Correlation between serum dosage of Calcium, Phosphorus, Alkaline Phosphatase and epifisary closing age in foals of low genetic grouping

Correlação entre as dosagens séricas de Cálcio, Fósforo, fosfatase Alcalina e idade de fechamento epifisário em potros do grupamento genético baixadeiro

DOI:10.34117/bjdv8n1-238

Recebimento dos originais: 07/12/2021
Aceitação para publicação: 14/01/2022

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ABSTRACT
The subsided region of Maranhão is characterized by its peculiar ecosystem, flooded fields in rainy seasons and an arid environment in the dry season. In this region, there is a remarkable record of the presence of a genetic group called by the local population, of horse "baixadeiro". The study aimed to determine, through radiographic evaluation, the age of closure of the radio growth plate of these animals, correlating radiographic findings with gender and serum dosages of calcium, phosphorus and alkaline phosphatase. In the study, 60 horses were used, with ages ranging from 18 to 30 months, divided into two equal groups according to gender. A portable X-ray device was used to obtain the images. The radiographic technique used was 53 kVp and 5 mAs, with a film focus distance of 70 cm in craniocaudal projection. Blood samples were performed by puncture of the external jugular vein and stored in Ependorff tubes, frozen until processing. Biochemical analyses were performed using specific kits to measure calcium, phosphorus, and alkaline...
According to the results obtained, the average age of closure of the growth plate was 26.7 months for the male and 24.5 in females. As for serum dosages, we observed that the average values found in calcium were higher in males, while the average values found for both phosphorus and alkaline phosphatase were higher in females.

**Keywords:** epiphysis, equine, x-ray

**RESUMO**

A região da baixada maranhense é caracterizada por seu ecossistema peculiar, campos alagados em épocas de chuvas e um ambiente árido na época de seca. Nessa região, existe um registro marcante da presença de um grupamento genético chamado pela população local de cavalo “baixadeiro”. O estudo teve como objetivo determinar, por meio de avaliação radiográfica, a idade de fechamento da placa epifisária do rádio desses animais, correlacionando os achados radiográficos com o sexo e as dosagens séricas de cálcio, fósforo e fosfatase alcalina. No trabalho utilizaram 60 equinos, com idades variando de 18 a 30 meses, divididos em dois grupos iguais de acordo com o sexo. Na obtenção das imagens, foi utilizado um aparelho de Raios-X portátil. A técnica radiográfica usada foi de 53 kVp e 5 mAs, com distância foco filme de 70 cm em projeção crânio-caudal. As colheitas de sangue foram feitas por punção da veia jugular externa e armazenadas em tubos tipo Ependorff, congelados até o processamento. As análises bioquímicas foram realizadas utilizando-se kits específicos para mensuração de cálcio, fósforo e fosfatase alcalina. De acordo com os resultados obtidos, a idade média do fechamento da placa epifisária foi de 26,7 meses para o macho e de 24,5 nas fêmeas. Quanto às dosagens séricas observamos que os valores médios encontrados no cálcio foram superiores nos machos, enquanto os valores médios encontrados tanto para o fósforo quanto para a fosfatase alcalina foram superiores nas fêmeas.

**Palavras-chave:** epífise, equino, raio-x

**1 INTRODUCTION**

Native or naturalized breeds are composed of animals that are for a long period under the action of natural selection in certain environments, to the point of presenting specific characteristics of these conditions (MCMANUS, et al. 2005). These breeds, through natural selection, have developed unique adaptation characteristics that may be demanded in the future, which gives them genetic value and justifies their preservation (EMBRAPA, 2020).

The genetic group known as "baixadeiro" has characteristics of adaptation to the environmental and nutritional conditions of the region of subsided Maranhão. As has been the case with other naturalized breeds, the horses of this group have been at risk of extinction due to crossings with exotic breeds prioritizing the increase in economic value and genetic pattern, resulting in deterioration of their genetic potential (SERRA, 2016).
The type of rearing livestock, feeding, nutritional supplementation and the challenges by pathogens interfere in the development of the animal (PRADO FILHO, 2004).

