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Mineral contents in bone and liver of sheep with or without periodontitis

[*Conteúdo de minerais em ossos e no fígado de ovinos com ou sem periodontite*]

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ABSTRACT

Due to the supposed involvement of minerals in cases of ruminant periodontitis, this study aimed to analyze the concentrations of phosphorus (P) in bone, and cobalt (Co), copper (Co), iron (Fe), zinc (Zn) and selenium (Se) in liver of a cohort of sheep affected or not by periodontitis. From an outbreak of the disease in 2011 in Pará state, Brazil, rib and liver samples were obtained from 22 sheep with periodontitis and seven samples from healthy animals. Based on the concentrations of the different minerals in the tissues, we concluded that there was no relationship between periodontal disease in sheep with any mineral deficiency status. In contrast, most of the minerals in the tissues were above or within the recommended concentrations in bone and liver. Within the various aspects which until now have been studied regarding periodontitis in ruminants, the results obtained here corroborate the fact that periodontal disease in sheep is an infectious disease and it is not a consequence of the deficiency or excess of mineral elements in the diet.

Keywords: minerals, periodontal disease, ruminants, sheep

RESUMO

Devido ao suposto envolvimento de minerais em casos de periodontite em ruminantes, este trabalho teve como objetivo analisar as concentrações de fósforo (P) nos ossos, além de cobalto (Co), cobre (Co), ferro (Fe), zinco (Zn) e selênio (Se), no fígado de ovelhas afetadas ou não por periodontite. De um surto da doença em 2011, no estado do Pará, Brasil, foram obtidas amostras de costelas e fígado de 22 ovelhas com periodontite e sete amostras de animais saudáveis. Com base nas concentrações dos diferentes minerais nos tecidos, conclui-se que não houve relação entre a doença periodontal em ovinos com qualquer estado de deficiência mineral. A maioria dos minerais estava acima ou dentro das concentrações recomendadas, seja no tecido ósseo, seja no hepático. Entre os diversos aspectos até agora estudados sobre a periodontite em ruminantes, os resultados obtidos corroboram o fato de a doença periodontal em ovinos ser uma doença infecciosa e não decorrente da deficiência ou do excesso de elementos minerais na dieta.

Palavras-chave: doença periodontal, minerais, ovinos, ruminantes

INTRODUCTION

Periodontitis is the immune-inflammatory response caused by the action of a complex oral microbiota in a susceptible host, which results in the loss of the tissues that support the teeth and their eventual exfoliation (Loesche, 1993; Schenkein, 2006). It is a disease that affects several species, including dogs (Peddle *et al.*, 2009), cats (Booij-Vrieling *et al.*, 2010), monkeys (Gaetti-Jardim *et al.*, 2012), sheep

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(Baker and Britt, 1984; Anderson and Bulgin, 1984; Spence *et al*., 1988; Ridler and West, 2007) and humans (Susin *et al*., 2005).

In Brazil, periodontitis in cattle (commonly known as “swollen face”) affects young animals (*i.e.* mainly calves) when teeth erupt (Döbereiner *et al*., 1974, 2000, 2004). This disease was first described in sheep in Brazil in 2016 (Silva *et al*., 2016) and the affected animals presented enlargement of the upper jaw, alveolar bone loss, and in final stages, loosening and loss of lower and upper molars; in the most severe cases, abscess formation and fistula were present, with drainage of purulent exudate. Consequently, these sheep had poor growth and low body condition, dying due to starvation. In the past, it has been hypothesized that the periodontal disease of cattle was a metabolic disease from a mineral imbalance, with the involvement of calcium (Ca) and phosphorus (P), as well as other minerals, with special emphasis on copper (Cu) (Camargo *et al*., 1981; Nazário, 1984; Soni, 1984). However, in later studies, Moraes *et al*. (1994) and Döbereiner *et al*. (2004) concluded that the swollen face in cattle was not a consequence of mineral deficiency or imbalance.

Due to the suspected involvement of mineral imbalance in cases of periodontitis in cattle, this study aimed to analyze the levels of phosphorus in the bone and of some micro minerals in liver of sheep from the same herd, affected or not by periodontal disease.

MATERIALS AND METHODS

The work described here was conducted in accordance with the requirements of the Federal University of Pará (UFPA) Animal Ethics Committee, and all procedures followed the UFPA guidelines for the use of animals for scientific purposes. All the procedures with the animals were approved by CEUA/UFPA, under CEUA protocol nº 3470240222.

