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Seasonal changes in enzyme activities and mineral concentrations in Holstein stallions blood plasma

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ABSTRACT

The aim of this study was to determine seasonal changes in the activity of some enzymes and concentration of some minerals in Holstein stallions, as the literature provides insufficient data on that matter. Blood was collected from the jugular vein, with heparin as the anticoagulant. Blood plasma activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamiltransferase (GGT), alkaline phosphatase (AP), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined. Also, concentrations of calcium, phosphorus, magnesium, iron and copper were measured. Most parameters determined in this study were highest in the summer period. A significant negative correlation between the concentration of calcium and inorganic phosphate was also determined. We can assume that seasonal changes in blood enzyme activities and blood mineral concentrations depend on muscle activity and balanced feed intake.

Key words: seasonal changes, blood enzyme activities, blood mineral concentration, Holstein stallion

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Introduction

Biological rhythms express adaptation to the annual changes in photoperiods and provide information on nutritional state, social status or stress (TESTER and FIGALA, 1990) and this information is of diagnostic importance. Activity and feeding are modulated by biological time patterns, such as reproduction or digestion (STOJEVIĆ et al., 2005;

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BERGER et al., 1999). The physiological responses of animals to environmental stress during the winter and summer and their energy balance show that seasonal heat and cold stress have profound effects on some serum biochemical parameters (ELDON et al., 1988; SOVERI et al., 1992; BENGOUMI et al., 1997). Seasonal variations are mainly related to the effects of the photoperiod, the climatic conditions and differences in the composition and characteristics of the diet (BERTONI, 1996). In summer, horses increasingly shift their search for food to the hours of night, to avoid disturbance by flying insects and high temperatures (KASEDA, 1983; DUNCAN, 1985; MAYES and DUNCAN, 1986; BOYD et al., 1988). BERGER et al. (1999) investigated diurnal and ultradian rhythms of behaviour in Przewalski horses raised in different zoos and kept in enclosures under semi-natural conditions, and determined that the level of activity was lowest in winter; whereas feeding was lowest in summer. They also determined that feeding was at its highest level in spring (April), but was low in summer (June/July). Stallion are seasonal breeders and demonstrate variations in many hormones: luteinizing hormone, follicle-stimulating hormone and testosterone (CLAY et al., 1988), prolactin (THOMPSON et al., 1986), 17β -estradiol and estrone (THOMPSON et al., 1978). The season is also known to influence seminal characteristics and sexual behaviour (SKINNER and BOWEN, 1968; PICKETT et al., 1970). Successful reproduction requires complete provisions of macro and microminerals, including copper (TUORMAA, 2000), calcium and magnesium (WONG et al., 2001).

The aim of this study was to determine seasonal changes in the activity of some enzymes and the concentration of some minerals in Holstein stallions, as the literature provides insufficient data on that matter. Also, since Holstein horses are a dominant force in international show jumping, it is of great importance to understand the metabolic and functional processes for correct management and veterinary controls. Seasonal variations have to be taken into consideration for a correct interpretation of blood chemistry values.

Materials and methods

Investigations were carried out on 10 Holstein stallions aged between 5 and 10 years. According to the horse breeding Annual Report in the Republic of Croatia, in 2010 the number of Holstein horses was 160 (64 stallions and 90 mares), with the largest number of animals bred in the area of the Koprivničko-križevačka County (64 animals). This study comprised 16 % of the stallion population in the Republic of Croatia. This specific group of horses, Holstein stallions, was chosen because at the time of sampling there was research in progress which included Holstein horses within the stud farm of the College of Agriculture in Križevci, Croatia. The stallions chosen for the study are a sporting horse breed, but they are kept as animals for the purpose of learning for students. The animals were kept in individual stalls with free access to the outside, in the same conditions, and fed by the same regime. The animals were fed twice a day with 4 kg of silage and 2 kg of oats. The animals had a vitamin mineral stone Biosaxon (Salinen, Austria) composed of (information supplied by the manufacturer): sodium 35 %, calcium 1.4 %, phosphorus 1.2 %, magnesium 0.3 %, with zinc, protein, manganese, iodine, selenium and cobalt as additives. In the spring and summer the animals were out on pasture. Water was made available *ad libitum*. All the animals were clinically healthy. In the sampling area the climate is continental and the type of pasture, on which horses were kept during the spring and summer periods, was pasture with mixed herbs.

