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Serum profiles of calcium, phosphorus, magnesium, vitamin D and parathyroid hormone in Caspian horses during different seasons

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ABSTRACT

Minerals play an essential role in the normal vital process. Calcium, phosphorous and magnesium are the most abundant minerals. Vitamin D and parathyroid hormone play a key role in regulation of their homeostasis as well. The aim of this study was to evaluate calcium, phosphorous, magnesium, vitamin D and parathyroid hormone on 30 Caspian horses of Guilan Province (south of the Caspian Sea) in two different seasons. So that, 15 stallions and 15 mares were sampled at the age groups ≤ 3 years and > 3 years respectively. Fasting blood samples were collected twice, in August and January 2015 from jugular vein of horses. Serum calcium, phosphorous and magnesium were measured by colorimetric methods, while 25(OH)D_3 and parathyroid hormone were measured by ELISA. The calcium (11.50 vs. 14.25 mg.dL^{-1}), magnesium (2.13 vs. 3.72 mg.dL^{-1}) and vitamin D (1.66 vs. 2.48 ng.ml^{-1}) levels were lower in winter than in summer ($P < 0.05$). The Caspian horses had higher phosphorous (4.52 vs. 3.26 mg.dL^{-1}) in winter than in summer ($P < 0.05$). Effect of sex on the measured parameters was not significant. Effects of age on the calcium, magnesium, vitamin D and parathyroid hormone levels were not significant, but ≤3 year - old horses had higher phosphorous (4.63 vs. 3.15 mg.dL^{-1}) than >3 year - old ones ($P < 0.05$). The vitamin D level of mares was higher (3.10 vs. 1.43 ng.ml^{-1}) in summer than in winter ($P < 0.05$). Effects of sex, season, age and their interactions on parathyroid hormone were not significant. In conclusion, calcium, phosphorous, magnesium and parathyroid hormone levels in Caspian horses were within their physiological range, but vitamin D was low without any signs of deficiency. The Caspian horses had lower calcium, magnesium and vitamin D levels in winter than in summer.

Key words: Caspian horse, Calcium, Phosphorous, Vitamin D, Parathyroid hormone.

INTRODUCTION

A brilliant national inheritance of Iran is Caspian horse, which is the firstborn species of horses, originated from the north of Iran. This species is the rarest one in worldwide, so that just near 300 horses can be found in Iran (Firouz 1972). Minerals have important roles in the normal functions of body. Calcium (Ca), phosphorous (P) and magnesium (Mg) are the most plentiful minerals among organic materials, and vitamin D plays an important role in controlling the homeostasis of these minerals (Piccione *et al*. 2008). However, many aspects of the homeostatic system in horses are different from those in most mammalian species (Piccione *et al*. 2008). In addition, homeostatic regulation mechanisms are not recognized in the serum of horse blood (Breidenbach *et al.* 1998; Cowan 2002; Harmeyer & Schlumbohm 2004). Vitamin D affects significantly regulating Ca and P, proliferation of cell, physiology of bone and integrity of epithelial tissue (Dittmer & Thompson 2011; Toribio 2011; Pozza *et al*. 2014). Vitamin D increases renal reabsorption of P and Ca, as well as absorption in gastrointestinal system (Rourke *et al*. 2010; Toribio 2010; Pozza *et al*. 2014). In addition, vitamin D controls osteoblast activity, an important function which its absence can result in skeletal irregularity (Dittmer & Thompson 2011; Pozza *et al*. 2014). Another functions of vitamin D which have been lately characterized, include anti-carcinogenic, anti-inflammatory effects and immunomodulation (Haroon & Fitzgerald 2012, Radlovic *et al*. 2012). Ultraviolet light can produce vitamin D_3 in the skin (Toribio 2011; Pozza *et al*. 2014). Variation in concentrations of serum vitamin D over different seasons has been reported in Scandinavian horses (Maenpaa *et al*. 1988; Pozza *et al*. 2014). Parathyroid hormone (PTH) is a hormone, which has a significant role in maintaining the serum levels of phosphate and Ca in the normal range and the control of this hormone is performed through the serum levels of Ca and calcitriol. Increasing PTH level usually causes Ca or vitamin D deficiency (Steingrimsdottir *et al*. 2005).