This study was conducted using samples collected during an outbreak of the periodontal disease, described in Silva *et al*. (2016), which occurred in 2011 in the municipality of Benevides, PA, Brazil ($1^{O}21'.41$”S; $48^{O}13'.31$”O, 21 m altitude). Samples of bone and liver were obtained from 22 sheep (adults, six males and 16 females, with mild or severe periodontitis) necropsied during our visits to the property and from seven healthy sheep (three males and four females from the same herd) at slaughter in the abattoir. The 29 animals were raised on pastures of *Megathyrsus* (*Panicum*) *maximum* cv. Massai. The herd received a commercial mineral mixture (CMM) containing calcium (max. 135 g/kg), phosphorus (min. 75 g/kg), sodium (158 g/kg), cobalt (62 mg/kg), sulfur (12 g/kg), iron (1500 mg/kg), selenium (15 mg/kg), manganese (2580 mg/kg), zinc (3100 mg/kg), fluorine (max. 720 mg/kg), iodine (62 mg/kg), magnesium (8 g/kg), vitamin A (250 IU), vitamin D (100 IU) and vitamin E (500 IU) and no animals showed clinical signs of any mineral deficiency.

The classification of the animals with mild or severe periodontal disease and sheep without periodontitis was performed after a clinical examination, according to Radostits *et al*. (2002) and Pugh (2004), with a detailed inspection of the animals' maxilla and mandible region.

The 12^{th} rib was completely removed, and the soft tissues were discarded to obtain a bone sample for P analysis. A fragment of bone tissue weighing approximately 5 - 6 grams was withdrawn from the proximal third of the rib and preserved in 10% formaldehyde. The bone samples were lyophilized for 72 h and partially defatted by successive washings (4 h) with petroleum ether. After partial degreasing, the samples were ground in a grinder type knife and submitted to the analysis of DM and ash. The bone ashes were then submitted to acid digestion and thereafter analyzed for P by colorimetric spectrophotometry.

The liver samples were obtained by removing 5 - 10 grams from the caudate lobe of the liver, using a stainless-steel knife. The liver samples were stored in plastic bags, identified and frozen at -80 °C until chemical analysis. To determine the levels of Cu, cobalt, iron, zinc and selenium, the liver samples were thawed and lyophilized. Subsequently, the samples were crushed in gral and pistil. For the determination of Cu, Co, Fe, Zn, and Se the crushed samples were weighed between 0.25 to 0.26 g and placed in a Teflon digestion tube (Xpress model). Then, 3 mL of 65% nitric acid, 1 mL of 30% hydrochloric acid and 1 mL of 30% hydrogen peroxide were added. The samples were left to rest for 2 hours for pre-digestion and digested for 50 minutes in a closed system by microwave radiation (MARSXpress, CEM Corp. Matthews, NC, USA). The Cu, Co, Fe and Zn were analyzed using the Induced Plasma Optical Emission Spectrometry (ICP OES) in the ICP-OES equipment (VARIAN's simultaneous axial Vista-MPX CCD) in an automatic sampling system (SPS-5). Selenium was determined using the Optical Emission Spectrometry technique with Hydride Generation coupling (HG-ICP OES) on the iCAP 6000-CCD simultaneous equipment (Thermo Scien¬tific, Madison, USA), axial configuration and equipped with a automatic sampling (CETAC-ASX 520). All analyzes were performed at the Toxicology Laboratory of the Environment Section of the Evandro Chagas Institute (SAMAM/IEC/ SVS/MS).

The data were initially tested for normal distribution using the Kolmogorov-Smirnov test and then subjected to analysis of variance. The significance level of 5% was adopted and the minimum significant difference was estimated by the Student Newman-Keuls test. The means were described with the respective standard errors.

RESULTS

Table 1 shows the levels of minerals in the tissues of sheep with or without periodontitis. The mean values of P, Co, Fe, Zn, and Se were similar amongst the three groups of sheep. The sheep affected by severe periodontitis had the highest liver Cu concentration.

Table 1. Phosphorus concentrations in bone and Cobalt, Copper, Iron, Zinc and Selenium levels in the liver of sheep (on dry matter basis) with or without periodontitis

	Without periodontitis Average ± SEM	Mild periodontitis Average ± SEM	Severe periodontitis Average ± SEM	*P* value
Animals	7	11	11	
P in bone (%DM)	18.1±0.79	18.3±0.69	17.9±0.65	0.88
Cobalt (mg/kgDM)	0.402^{b}±0.21	0.332^{c}±0.22	0.621^{a}±1.02	0.015
Copper (mg/kgDM)	364.7^{b}±143.4	392.3^{b}±190.6	682.3^{a}±353.1	0.02
Iron (mg/kgDM)	655.4±347.0	715.5±367.5	632.8±251.5	0.32
Zinc (mg/kgDM)	203.9±192.5	142.3±90.5	161.5±144.5	0.11
Selenium (mg/kgDM)	0.95±0.66	1.04±0.67	0,77±0.46	0.16

SEM = Standard Error of the Mean
Different letters in the same row indicate statistical difference (P<0.05)

DISCUSSION

The mean values of P in bone ash were similar amongst the three groups of sheep (Table 1). This demonstrates the fact that the animals with any stage of periodontitis were not able to change the composition of P in their bones nor that they were deficient in this mineral, since the P values of healthy animals are generally between 15 to 18% of the bone ashes, with values below 12 – 13% suggesting some degree of P deficiency. As the sheep were being routinely supplemented with a good mineral mixture (containing 75g of P per kg) it was to be expected that they were not deficient in P.

