

Serum and liver copper, iron, molybdenum and zinc concentration in goats and sheep in the state of Paraíba, Brazil¹

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ABSTRACT- Silva T.R., Soares P.C., Dantas A.F.M., Marques A.V.S., Filho E.F.O., Aguiar G.M.N., Marques A.L.A. & Riet-Correa F. 2018. **Serum and liver copper, iron, molybdenum and zinc concentrations in goats and sheep in the state of Paraíba, Brazil.** *Pesquisa Veterinária Brasileira* 38(7):1313-1316. Hospital Veterinário, Universidade Federal de Campina Grande, Campus de Patos, Patos, PB 58700-970, Brazil. E-mail: tatianerodrigues.vet@gmail.com

This study aimed to determine Cu, Fe, Mo, and Zn liver and serum concentration in sheep and goats raised in the rangelands of the semiarid region of the state of Paraíba, Brazil, during the dry and rainy seasons, and to establish if Cu deficiency is primary or secondary to high ingestion of Mo or Fe. Cu, Zn, Mo and Fe concentrations were determined by atomic absorption spectrometry coupled to mass (ICP-Plasma) in 253 liver and serum samples randomly selected in a slaughterhouse. The mean serum concentrations of Cu in the goats and sheep were $11.82 \pm 3.28 \mu\text{mol/L}$ and $10.97 \pm 3.61 \mu\text{mol/L}$ respectively. The liver Cu concentrations were $160.37 \pm 11.77 \text{mg/kg}$ in goats and $152.12 \pm 13.16 \text{mg/kg}$ in sheep. The mean serum Fe concentrations were $16.38 \pm 4.51 \mu\text{mol/L}$ in goats and $25.41 \pm 9.76 \mu\text{mol/L}$ in sheep. The mean Fe concentrations in the liver were $189.37 \pm 6.51 \text{mg/kg}$ in goats and $313.70 \pm 12.89 \text{mg/kg}$ in sheep. The mean serum concentrations of Mo were $0.14 \pm 0.04 \mu\text{mol/L}$ in goats and $0.29 \pm 0.06 \mu\text{mol/L}$ in sheep. The mean Mo concentrations in the liver were $6.09 \pm 0.23 \text{mg/kg}$ in goats and $6.22 \pm 0.15 \text{mg/kg}$ in sheep. The mean serum Zn concentrations were $8.30 \pm 1.91 \mu\text{mol/L}$ in goats and $8.63 \pm 2.22 \mu\text{mol/L}$ in sheep. The mean Zn concentrations in the liver were $132.80 \pm 3.39 \text{mg/kg}$ in goats and $130.70 \pm 2.99 \text{mg/kg}$ in sheep. These results show low or marginal serum and liver concentrations of Cu and Zn, indicating that these minerals should be supplemented. The normal or even low concentrations of Mo and the high concentration of Fe suggest that Cu deficiency may be primary, due to low Cu ingestion, or secondary due to high Fe ingestion.

INDEX TERMS; Copper, iron, molybdenum, zinc, mineral nutrition, goats, sheep, small ruminants, trace mineral deficiencies, Brazilian semiarid.

RESUMO.- [Concentrações sérica e hepática de cobre, ferro, molibdênio e zinco em ovinos e caprinos no estado da Paraíba.]. Este estudo teve como objetivo determinar Cu,

Fe, Mo e Zn no fígado e soro em ovinos e caprinos criados nas pastagens da região semiárida do Estado da Paraíba, Brasil, nas estações seca e chuvosa, e estabelecer se a deficiência de Cu e primária ou secundária a alta ingestão de Mo ou Fe. Cu, Zn, Mo, e Fe foram determinados por espectrometria de absorção atômica com plasma indutivamente acoplado (ICP-OES) em 253 amostras de fígado e soro selecionados aleatoriamente em um matadouro. As concentrações séricas de Cu nos caprinos e ovinos foram $11,82 \pm 3,28 \text{mmol/L}$ e $10,97 \pm 3,61 \text{mmol/L}$, respectivamente. As concentrações de Cu no fígado foram de $160,37 \pm 11,77 \text{mg/kg}$ em caprinos e $152,12 \pm 13,16 \text{mg/kg}$ em ovinos. As concentrações séricas de Fe foram de $16,38 \pm 4,51 \text{mmol/L}$ em caprinos e $25,41 \pm 9,76 \text{mmol/L}$ em ovinos. As concentrações de

