2012).

Serum protein electrophoresis is a useful diagnostic tool in equine medicine. Serum electrophoresis from healthy horses is characterized by the absence of the prealbumin region and by six different bands: albumin, α_1 -globulins, α_2 -globulins, β_1 -globulins, β_2 -globulins and γ -globulins (CARAPETO, 2006). Albumin is important in regulating blood volume by maintaining the oncotic pressure of the blood compartment (FELDMAN et al., 2002). Globulins are a heterogeneous group of proteins that include antibodies and other inflammatory molecules, haemostatic and fibrinolytic proteins, and carriers of lipids, vitamins and hormones (ALBERGHINA et al., 2010). In horses, changes in concentrations of total proteins and their fractions could be attributed to different causes, including poor performance, depression, fever, mass loss, diarrhoea, abdominal pain, and polyuria (COLHAN et al., 1999). Only a few studies have dealt with the assessment of physical, hematological, and biochemical parameters (RAMMERSTORFER et al., 1998; KÄSTNER et al., 1999; RAMMERSTORFER et al., 2001; VAZZANA et al., 2014) in reining horses during exercise, however no study has dealt with changes in serum total protein. Therefore, the objective of the present study was to evaluate the modifications of serum total proteins and their fractions in Quarter Horses during a simulated reining session.

Materials and methods

Animals and housing. The trial was carried out in a horse training centre in Sicily, Italy (latitude 38° 10' 35"N; longitude 13° 18' 14"E). Eight Quarter Horses (5-15 years; 3 male, 5 female, mean body mass 480 ± 60 kg) were used. All animals were clinically healthy and free from internal and external parasites. Their health status was evaluated on the basis of a thorough clinical exam. The horses were kept under natural photoperiod and ambient temperature, in individual stalls with free access to water and were fed four times a day with commercial feed and alfalfa hay. All horses performed reining pattern 5 of the National Reining Horse Association, consisting of 2 large circle gallops, 1 small circle lope, 1 stop, 4 spins, 2 large circle gallops, 1 small circle lope, 1 stop, 4 spins, 1 large circle gallop, lead change, 1 small circle lope, 1 large circle gallop, lead change, 1 large circle gallop, lead change, 2 rollbacks, 1 stop and backing. On the day of the reining

session, the mean environmental temperature and relative humidity were 23.4 °C and 64 %, respectively.

Blood sampling and analysis. Blood was collected by jugular venipuncture in vacuum tubes containing no anticoagulant (Terumo Corporation Japan), at 5 time points: before exercise (T0), immediately after the exercise (T1), and during the recovery period at 1h (T2), 2h (T3) and 24h after the exercise (T4). Blood samples were transported to the clinical laboratory in a cooler with an ice block (4-6 °C) within 30 minutes. Following standing at room temperature for 20 min, the tubes were centrifuged at 3.000 rpm for 10 min and the obtained serum was stored at -25 °C until analysed. The concentration of serum total proteins (TP) was determined by the biuret method (KAPLAN, 2003) using a commercially available kit (Total Protein, Gesan, Campobello di Mazara, Italy), by means of an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy) at a wavelength 540 nm. The protein fractions were performed using an automated system (Sel Vet 24, SELEO Engineering, Naples, Italy) according to the procedures described by the manufacturer previously used in horses (ALBERGHINA et al., 2013). For each sample, 25 µL of serum were applied to numbered sample wells. Each holder accommodates up to 24 samples. Films were subjected to electrophoresis for 28 minutes at 450V. After electrophoresis, the films were simultaneously fixed using an automated system, stained in red stain acid solution for 10 minutes, and then dried at 37 °C. After destaining in acetic acid and drying completely for 15 minutes, the films were scanned on a densitometer, and the electrophoretic curves plus the related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, then absolute concentration (g/dL) and albumin/globulin ratio (A/G) were calculated using the total protein concentration. The major protein fractions were divided, according to the recommendation by the manufacturer, from cathode to anode as albumin, α_1 , α_2 , β_1 , β_2 , and γ -globulins, respectively.