Blood samples were taken at the same time (8-9 am) by puncture of the jugular vein (vena jugularis externa). Blood samples were taken once per season for one year. Seasons were defined as follows: spring (March, April, May), summer (June, July, August), autumn (September, October, November) and winter (December, January, February). Heparinized plasma was obtained by centrifugation at 1600 g for 20 min at 20 °C. Samples were stored at -20 °C and analysed at the end of the sampling period. In these samples the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamiltransferase (GGT), alkaline phosphatase (AP), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined. Also, concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe) and copper (Cu) were measured. Samples were analyzed using a gamma Heλios UV visible spectrophotometer, Thermo Spectronic UK, with ready-made kits of “Herbos Diagnostics Ltd.” Sisak. Our laboratory does not have reference levels for the measured parameters since we are not a clinical but a preclinical department. Indicators were determined in a scientific and not a routine laboratory.

Statistical analysis of the results was performed using the computer statistical package Statistica, version 9 (StatSoft, Inc., Tulsa, SAD). The normality of distribution was checked using Kolmogorov-Smirnov and Shapiro-Wilk’s W test. Results are presented as mean ± standard deviation (m ± SD). The significance of differences within seasons was checked by the repeated measured analysis of variance and Tukey’s test for unequal sample size in the case of normal distribution. Friedman ANOVA test and Wilcoxon post hoc analysis were employed when the distribution was not Gaussian (PETRIE and WATSON, 2006). The correlation between study parameters was tested by Spearman Rank Order Correlations. The level of statistical significance was set at $P<0.05$.

Results

Using the Kolmogorov-Smirnov and Shapiro-Wilk’s W test, we determined non Gaussian distributions of the results for the CK, AST, ALT activities and for iron and copper concentrations.

As shown in Fig. 1, the highest GGT and CK activities in the blood plasma were measured in the summer and GGT activity was significantly higher than the activity measured in the spring ($P = 0.043$); CK activity in the summer was significantly higher than the measured activity in the spring (P = 0.046) and in the winter (P = 0.028). As shown in Fig. 1, the activity of ALT measured in the summer was significantly lower compared with the activity measured in the winter (P<0.001), in the spring (P = 0.009) and in the autumn (P<0.001). Significantly higher AP activity was measured in the summer compared to the other seasons (P<0.001; Fig. 2). Activity of AST was significantly higher in the summer than the values measured in the winter (P = 0.043; Fig. 2). Activity of LDH showed no significant seasonal variation.

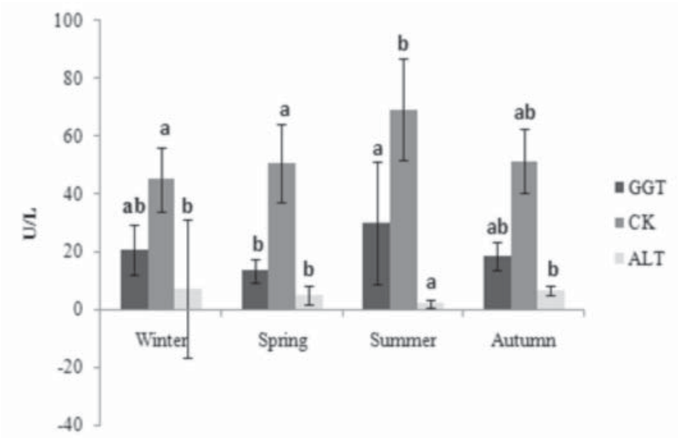

Fig. 1. Seasonal changes of GGT, CK and ALT activities in Holstein stallion blood plasma. Values are shown as mean ± standard deviation; a, b: P<0.05 between seasons.