Difference in the levels of Ca, P, Mg, vitamin D and PTH in summer and winter are not clear in Caspian horses. There is no report about normal values of mentioned parameters in Caspian horses so far. This study was aimed to measure the serum levels of Ca, P, Mg, 25(OH)D_3 and PTH in Caspian horses during summer and winter and also to study the impact of age, gender, season and their interactions on these parameters.

MATERIALS AND METHODS

Animals and Blood Sample Collection

Blood samples were collected from healthy Caspian horses (n = 30; stallions = 15, mares = 15) in Guilan Agricultural and Natural Resources Research Center (latitude: 37°18′N, longitude: 49°65′E). Fasting blood samples were collected twice in August (day length 14h) and January (day length 10h) from jugular vein. Based on age, the horses were divided into two groups: equal to and less than 3 years (≤ 3 years) and greater than 3 years (> 3 years).

The studied Caspian horses exhibited excellent physical and health conditions without any history of disease, and had not received any treatment for one month before sampling. The animals did not work during collecting samples. Horses were fed with grass and alfalfa hay with 4h.day^{-1} access to grazing (similar diets and amounts were used in both summer and winter). All horses were individually kept in stalls and permitted unlimited access to water over the experiment.

Blood sample collection was carried out for all animals in the morning (07:00–09:00h). Blood samples were poured in tubes without additives, permitted to clot, centrifuged at 3000 rpm for 10 min at 4°C, and serums were kept at -80°C prior analysis.

Serum analysis

Serum Ca (Darman Kave Kit, Esfahan, Iran), P (Ziest Chem kit, Tehran, Iran), and Mg (Pars Azmun kit, Karaj, Iran) concentrations were measured by colorimetric methods (Piccione *et al*. 2008). Serum 25(OH)D_3 (Euroimmun kit, Lubeck, Germany) and PTH (horse PTH ELISA kit, Shanghai Crystal Day Biotech. Co. LTD, Shanghai, China) levels were determined using an enzyme-linked immunosorbent assay (ELISA) test (Pozza *et al*. 2014).

Statistical analyses

The following model was used for statistical analysis of data:

$Y_{ijkl}=\mu + Sex_i + Age_j + Season_K + (Sex \times Age)_{ij} + (Sex \times Season)_{ik} + (Age \times Season)_{jk} + (Sex \times Age \times Season)_{ijk} + e_{ijkl}$

Where, Y_{ijkl} = Concentration of blood parameters; μ = The mean of blood parameters; Sex_i = Animal sex (two levels; male or female); Age_j = Animal age (two levels; less or older than three years old); $Season_k$ = Season (two levels; summer or winter); e_{ijkl} = Random residual effect. The repeated measurements of blood parameters were analyzed using the Mixed procedure of SAS program (version 9.2) with the error covariance structure of first - order heterogeneous autoregressive structure. Differences among least-squares means were tested using Tukey adjustment method. Acceptable significant levels were declared at P < 0.05.

RESULTS

The Ca, P, Mg, 25(OH)D_3 and PTH concentrations were found to be 14.25 $mg.dL^{-1}$; 3.26 $mg.dL^{-1}$; 3.72 $mg.dL^{-1}$; 2.48 $ng.ml^{-1}$ and 73.23 $pg.ml^{-1}$ in summer, respectively, while their values were 11.50 $mg.dL^{-1}$; 4.52 $mg.dL^{-1}$; 2.13 $mg.dL^{-1}$; 1.66 $ng.ml^{-1}$ and 70.16 $pg.ml^{-1}$ in winter, respectively.

Ca, P and Mg

Serum Ca, P and Mg concentrations are shown in Table 1. The effect of sex on the measured parameters was not significant (P > 0.05). Ca and Mg concentrations were lower, while P level was higher in winter than in summer (P < 0.05). Serum Ca and Mg levels were not different between ≤ 3 and > 3 year - old horses, while serum P was lower in >3 year - old horses. Interactions of sex × age on Ca and Mg concentrations were not significant, while serum P in mares and stallions > 3 year - olds were lower than mares and stallions ≤ 3 year ones (p < 0.05).

Interactions of sex × season and age × season on the Ca, P and Mg concentrations were significant (p < 0.05), while interactions of sex × age × season on the Ca, P and Mg concentrations were not significant (p > 0.05).