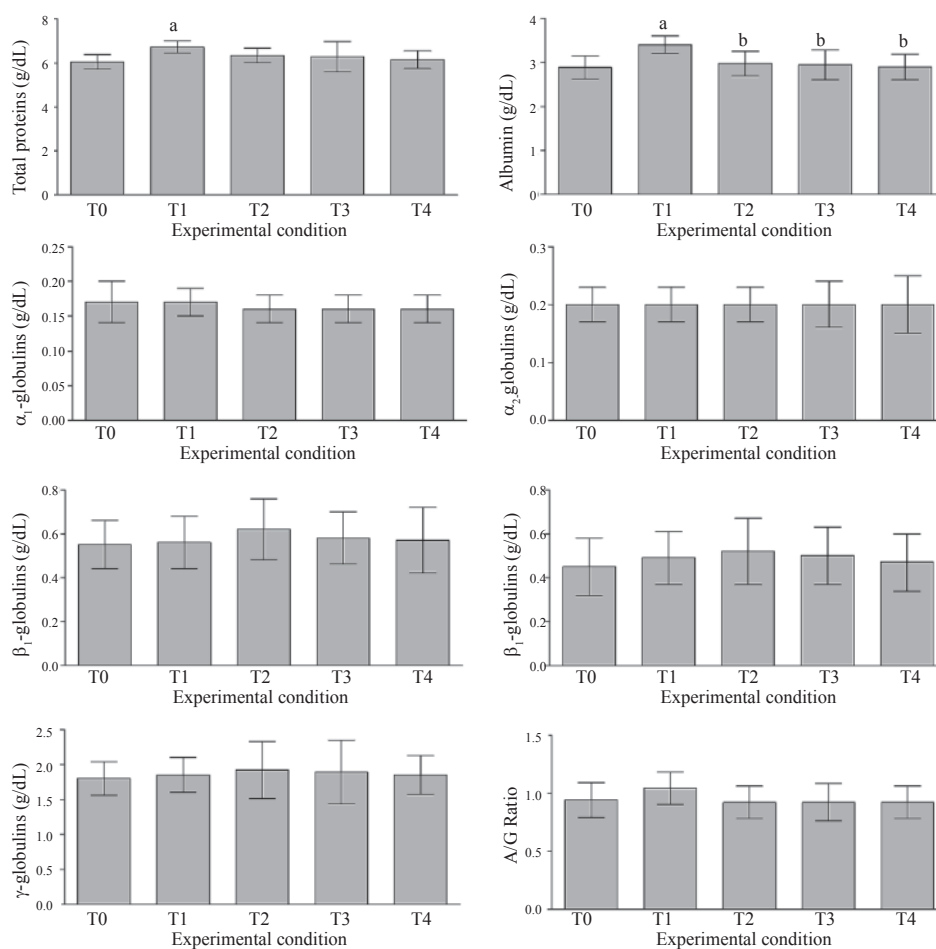
Statistical analysis. All data were expressed as means \pm standard deviation (SD). Descriptive statistics were estimated for the serum total proteins and their fractions. The fixed effects of time (T0-T4) were analyzed with the univariate general linear model of Statistica 7 software (STATISTICA 7, Stat Soft Inc., USA); the Bonferroni's test was used as post hoc comparison. P value <0.05 was considered statistically significant.

Results

The mean values of the electrophoretic serum protein profile obtained in the present study were within the physiological range reported for horses (Fig. 1). The general linear model showed the statistically significant effect of time on TP (P<0.05) and Alb (P<0.05).

The application of Bonferroni's post-hoc comparison showed a statistically significant increase in TP values at T1 (P<0.05) compared to T0. Albumin concentrations exhibited

higher values at T1 ($P < 0.05$) compared to T0, T2, T3 and T4 (Fig. 1). No statistical significance was found in globulins fractions under the experimental conditions. Fig. 2 shows representative serum protein electrophoretograms observed in a horse during experimental conditions.



Significance: ^a vs T0; ^b vs T1

Fig. 1. Mean values ± standard deviation (SD), together with the relative statistical significance of serum total proteins, their electrophoretic fractions and A/G ratio obtained before exercise (T0), immediately after exercise (T1), and in recovery at 1h (T2), 2h (T3) and 24h after exercise (T4) in eight Quarter Horses