To evaluate the bone maturity of horses through radiography, the distal epiphysis of the radius is the region of choice, because it presents a more accurate indication of skeletal evolution (STASHAK, 2002).

With the practice of biochemical studies, it was noted that blood serum reflects cellular integrity and organic function but may undergo variations in standard values that may be related to organic dysfunctions, as well as irregular extravasation of cellular constituents to serum and deficiencies in the production, absorption and excretion of serum components (RADIN, 2003).

Given the need to obtain data on the age of closure in horses, especially the "baixadeiro" group that need information about their bone development, for the beginning of service activities, the present study aimed to determine through radiographic evaluation the age of closure of the distal growth plate of the radio, correlating radiographic findings with gender and serum dosages of calcium, phosphorus and alkaline phosphatase.

2 MATERIAL AND METHODS

We used 60 horses of the genetic group "baixadeiro", divided into two equal groups according to gender and age ranging between 18 and 30 months (age was obtained through observation and dental evaluation). Kept in extensive rearing regime, without nutritional, reproductive or sanitary control, feeding on native pasture. They were submitted to a previous clinical examination for the selection of healthy animals who were able to participate in the experiment. They come from properties located in the cities of Pinheiro and Viana, state of Maranhão, located in the region of subsided Maranhão.

The study was approved by the Ethics and Animal Experimentation Committee of the Veterinary Medicine Course, Maranhão State University, with protocol number 19/2011, approved on 08/31/2011.

The foals were x-rayed only once and divided into three groups according to the radiographic classification of closure of the distal epiphysis of the radio following the STASHAK classification (2002).

In the study of the closure of the distal growth plate of the radius, a portable X-ray device, model FNX Jockey 90, chassis of size 24x30cm, containing screen and radiographic film of the same size was used. The radiographic technique was 53 kVp and
5 mAs, with a film focus distance of 70 cm. The craniocaudal radiographic projection was used for the study and the revelation was performed manually.

Blood samples were collected by puncture of the external jugular vein to obtain the serum, transferred, and stored in Ependorff tubes, frozen until processing. Biochemical analyses were performed using specific kits to measure calcium, phosphorus, and alkaline phosphatase, following the technical recommendations of the manufacturers.

For the association between the degree of closure of the growth plate, gender and age group, the χ²-test was used. To evaluate the interdependence between the serum dosages within the age group and the groups determined by the degree of closure of the growth plate, a nonparametric and non-paired ANOVA was performed. Radiographic findings were correlated with the serum dosages and with the gender and age group of the animals and their possible interdependencies, using the student's t-test.

3 RESULTS AND DISCUSSION

With the determination of the age of closure of the growth plate, it was observed that the average closing age in males was 26.7 months while in females it was 24.5 months (Table 1).

Table 1: Average and standard deviations of age (months) of the closure of the growth plate of horses of the "baixadeiro" group according to gender. Maranhão, 2012

<table>
<thead>
<tr>
<th>GENDER</th>
<th>AGE IN MONTHS</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE</td>
<td>26.7</td>
<td>± 4.87</td>
</tr>
<tr>
<td>FEMALE</td>
<td>24.5</td>
<td>± 4.20</td>
</tr>
</tbody>
</table>

The results are close to those found by VULCANO et al. (2000), 24 months in males and 23 in English Thoroughbred females; approximately 26 months in females and at 28 months in males of the Brazilian equestrian breed (GODOY et al., 2004), according to Moraes et al. (2017) and Pimentel et al. (2017), which accompanied the biometric development of Creole foals and observed their growth occurs until three years of age, with the peak growth in the first year. Differing from the data described by Llavet et al. (2020), who when studying Creole horses, observed a later growth rate, approximately 53 months old, and are medium-sized animals.

It was also observed that the growth closure in males occurred later in relation to females, corroborating the description made by Godoy et al. (2004), which when studying
equine horses of the Brazilian equestrian breed (BH), verified that the growth closure in males occurs later when compared with females. Describing the precocity of physis closure in females due to hormonal factors. However, in studies with Creole horses, Llavet et al. (2020) did not observe the relationship between gender and the age of phesynic closure.