The sheep affected by severe periodontitis had the highest liver Co concentration (Table 1), but all groups had levels of hepatic Co above the values considered adequate. Assuming 28% of DM in the liver, Radostitis (2002) considers values below 0.07mg per kg of DM of the liver as animals clinically deficient in Co and that values above 0.10mg per kg of DM of the liver are from sheep not deficient. Moraes *et al.* (1994) investigated the possibility of any mineral imbalance in the ingested forage could be related to the etiology of periodontal disease in cattle, found similar results, with adequate levels of Co in healthy and/or affected cattle by periodontitis.

The sheep affected by periodontitis had the highest liver Cu concentration (Table 1). The hepatic Cu levels were higher than the reference values *i.e.* 100 to 250mg/kgDM or 6400 to 16000 µmol/kgDM) for non-deficient animals and for those not subject to poisoning by this element (Table 1). Marques *et al.* (2011), when describing the hepatic concentrations of this micromineral, in healthy sheep, found mean values of 158.5±83.0mg/kgDM (10144µmol/kgDM). On the other hand, the

United Kingdom's Animal and Plant Health Agency (APHA) established that values greater than 8000 µmol of Cu in hepatic DM (125mg/kgDM) should be considered as a risk of Cu poisoning, even in the absence of clinical signs (Kendall *et al*., 2015).

The average levels of hepatic Fe did not differ between the three groups (Table 1). These values were higher than the value of 245±108mg/kgDM, described by Silva Júnior *et al*. (2015) and the levels of 156.1 to 210.5mg/kgMS, verified by Marques *et al.* (2011). These higher values are possibly related to high Fe intake, either in the mineral mixture or in the forage consumed by the animals.

The liver Zn concentrations were superior to the values found by Silva Júnior *et al*. (2015), Tokarnia *et al*. (1999) and by Antonelli *et al*. (2016). These authors found 118, 101 – 128, and 115-143mg of Zn per kgDM, respectively. According to Fick (1979), the reference values for hepatic Zn in healthy ruminants are between 84 to 132mg/kgDM.

Of all minerals investigated in this study, Se was the one that had, in a greater number of animals, liver levels below the reference level (1.2 to 2.0mg/kgDM) described by Miles *et al*. (2011) and by Radostits *et al.* (2002). Moraes *et al*. (1986) in the state of Mato Grosso observed, in a herd of cattle affected by periodontitis, that 33% (4/12) of the liver samples had low Se concentration. This author also evaluated samples of Liver from healthy cattle (*i.e.*, without periodontal disease), where 20% had Se levels below ideal. However, liver Se concentration is within the adequate range described by Van Vleet (1980). When the data of this author are expressed in a liver sample containing 25% DM, the values above 0.80 mg of Se per kg of DM are from healthy animals, between 0.40 and 0.80 mg are from subclinical deficient animals and less than 0.40 mg came from animals clinically deficient in Se.

Based on the analysis of the different minerals in the sheep tissues, there was no relationship between periodontal disease and the issue of mineral deficiency or imbalance in the diet. On the contrary, most of the minerals were above or within the reference values described by different authors. Therefore, the mineral supplementation carried out on the property was adequate to meet the sheep minerals requirements. On the other hand, it is necessary to warn that, in the studies of analysis of minerals in liver, there is great variation (*i.e.,* standard deviation) in the data; either as a function of the status of the animal itself, or as a function of the nature of the analytical procedure adopted by the laboratory.

Although investigations into the pathogenesis of periodontitis have traditionally centered on the role of bacterial infection, over the past decades there has been increasing interest in the host response factors that drive periodontal disease (Cochran, 2008). In humans, it is now understood that the immune and inflammatory responses are critical to the pathogenesis of periodontitis and are shaped by several host-related factors, both intrinsic (*e.g.,* genetics) and induced (*e.g.,* pollutants). For Döbereiner *et al.* (2004), Moraes *et al*. (1994) and Rosa and Döbereiner (1994), there is no evidence of a relationship between the periodontitis of cattle and mineral imbalance or deficiency; these authors demonstrated that the bone changes are of secondary origin and are not caused by a deficiency of P or other minerals. Therefore, by analogy based on the findings of the present study, the issue of mineral imbalance or deficiency should not be considered as a triggering factor for periodontal disease in sheep.

CONCLUSION

Based on the findings described in this study, the mineral supplementation of the sheep was adequate and met the requirements of the animals, which was evidenced by the analysis of P in the bone and of several microminerals in the liver. Thus, it is not possible to associate periodontal disease of sheep with cases of mineral deficiency or imbalance in the diet. This fact reinforces the hypothesis that periodontitis of sheep is of infectious origin, as well as the periodontal disease of cattle.

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