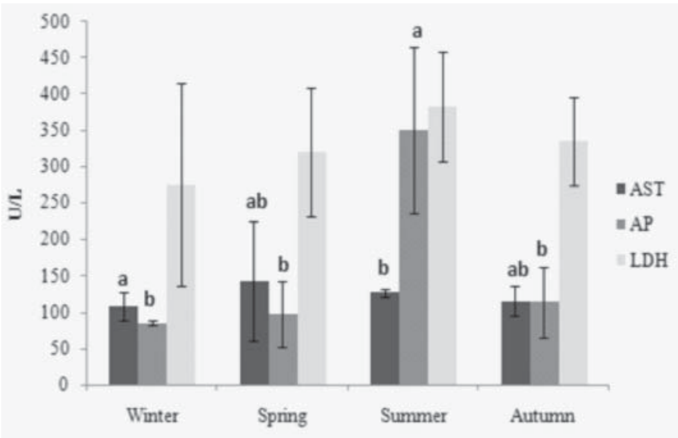

Fig. 2. Seasonal changes of AST, AP and LDH activities in Holstein stallion blood plasma. Values are shown as mean ± standard deviation; a, b: P<0.05 between seasons.

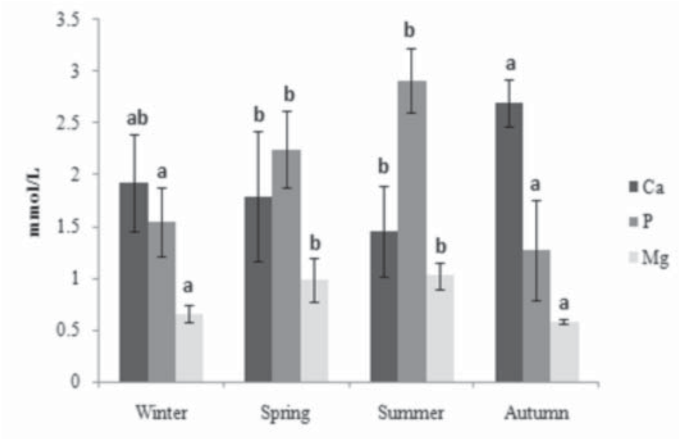

Fig. 3. Seasonal changes of Ca, P and Mg concentrations in Holstein stallion blood plasma. Values are shown as mean ± standard deviation; a, b: P<0.05 between seasons.

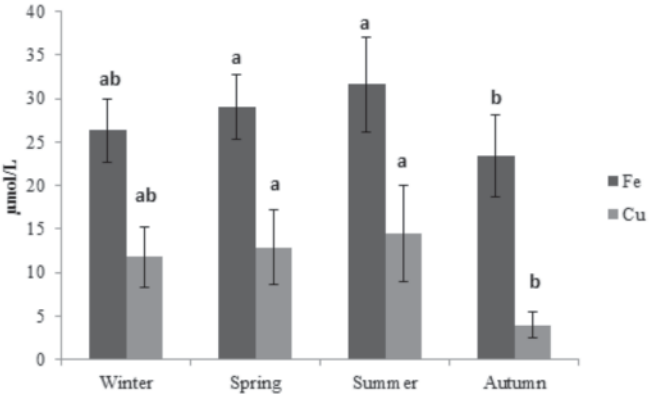

Fig. 4. Seasonal changes of Fe and Cu concentrations in Holstein stallion blood plasma. Values are shown as mean ± standard deviation; a, b: P<0.05 between seasons.