When analyzing the serum calcium, phosphorus and alkaline phosphatase dosages, in relation to the degree of closure of the epostille plaque to the age group of horses, it was observed that there were no statistically significant differences (p > 0.05) between the averages of each serum dosage of the studied elements.

Comparing the calcium, phosphorus and alkaline phosphatase dosages in relation to the sex of the animals, a statistically significant difference (p < 0.05) was observed between the means in all variables analyzed. It was found that the mean calcium in males was higher than that found in females, while the values found of phosphorus and alkaline phosphatase showed higher averages in females than in males (Table 2).

Table 2: Average values and respective standard deviations of serum calcium, phosphorus and alkaline phosphatase (mg/dL) dosages of horses of the “baixadeiro” group, distributed according to gender. Maranhão, 2012

<table>
<thead>
<tr>
<th>SERUM DOSAGE</th>
<th>GENDER</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg/dL)</td>
<td>MALE</td>
<td>FEMALE</td>
<td>P</td>
<td>KW</td>
</tr>
<tr>
<td>9.29± 1.98</td>
<td>8.03± 2.05</td>
<td>0.0151</td>
<td>2.583</td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>6.31b± 1.45</td>
<td>7.16a± 1.28</td>
<td>0.0420</td>
<td>2.128</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>187.24b± 22.01</td>
<td>203.82a± 19.08</td>
<td>0.0024</td>
<td>3.331</td>
</tr>
</tbody>
</table>

a, b – different letters on the same line indicate significant statistical differences between AVERAGES. ANOVA with comparison of means by unpaired t-test;

This statistical difference between the means of serum calcium and phosphorus dosages between the sexes can be explained through the interaction of these minerals, where high concentrations of Ca may lead to P deficiency, even if the P intake is adequate, as mentioned above, where males have a low P rate and high Ca. Different from what occurs in females, where we observed high rates of P and low rates of Ca, where the same author justifies that the dependence relationship of the two minerals can be inverse (TAMIN & ANGEL, 2003), with the regulation of these elements directly linked hormonal regulation (GEOR et al., 2013)

The statistical difference between the P serum P dosages in males and females observed in the study were described by Cunha (1991); Mcdowell (1992), who in his studies observed that this micromineral can be influenced by several factors, including: breed, growth rate and productivity, training stress, performance, age, quality of bulky
foods, environmental condition, level of sweating and other minerals in the feed, as well as the age of animals (NRC, 2007).

The difference in AF values found in females when compared to males is relevant data, because according to Costa et al. (2009), this enzyme may be hormonally influenced.

The average serum calcium dosages obtained in this study ranged from 8.25 to 8.80 mg/dL, corroborating Melo et al. (2021), which when studying healthy horses submitted to enema, obtained calcium values ranging from 8 to 10.5 mg/dL. The present results differ from those found by Campelo et al. (2008), which when studying mares, found values ranging from 1 to 1.4 mg/dL, values that are lower when compared to the results obtained in this study, a difference that may be related to the age of the animals studied, management and region where the animals were studied. Also differing from data obtained by (BARRÊTO-JÚNIOR et al, 2017), who when evaluating horses before and during treatment for hypocalcemia, observed values of 1.5 to 4.5 mg/dL of calcium and 0.47 to 1 mg/dL of phosphorus. On the other hand, Vitti et al. (2008), Costa et al. (2017) and Ribeiro et al. (2021), when studying ponies and horses, obtained 10.3 to 11.94 mg/dL of calcium and 9 to 10.5 mg/dL of phosphorus, respectively, presenting higher values than those found in the animals studied. These results may be related to food management and supplementation to which the animals are submitted.

Regarding the means of the phosphorus serum dosages, the values ranged from 6.40 to 6.72 mg/dL according to the degree of closure, a result that was lower than those of Mundin et al. (2004), where the values found ranged from 9.18 to 16.5 mg/dL. However, the serum P dosages in the present study were higher than the average found by Vitti et al. (2008), which obtained the average dosage of 4.98 mg/dL; Campelo et al. (2008), who, when studying mares, obtained values of 3 to 4.5 mg/dL. Such differences may be related to age, management, and region where the study was conducted.