Table 1. Effects of sex, season, age and their interactions on the serum Ca, P and Mg in Caspian horses.

Effect	Calcium ($mg.dL^{-1}$)	Phosphorus ($mg.dL^{-1}$)	Magnesium ($mg.dL^{-1}$)
Main Effects			
Effect of sex			
Male	13.09 ± 0.43*	3.66 ± 0.18	3.04 ± 0.20
Female	12.66 ± 0.44	4.12 ± 0.18	2.81 ± 0.20
Effect of season			
Summer	14.25^a ± 0.38	3.26^b ± 0.17	3.72^a ± 0.20
Winter	11.50^b ± 0.38	4.52^a ± 0.17	2.13^b ± 0.21
Effect of age			
≤ 3 Years	12.59 ± 0.42	4.63^a ± 0.18	3.01 ± 0.19
> 3 Years	13.17 ± 0.45	3.15^b ± 0.19	2.84 ± 0.20
Interactions			
Sex × Age			
Male × ≤ 3 Years	12.80 ± 0.59	4.33^a ± 0.25	3.08 ± 0.27
Male × > 3 Years	13.38 ± 0.63	2.99^b ±0.27	3.01± 0.29
Female × ≤ 3 Years	12.38 ± 061	4.93^a ± 0.26	2.94 ± 0.28
Female × > 3 Years	12.95 ± 0.63	3.31^b ± 0.27	2.69 ± 0.29
Sex × Season			
Male × Summer	14.42^a ± 0.54	2.84^b ± 0.23	4.03^a ± 0.29
Male × Winter	11.76^b ± 0.54	4.47^a ± 0.23	2.06^c ± 0.29
Female × Summer	14.08^a ± 0.54	3.68^b ± 0.23	3.42^b ± 0.29
Female × Winter	11.25^b ± 0.55	4.56^a ± 0.24	2.20^c ± 0.30
Season × Age			
Summer × ≤ 3 Years	14.38^a ± 0.52	4.48^a ± 0.23	3.70^a ± 0.28
Summer × > 3 Years	14.12^a ± 0.55	2.04^b ± 0.24	3.74^a ± 0.30
Winter × ≤ 3 Years	10.80^b ± 0.53	4.78^a ± 0.23	2.31^b ± 0.29
Winter × > 3 Years	12.21^a ± 0.55	4.26^a ± 0.24	1.95^b ± 0.30

*Least squares means ± SE.

$^{a-c}$ Least squares means with different superscripts differ significantly (P < 0.05).

Vitamin D and PTH

Concentrations of 25(OH)D_3 and PTH are presented in Table 2. Effects of sex and age on 25(OH)D_3 were not significant ($P > 0.05$).

The 25(OH)D_3 level was higher in summer than in winter ($P < 0.05$). Interactions of sex × age were not significant concerning to the vitamin D and PTH values. The vitamin D level in mares was higher in summer than in winter ($p < 0.05$). Interactions of season × age were significant.

So that, ≤ 3 year - old horses in winter had lower vitamin D than ≤ 3 as well as > 3 year - olds in summer. Effects of sex, season, age and their interactions on PTH values were not significant. Interaction of sex × age × season on the Vitamin D and PTH levels were not significant ($P > 0.05$).

Table 2. Effects of sex, season, age and their interactions on the serum vitamin D and PTH levels in Caspian horses.

Effect	Vitamin D ($ng.ml^{-1}$)	PTH ($pg.ml^{-1}$)
Main Effects		
Effect of sex		
Male	1.88 ± 0.27*	78.34 ± 10.45
Female	2.26 ± 028	65.05 ± 10.48
Effect of season		
Summer	2.48^{a} ± 0.27	73.23 ± 7.72
Winter	1.66^{b} ± 0.28	70.16 ± 7.76
Effect of age		
≤ 3 Years	1.91 ± 0.27	60.25 ± 10.13
> 3 Years	2.24 ± 0.28	83.14 ± 10.79
Interactions		
Sex × Age		
Male × ≤ 3 Years	1.80 ± 0.37	64.69 ± 14.28
Male × > 3 Years	1.97 ± 0.40	92.00 ± 15.26
Female × ≤ 3 Years	2.02 ± 0.39	55.81 ± 14.36
Female × > 3 Years	2.50 ± 0.40	74.29 ± 15.26
Sex × Season		
Male × Summer	1.87^{b} ± 0.38	81.61 ± 10.92
Male × Winter	1.90^{b} ± 0.38	75.08 ± 10.92
Female × Summer	3.10^{a} ± 0.38	64.86 ± 10.92
Female × Winter	1.43^{b} ± 0.40	65.24 ± 11.04
Season × Age		
Summer × ≤ 3 Years	2.45^{a} ± 0.37	57.75 ± 10.55
Summer × > 3 Years	2.52^{a} ± 0.40	88.71 ± 11.28
Winter × ≤ 3 Years	1.37^{b} ± 0.38	62.75 ± 10.67
Winter × > 3 Years	1.95^{ab} ± 0.40	77.57 ± 11.28