As shown in Fig. 3, the highest concentration of calcium was found in the autumn and it was significantly higher than concentrations measured in the spring (P = 0.042) and in the summer (P = 0.003). The concentrations of inorganic phosphate and magnesium were significantly lower in the autumn and in the winter compared to concentrations measured in the spring and summer (P = 0.002, P<0.001, and P<0.001, P = 0.002 respectively; Fig. 3). The concentrations of copper and iron in the stallions' blood plasma were significantly lower in the autumn compared to levels measured in the summer (P = 0.043; Fig. 4) and spring samplings (P = 0.043; Fig. 4).

In the blood plasma of Holstein stallions significant positive correlations of AST and LDH activities ($r = 0.399$, $P<0.05$) and AST and CK activities ($r = 0.495$, $P<0.05$) were determined. A significant negative correlation between the concentration of calcium and inorganic phosphate ($r = -0.561$, $P<0.05$) was also determined.

Discussion

Since seasonal variations have to be taken into consideration for a correct interpretation of blood chemistry values, we determined seasonal changes in the concentrations of some enzymes and some minerals in Holstein stallions. The enzymes CK, LDH and AST serum activities are considered to be the main indicators of myocyte stability. Activity of CK is proportional to physical activity (BOFFI et al., 2002). In this study CK activities were within the physiological range (<50 U/L, PRITCHARD et al., 2009) for all measured seasons, except in the summer, when CK activity was higher (69.25 ± 17.35 U/L). In this study higher CK activity in the plasma of the Holstein stallions was measured in the summer than in spring and winter. Also, in this study, higher AST activity was determined in the summer compared to the winter sampling. The values measured in this study were lower compared to the reference values for AST in horses (KANEKO et al., 2008) (226-366 U/L). This difference could be due to the different laboratory methods used to determine the individual biochemical indicators. During the summer, the physical activity of these animals was more expressed, since they were kept on pasture, and the values of CK and AST activity were higher (Fig. 1 and 2), in accordance with the higher muscle activity (LACERDA et al., 2006). Also, WEIGERT et al. (1980) found 60 % higher AST activity in horses exposed to greater muscle activity. In this study, a significant correlation between CK and AST activities ($r = 0.49$; $P<0.05$) was established. LACERDA et al. (2006) found a significant correlation between CK and LDH ($r = 0.59$; $P<0.001$).

In this study, LDH activity was within the reference range for horses (KANEKO et al., 2008; 162-412 U/L). Also LDH activity was significantly related to AST activity ($r = 0.40$; $P<0.05$). The highest LDH activity in this study was measured in the summer (382.35 ± 76.31 U/L) and the lowest in the winter (275.3 ± 139.08 U/L) with no significant difference. The effect of exercise type on CK, AST and LDH values has been reported in horses, indicating that higher intensity exercises are related to higher enzyme serum levels (CÁS, 1998).

The activities of enzymes in the horses' blood vary in different horse breeds: Croatian Coldblood, Posavina horse breeds (PAĐEN et al., 2014), Percheron (AOKI and ISHII, 2012). In the literature, GGT activity ranges from 4.3-13.3 U/L (KANEKO et al., 2008), 5.57-19.49 U/L (GUPTA et al., 2002), to 31.8 ± 12.4 U/L (LACERDA et al., 2006). According to those values, GGT activities in this study were within the range mentioned (spring period 13.48 ± 4.30 U/L, summer period 29.92 ± 21.08 U/L, autumn period 18.64 ± 5.13

U/L and winter period 20.92 ± 8.54 U/L). HASKOVIĆ and SULJEVIĆ (2011) determined seasonal differences in GGT activity in male Bosnian mountain horses, with significantly higher activity measured in the autumn. Seasonal differences were also identified in this study, but in contrast to the results of HASKOVIĆ and SULJEVIĆ (2011), we found significantly higher GGT activity in the summer compared to the spring. In horses, high levels of serum GGT activity are associated with a variety of hepatic disorders, such as toxic hepatic failure, subclinical hepatopathy and hyperlipemia (AOKI and ISHII, 2011). In this study, all the horses were clinically healthy, and GGT activity in the summer showed greater individual differences, although the reason for this increase remains unclear.