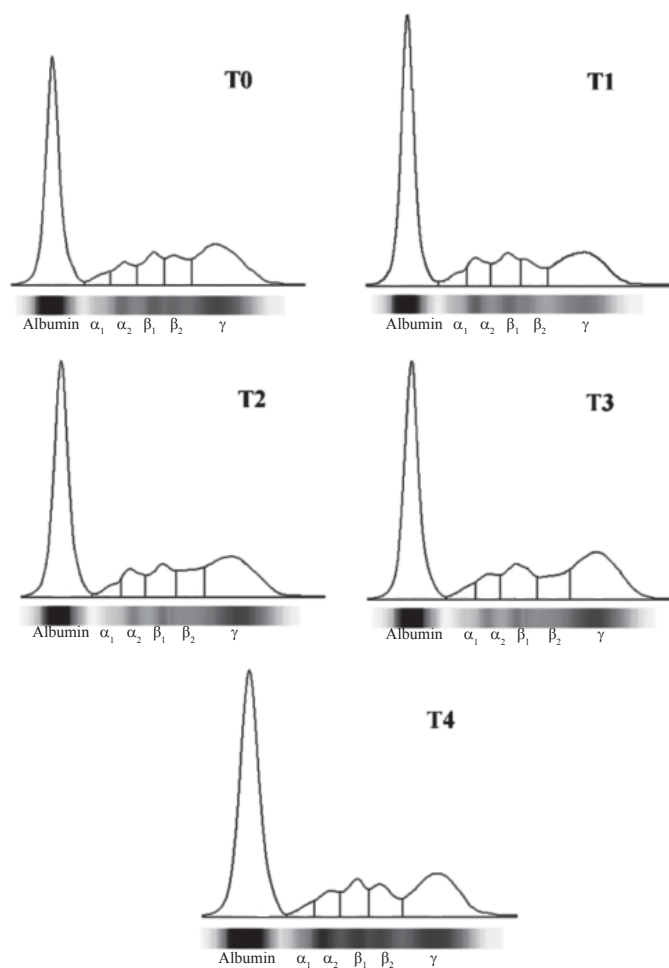


Fig. 2. Representative serum protein electrophoretograms observed in a horse before exercise (T0), immediately after exercise (T1), and in recovery at 1h (T2), 2h (T3) and 24h after exercise (T4)

Discussion

Physical exercise induces different physiological and biochemical adaptations in horses (BALOGH et al., 2001). In the present study, significant changes occurred in TP and Alb values immediately after a reining session (T1). In horses, most studies have shown an increase in TP as the result of various racing exercises, competitions and training programs (ROSE et al., 1983; ART et al., 1990; JABLONSKA et al., 1991; SOMMARDABL et al., 1994; DAHALBORN et al., 1994; SANTOS et al., 2001; SCHOTT et al., 2006; FAZIO et al., 2011; JANICKI et al., 2013). As shown in previous studies (PICCIONE et al., 2007; ZOBBA et al., 2011), in horses during exercise splenic contraction forces a large mass of erythrocytes into the circulation, suddenly increasing circulating blood volume, and this is accompanied by a rise in plasma proteins. The increase of TP during exercise might be due to a reduction in extracellular fluids and haemoconcentration (ART et al., 1990; JABLONSKA et al., 1991). During maximal exercise, there is a redistribution of fluids and electrolytes from the vascular compartment to the tissue extracellular fluid spaces, with a resulting increase in TP and Alb (FAZIO et al., 2011). Alb is a major storage reservoir of proteins and transporters of amino acids. It is the most osmotically active plasma protein due to its abundance and small size, and accounts for about 75 % of the osmotic activity of plasma (KANEKO et al., 1997). The increase in plasmatic proteins reflects an increase in TP due to the exchange between fluid intra- and extracellular. No statistical difference in globulin fractions was found between times under the experimental conditions. This result differs from PICCIONE et al. (2007), who found a significant increase after the race vs rest in α_1 - and α_2 - globulins values of Thoroughbred horses. However, it has been shown that fluid movement within the organism does not influence β - and γ - globulins that are mainly constituted from elements of the immune system and do not undergo statistically significant modifications (PICCIONE et al., 2007).

The present study shows that reining exercise affects electrophoretic parameters by increasing TP and Alb levels immediately after exercise. These results provide insight into the reining horse's physiological response to exercise, allowing better evaluation of the athletic performance of this sport horse.

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SAŽETAK

Cilj ovog istraživanja bio je procijeniti učinak natjecanja u obuzdavanju konja na ukupne serumske proteine i na njihove pojedine frakcije. U istraživanju je osam konja Quarter pasmine u dobi od 5 do 15 godina bilo podvrgnuto vježbi 5. stupnja obuzdavanja prema Nacionalnoj udruzi za obuzdavanja konja. Uzorci krvi bili su uzeti od svakog konja prije vježbe (T0), neposredno nakon vježbe (T1), zatim nakon jednog sata (T2), dva sata (T3) i 24 sata (T4) odmora. U svim uzorcima seruma određena je koncentracija ukupnih proteina, albumina (Alb), α_1 -globulina, α_2 -globulina, β_1 -globulina, β_2 -globulina i γ -globulina te je određen omjer između albumina i globulina (A/G). Jednosmjerna analiza varijance (ANOVA) pokazala je statistički značajan učinak vremena na ukupne proteine i albumin. Primjena Bonferronijeve post-hoc usporedbe pokazala je statistički značajno povećanje vrijednosti ukupnih proteina neposredno nakon vježbe u odnosu na one prije vježbe. Koncentracija albumina bila je povećana neposredno nakon vježbe u usporedbi s koncentracijom prije vježbe te onom jednog sata, dva sata i 24 sata nakon vježbe. Istraživanje pokazuje kako natjecateljsko obuzdavanje konja utječe na elektroforetske pokazatelje povećanjem razine ukupnih proteina i albumina neposredno nakon vježbe. Rezultati pružaju uvid u fiziološki odgovor na vježbu što omogućuje bolju prosudbu atletskih sposobnosti ove pasmine športskog konja.

Ključne riječi: ukupni serumski proteini, elektroforetski profil, natjecateljsko obuzdavanje, "Quarter" konji