* Least squares means ± SE.

^{ab} Least squares means with different superscripts differ significantly ($P < 0.05$).

DISCUSSION

Although the biochemistry of serum and hormone of the horse is well recognized, particularly for the European and American types, and different text books explained them in detail (Eades & Bounous 1997; Meyer & Harvey 2004), but there are limited information about the ancient types (including Caspian horse) in their indigenous geographical areas. The present study is reported for the first time concerning to the Caspian horses. In our study, Ca, P, Mg and PTH concentrations were obtained in the horses' physiological range (Kaneko *et al*. 1997) while vitamin D was observed less compared to other studies (Maenpaa *et al*. 1988; Piccione *et al*. 2008; Pozza *et al*. 2014). Serum Ca was lower in winter than in summer and effects of sex and age on these parameters were not significant, which is in agreement with previous equine studies (Pozza *et al*. 2014). Effect of age was only significant on P, among the measured parameters. Osteocalcin in bone matrix increases with aging and increasing bone formation in growing horses. This protein is homeostasis for blood P

(Markowitz 1994). Serum Mg was lower in winter than in summer, but was within the physiological range and was not considered as deficiency. Effect of age on serum vitamin D level was not significant. Pozza *et al*. (2014) reported that serum 25(OH)D_3 concentrations were lower in foals than in yearling and adult horses. They initially stated that vitamin D placental translocation affects preterm neonates and the key source of 25(OH)D_3 in the immediate postpartum period is milk, which contains a low concentrations of 25(OH)D_3. Next, neonatal foals have limited exposure to ultraviolet light, especially in the Northern hemisphere. The reason for this difference could be that we had two age groups (≤ 3 years and > 3 years) and the number of newborn foals was low. Serum 25(OH)D_3 was lower in winter than in summer. Seasonal variations in serum concentrations of 25(OH)D_3 have been recorded in horses, with greater levels in summer compared to winter (Maenpaa *et al*. 1987, 1988; Pozza *et al*. 2014). The role of vitamin D in the calcium homeostasis is well known in humans, but there was an uncertainty for this mechanism in horses (Harmeyer & Schlumbohm 2004; Piccione *et al*. 2008). Despite low or unrecognizable concentrations of vitamin D metabolites, horses have high concentrations of extracellular Ca (Maenpaa *et al*. 1987, 1988; Breidenbaeh *et al*. 1998; Pozza *et al*. 2014). The Caspian horses in the present study had access to grazing and were exposed to sunlight, but they had 25(OH)D_3 levels less than those observed in other horse types (Van Schoor & Lips 2011). The presence of ultraviolet light affects converting 7-DHC to provitamin D_3 in the skin that then endures thermal isomerization to vitamin D_3 over three days. Ultraviolet radiation is lower over winter months, when hours of daylight are low, leading to decreased vitamin D synthesis (Dittmer & Thompson 2011). Horses have higher serum calcium and lower 25(OH)D_3 levels compared to other animal species,. Actually, the vitamin D metabolite levels in horses are lower than those at which rickets happen in other animals, proposing a variation over species and probably showing distinct mechanisms for metabolism of phosphate and Ca in horses (Dittmer & Thompson 2011).

The results of the present study revealed that ponies fed enough P and Ca, require vitamin D in the diet for normal development of their bone if they have no sunlight; supplemental vitamin D is not required with plentiful sunlight (EL Shorfa *et al*. 1979).