Significantly lower ALT activity in this study was measured in the summer period (2.13 ± 1.36 U/L) compared with the other seasons (Fig. 1). The highest ALT activity was measured in the summer (7.37 ± 3.98 U/L). The measured activities were within the range specified by KANEKO et al. (2008) (3-23 U/L). There is little hepatic ALT activity in large domestic animals and ALT activity is found in several body organs, but the magnitude of activity in horses per gram of tissue differs little in the liver when compared to muscle (NOONAN, 1981). The low activities of ALT during the year in this study could indicate the small effect of the different seasons on enzyme activity.

In the literature AP activity varies, similar to the variation in GGT activity. AP activity ranges from 97-156 U/L (GUPTA et al., 2002), <250 U/L (PRITCHARD et al., 2009) to 143-395 U/L (KANEKO et al., 2008). Activities in this study were within the mentioned reference ranges. Seasonal changes in the activity of AP were determined in humans (DEVGUN et al., 1981) as well as in sheep (YOKUS et al., 2006) and Bosnian mountain horses (HASKOVIĆ and SULJEVIĆ, 2011). The stallions in this study had significantly higher AP activity in the summer (350.35 ± 113.96 U/L) compared to all other seasons. As the changes in AP activity are associated with mineral metabolism (ŠTRAUS, 2009), this high activity of AP is probably the result of the lower calcium concentration, which was the lowest in the summer period. It is known that the reference intervals for enzyme activities are very broad (KANEKO et al., 2008), and this explains the large standard deviations in enzyme activities in this study.

The calcium concentration in this study ranged in all seasons combined from 0.95-2.94 mmol/L, and it was lower than the reference values established by KANEKO et al. (2008) (2.80-3.40 mmol/L). The lowest values were measured in the summer (1.46 ± 0.44 mmol/L), and they were significantly lower in comparison to the values measured in the spring and autumn. GROMADZKA-OSTROWSKA et al. (1985), over three years of investigation of mineral concentrations in Shetland ponies, determined that calcium concentrations are seasonally variable. In contrast, other studies in dairy cows (MTUI et al., 2007) and in healthy humans (DEVGUN et al., 1981) found no seasonal changes in calcium serum levels. In this study, we determined a significant negative correlation between the concentrations of calcium and inorganic phosphate ($r = -0.56$, $P<0.05$), which indicates a reciprocal relationship between those two actions. Low levels of calcium in healthy horses may be caused by inadequate absorption in the intestines. High levels of phosphate in the food may interfere with calcium absorption in horses (GOFF, 2000), and the highest phosphate levels in this study were found in the summer, which coincided with the lowest levels of calcium (Fig. 3).

The concentration of inorganic phosphate in this study was significantly higher in the summer (2.91 ± 0.31 mmol/L) and lowest in the autumn (1.27 ± 0.49 mmol/L). SHIRLEY et al. (1967) determined that inorganic phosphate depends on the season, with higher values in September than in March. MTUI et al. (2007) demonstrated increased plasma concentrations of phosphorus in the dry season of the year. If the food is rich in phosphorus, there is an increase in the concentration of phosphate in the blood, as well as an increase in alkaline phosphatase activity (PAYNE and PAYNE, 1987). In this study, the highest AP activity was determined in the summer, and the reason for the increased inorganic phosphate concentration is probably the increased intake and increased absorption in the gastrointestinal tract.

The concentration of magnesium in horse blood is 0.77-1.15 mmol/L (KANEKO et al., 2008; BERLIN and AROCH, 2009; LACERDA et al., 2006). The concentration of magnesium in this study was significantly higher in the summer (1.04 ± 0.13 mmol/L) than in the autumn (0.58 ± 0.03 mmol/L). Contrary to our finding, GROMADZKA-OSTROWSKA et al. (1985) and MTUI et al. (2007) observed no seasonal changes in magnesium concentrations. Variations in blood magnesium are nearly all related to changes in dietary magnesium intake (GOFF, 2004). Specifically, maintaining magnesium homeostasis has no direct homeostatic control (GOFF, 2004), so we may assume that the lower values in the autumn period are a consequence of the insufficient supply of magnesium in feed intake.