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Fe no fígado foram $189,37 \pm 6,51$ mg/kg em caprinos e $313,70 \pm 12,89$ mg/kg em ovinos. As concentrações séricas de Mo foram de $0,14 \pm 0,04$ mmol/L em caprinos e $0,29 \pm 0,06$ mmol/L em ovinos. As concentrações de Mo no fígado foram $6,09 \pm 0,23$ mg/kg em caprinos e $6,22 \pm 0,15$ mg/kg em ovinos. As concentrações séricas de Zn foram $8,30 \pm 1,91$ mmol/L em caprinos e $8,63 \pm 2,22$ mmol/L em ovinos. As concentrações de Zn no fígado foram $132,80 \pm 3,39$ mg/kg em cabras e $130,70 \pm 2,99$ mg/kg em ovelhas. Estes resultados mostram concentrações baixas ou marginais de Cu e Zn no soro e fígado indicando que estes minerais devem ser suplementados. As concentrações normais ou mesmo baixos de Mo e as altas concentrações de Fe sugerem que a deficiência de Cu pode ser primária, devido à baixa ingestão de Cu, ou secundária a alta ingestão de Fe.

TERMOS DE INDEXIZAÇÃO: Cobre, ferro, molibdênio, zinco, ovinos, caprinos, nutrição mineral, pequenos ruminantes, deficiência mineral, semiárido brasileiro.

INTRODUCTION

Knowledge about mineral deficiencies in ruminants in northeastern Brazil is scarce. Enzootic ataxia due to copper deficiency was reported in lambs from Piauí (Tokarnia et al. 1966) and Rio Grande do Norte (Sousa et al. 2009), in goats in Paraíba (Guedes et al. 2007), and in lambs and goats in Pernambuco (Santos et al. 2006). Deficiencies of Cu and Co in cattle and sheep in the states of Maranhão and Piauí and Co deficiency in cattle in Ceará are also reported (Tokarnia et al. 1968). Low concentrations of Zn and Mn were found in the livers of cattle and sheep in different regions of northeastern Brazil (Moraes et al. 1998). Low levels of Cu and Zn were determined in the serum and livers of sheep and goats slaughtered in the State of Pernambuco (Marques et al. 2011). White muscle disease due to Se and vitamin E deficiency was reported in feedlot sheep (Riet-Correa 2004, Amorim et al. 2005). Phosphorus deficiency was reported in goats reared in pastures in Paraíba (Silva et al. 2011), and an outbreak of botulism associated with osteophagia as a result of P deficiency was reported in Piauí (Riet-Correa et al. 2012).

Despite those reports, the need is clear to acquire a better knowledge of the occurrence and geographical distribution of the different mineral deficiencies in small ruminants in the Brazilian semiarid region, which has a population of 10,110,352 sheep and 8,538,255 goats. This information is necessary for the appropriate formulation of mineral supplements at the lowest cost. This study aimed to determine the concentrations of Cu, Fe, Mo, and Zn in liver and serum samples from sheep and goats raised in the semiarid region of the State of Paraíba in the dry and rainy seasons and to establish if copper deficiency is primary, due to the ingestion of low levels of copper, or secondary to the ingestion of high levels of Mo or Fe.

MATERIALS AND METHODS

The samples of liver and blood were taken from animals slaughtered in a slaughterhouse in Patos, in the State of Paraíba. During collection, a survey was conducted to characterize the sex, age, nutritional score and municipality of origin. At all, 253 liver and serum samples were randomly collected from the municipalities of Patos, Nova Olinda,

Coremas, Santa Terezinha, São José das Espinharas, and Catingueira, all belonging to the semiarid region of Paraíba, as established by the Institute of Agricultural Defense in the State of Paraíba, Brazil.

The sampling was conducted during the final third of the rainy season, when forage was available in the pastures, and during the final third of the dry period, with a shortage of forage in the pastures. The sampling periods were defined following the normal rainfalls in the region as informed by the National Institute of Meteorology.

To obtain the serum, blood samples were collected by jugular venipuncture in Vacutainer tubes without anticoagulant. The blood samples remained at rest at room temperature to retract the clot and then were centrifuged for 15 minutes at 500G. The serum aliquots were then stored in Eppendorf tubes at -20°C for subsequent mineral analysis. Liver samples of approximately 50g of liver were obtained using stainless steel knives. These samples were placed on filter paper to remove excess blood and then placed in plastic bags, properly identified and stored in a freezer at -20°C .