Effects of sex, season, age and their interactions on serum PTH levels were not significant and were within the normal range for the horses. Toribio *et al*. (2005) reported that the parathyroid gland response was variable among horses; some horses responded to hypocalcemia with an increase in serum PTH concentration, whereas other horses did not.

The high blood levels of Ca in horses are evolutionary modifications that partly result from the low levels of vitamin D metabolites, which would decline their restraining influence on chief cells in parathyroid, and consequently increase the sensitivity of these cells to extracellular Ca (Toribio *et al*. 2003b; Pozza *et al*. 2014).

This, in theory, would change the set-point of PTH-Ca to the right, indicating that greater concentrations of Ca would be needed to restrain secretion of PTH, therefore, increasing the resting concentration of Ca.

The results of some studies indicated that parathyroid cells in horses which have an increased set-point of PTH-Ca have lower sensitivity to concentrations of extracellular Ca when compared th another species (Toribio *et al*. 2003a, 2003b; Pozza *et al*. 2014).

CONCLUSION

The results indicated that the Ca, P, Mg and PTH levels in Caspian horses were within the physiological range for the horses, but vitamin D was low without signs of deficiency.

The Caspian horses had lower levels of Ca, Mg and vitamin D in winter than in summer.

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وضعیت کلسیم، فسفر، منیزیم، ویتامین D و هورمون PTH در سرم اسب‌های کاسپین در فصول مختلف

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چکیده

مواد معدنی نقش اساسی در فرآیندهای حیاتی دارند و کلسیم، فسفر و منیزیم از فراوان‌ترین مواد معدنی هستند. ویتامین D و هورمون پاراتیروئید نقش مهمی در تنظیم هومئوستاز دارند. هدف از این تحقیق ارزیابی کلسیم، فسفر، منیزیم، ویتامین D و هورمون پاراتیروئید روی ۳۰ اسب کاسپین استان گیلان در دو فصل مختلف بود. بطوریکه از ۱۵ اسب نر و ۱۵ اسب ماده در گروه‌های سنی زیر ۳ سال و بالای ۳ سال نمونه برداری شد. نمونه‌های خون بصورت ناشتا و دو بار در ماه‌های شهریور و بهمن ۱۳۹۳ از ورید وداج اسب‌ها جمع‌آوری شد. کلسیم، فسفر و منیزیم به روش رنگ سنجی و 25(OH)D3 و هورمون پاراتیروئید به روش ELISA اندازه‌گیری شد. میزان کلسیم (۱۱/۵۰ در برابر ۱۴/۲۵ میلی‌گرم در دسی‌لیتر)، منیزیم (۲/۱۳ در برابر ۳/۷۲ میلی‌گرم در دسی‌لیتر) و ویتامین D (۱/۶۶ در برابر ۲/۴۸ نانوگرم در میلی‌لیتر) در زمستان کمتر از تابستان بود (p< ۰/۰۵). اسب‌های کاسپین در زمستان نسبت به تابستان فسفر (۴/۵۲ در برابر ۳/۲۶ میلی‌گرم در دسی‌لیتر) بیشتری داشتند (p< ۰/۰۵). اثر جنس روی عوامل اندازه‌گیری شده معنی‌دار نبود. اثر سن بر میزان کلسیم، منیزیم، ویتامین D و هورمون پاراتیروئید معنی‌دار نبود اما اسب‌های زیر ۳ سال نسبت به اسب‌های بالای ۳ سال فسفر (۴/۶۳ در برابر ۳/۱۵ میلی-گرم در دسی‌لیتر) بیشتری داشتند (p< ۰/۰۵). میزان ویتامین D (۳/۱۰ در برابر ۱/۴۳ نانوگرم در میلی‌لیتر) در اسب‌های ماده در تابستان بیشتر از زمستان بود (p< ۰/۰۵). اثر جنس، فصل، سن و اثر متقابل آنها بر هورمون پاراتیروئید معنی‌دار نبود. در نهایت می‌توان گفت سطوح کلسیم، فسفر، منیزیم و هورمون پاراتیروئید اسب‌های کاسپین در محدوده فیزیولوژیکی آنها قرار دارد اما اسب‌ها بدون هیچ نشانه‌ای، دارای کمبود ویتامین D بودند. میزان کلسیم، منیزیم و ویتامین D اسب‌های کاسپین در زمستان کمتر از تابستان بود.

*مؤلف مسئول