In the evaluations of alkaline phosphatase, the values obtained ranged from 184.20 to 196.53 U/L, differing from Campelo et al. (2008) and Souza et al. (2016), which found a variation from 200 to 313.90 U/L and higher than the values observed by Braga et al. (2021), which when studying the biological effects of aflatoxin b, obtained values ranging from 52 to 84 U/L. The differences verified with the authors may be related to the methodology used.

The average values of Ca found in the present study corroborate the results of Lewis et al. (2000) that when studying lactating mares and foals at weaning, observed
that the concentrations of this mineral would be lower, caused by the higher demands of this mineral in these phases.

The serum AF dosages did not vary statistically when compared to age, a result that corroborates with Benesi et al. (2009) who observed that after six months of age the values remained with small oscillations reaching averages like those of adult animals.

When testing the correlation between the degree of closure of the bone epiphysis with the serum variables (calcium, phosphorus and alkaline phosphatase), as well as the characteristics of the animals (gender and age group) and the possible interdependences, there was a correlation between alkaline phosphorus/phosphatase and alkaline calcium/phosphatase, which presented partial and positive dependence (p < 0.05), as demonstrated in the dispersion diagram (Figure 1) and negative partial dependence (Figure 2) respectively.

Figure 1: Dispersion diagram and regression equation, regression analysis and correlation between phosphorus (x) (6.64 ± 1.44 mg/dL) and alkaline phosphatase (y) (193.78 ± 24.36 mg/dL), of horses of the “baixadeiro” group, regardless of the degree of closure of the growth plate, gender and age group. r = 0.459; $r^2 = 0.212$; p = 0.0002; CV% = 0.1017%

When observing the dispersion diagram of the regression analysis and correlation between the dosage of phosphorus (x) (6.64 ± 1.44 mg/dL) and alkaline phosphatase (y) (193.78 ± 24.36 mg/dL), regardless of the degree of closure of the growth plate, gender and age group can verify the existence of correlation between the variables (r = 0.459) with partial and positive dependence, with negligible determination ($r^2 = 0.212$), and this regression is extremely significant by the t-test (p = 0.0002).

This statistical dependence may be related to the fact that, for alkaline phosphatase to start its activity, it needs a medium alkaline and P is an important mineral in contributing to establish and maintaining osmotic pressure and acid-base balance of animals (MURAKAMI, 2006).
When observing the dispersion diagram of the regression analysis and correlation between the calcium (x) (8.52 ± 2.10 mg/dL) and alkaline phosphatase (y) (193.78 ± 24.36 mg/dL), of “baixadeiro” group horses, regardless of the degree of closure of the growth plate, gender and age group, it can be verified the existence of correlation between the variables \((r = -0.2664)\) with partial and negative dependence, with significant determination \((r^2 = 0.71)\), and this regression was significant by the t-test \((p = 0.0396)\).

This negative dependence can be explained, because according to Kaneko (1997), phosphataemia apparently does not depend on hormonal factors, but is closely linked to calcium regulatory mechanisms, and the decline in phosphorus concentrations, which may be related to higher calcium intake of food origin than to resorption.

4 CONCLUSION

Given the conditions of extensive breeding, food, and sanitary management in which the animals live, the results obtained in this study lead us to conclude that:

- The age of closure of the distal epiphysis of the radio in horses of the "baixadeiro" group was approximately 27 months in males and 25 months in females;
- As for serum dosages, we observed that the average values found in calcium were higher in males, while the average values found for both phosphorus and alkaline phosphatase were higher in females;
- It is indicated the beginning of the work activities of the horses of the “baixadeiro” group at 25 months in females and 27 months in males, age at which these animals have already ceased their bone growth.
THANKS

To FAPEMA research funding institution, Maranhão State University, for the availability of the necessary equipment and infrastructure, and to the breeders of horses of the "baixadeiro" group, for the willingness to give the animals for the experiment.
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