Since iron is an essential nutrient required in a wide variety of metabolic processes, it is of great importance to determine its concentration in the blood (PONKA et al., 1998). The physiological concentration of iron in horse blood serum is 13.1-25.1 μmol/L (KANEKO et al., 2008). Samples were taken in the morning hours, since the concentration of iron may vary up to 70 % during the day (PAYNE and PAYNE, 1987). In this study, a significantly higher iron concentration was measured in the summer (31.61 ± 5.42 μmol/L) in comparison to concentrations measured in the autumn (23.41 ± 4.70 μmol/L). BIRICIK et al. (2005) and GROMADZKA-OSTROWSKA et al. (1985) found seasonal variations in horse blood iron concentrations. In this study both iron and copper concentrations were lower in the autumn compared with the levels measured in the summer, and the lower iron concentration could be explained by the lowering of copper concentration, because the copper-containing proteins hephaestin and ceruloplasmin are required for normal iron transport (WESSLING-RESNICK, 2006).

A significantly higher copper concentration in this study was measured in the summer (14.45 ± 5.57 μmol/L) compared to the activity measured in the autumn (3.97 ± 1.49 μmol/L). BIRICIK et al. (2005) also found serum copper levels were the highest in the summer months in Standardbred mares. Also, AUER et al. (1988) found lower copper concentrations in August in comparison with concentrations measured in February. The normal range of copper in the blood of most healthy animals is between 7.9 and 23.6 μmol/L (MASON, 1979). Copper deficit may result from malabsorption, caused by excess of calcium, iron, molybdenum, protein (insoluble compounds with sulphur). Since there are doubts about which method is the most appropriate for determination of the copper status in the body (copper or ceruloplasmin concentrations) further research is warranted.

In conclusion, we found significant seasonal changes in enzyme activities and mineral concentrations in healthy Holstein stallion blood. We can assume that seasonal changes may occur due to the greater muscle activity of the horses, especially in the summer period. Our findings also suggest the possible influence of feed intake with regard to the way the animals are kept.

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VRANKOVIĆ, L., J. ALADROVIĆ, B. BEER-LJUBIĆ, M. ZDELAR-TUK, Z. STOJEVIĆ: Sezonske promjene aktivnosti enzima i koncentracije minerala u krvnoj plazmi pastuha holštajnske pasmine. Vet. arhiv 85, 235-246, 2015.

SAŽETAK

Holštajnski konj dominantna je pasmina u međunarodnom preponskom jahanju pa je od velike važnosti razumjeti metaboličke procese za pravilno upravljanje uzgojem životinja kao i za kontrolu zdravlja stada. Cilj ovog istraživanja bio je utvrditi sezonske promjene u aktivnosti nekih enzima i koncentracije nekih minerala u krvnoj plazmi pastuha holštajnske pasmine. Krv za analizu uzeta je punkcijom vratne vene u epruvete s heparinom kao antikoagulansom. U uzorcima su određene aktivnosti aspartat aminotransferaze (AST), alanin aminotransferaze (ALT), γ-glutamil transferaze (GGT), alkalne fosfataze (AP), laktat dehidrogenaze (LDH) te kreatin kinaze (CK). U krvi su određene i koncentracije kalcija, fosfora, magnezija, željeza i bakra. Većina pokazatelja istraživana u ovom radu bila je najviša u ljetnom razdoblju. Utvrđena je i značajna negativna korelacija između koncentracije kalcija i anorganskog fosfora. Možemo pretpostaviti da su sezonske promjene u aktivnosti enzima i koncentracije minerala u krvi ovisne o mišićnoj aktivnosti i o uravnoteženom unosu hrane.

Ključne riječi: sezonske promjene, aktivnost enzima, koncentracija minerala, krv, holštajnski pastusi