For the determination of minerals in the serum, the samples were diluted 6-20:1-fold with Milli-Q water, according to Solaiman et al. (2001). The liver samples were fragmented using a scalpel blade, placed in a watch glass and dried in an oven at 103°C for 24 hours to obtain dry matter. After this procedure, all the samples were weighed on an analytical balance. All weights were recorded, and the samples were placed in tubes containing boro-silicate nitric acid-perchloric acid (4:1 v/v) and maintained at rest for 12 hours. Next, the tubes were placed in a block digester at 150°C . Upon completion of digestion, 10mL 0.1 N hydrochloric acid was added, and the solution was deposited on the plastic container, hermetically sealed and forwarded to the laboratory for analytical procedures (Tebaldi et al. 2000). Cu, Mo, Fe and Zn levels were determined by atomic absorption spectrometry coupled to mass (ICP - Plasma), using the device model Spectra - 200G (Miles et al. 2001).

For statistical analysis, the variables were described by the mean and standard deviation. The data were analyzed by the computational program Statistical Analysis System Institute, SAS, (2000), using the GLM procedure (General Linear Model) of SAS. The data were subjected to analysis of variance (F test), separating the effect of periods, species, and gender as causes of variation. Where the F test was significant, the treatment means were compared using Duncan. All statistical analysis was conducted at 5% significance. The following model was used: $Y_{ij} = P + E + S + E_{ij}$, where: Y_{ij} = the observed, P = effect of length, E = effect of species, S = effect of sex, E_{ij} = error.

RESULTS AND DISCUSSION

Table 1 shows the serum and hepatic concentrations of Cu, Fe, Mo and Zn according to season, species and sex of ruminants slaughtered in the semiarid region of Paraíba.

The mean serum concentrations of Cu in goats (11.82 ± 3.28 $\mu\text{mol/L}$) and sheep (10.97 ± 3.61 $\mu\text{mol/L}$) were not influenced by season ($P > 0.1462$), species ($P > 0.2166$), and sex ($P > 0.0516$). Also the mean hepatic Cu concentrations in goats (160.37 ± 11.77) and sheep (152.12 ± 13.16) were not influenced by the season ($P > 0.4245$), species ($P > 0.6452$) or sex ($P > 0.4240$). The normal serum Cu values differ among authors but in general may range from 10.16 to 31.25 $\mu\text{mol/L}$, and the hepatic levels range from 150 to 500mg/kg (Grace 1983, Oregui & Bravo 1993, Pott et al. 1999). The serum and liver concentrations of Cu found in this study suggest a marginal deficiency of this element and are similar to those observed in Pernambuco by Marques et al. (2011). Copper deficiency is one of the most important mineral deficiencies in Brazil, and low values of this element are found in the livers of ruminants

and in forage in various Brazilian regions (Tokarnia et al. 1999, McDowell 1999). In addition, various clinical forms of copper deficiency in ruminants have been diagnosed in all regions, including the Northeast (Riet-Correa 2004). The marginal serum and liver Cu concentrations observed herein suggest that in the semiarid region of northeastern Brazil, in addition to economic losses caused by outbreaks of enzootic ataxia in sheep and goats (Tokarnia et al. 1966, Sousa et al. 2009, Guedes et al. 2007, Santos et al. 2006), even greater economic losses may occur due to subclinical deficiency, which is associated with reduced growth and reproductive failure (McDowell 1992).

The serum Fe concentrations in goats ($16.38 \pm 4.51 \mu\text{mol/L}$) were significantly lower ($P < 0.0001$) than in sheep ($25.41 \pm 9.76 \mu\text{mol/L}$) and were significantly higher ($P < 0.0001$) in the rainy season ($11.61 \pm 2.98 \mu\text{mol/L}$) than in the dry season ($10.97 \pm 3.61 \mu\text{mol/L}$). Additionally, no significant variations were found between males and females ($P > 0.3159$). Serum Fe in small ruminants may vary from 34.6 to 37.45 $\mu\text{mol/L}$ (Radostits et al. 2007, Suttle 2010); thus, the results of this study demonstrate low serum concentrations of this element, which appeared even more marked in the goats ($16.38 \pm 4.51 \mu\text{mol/L}$) than in the sheep ($25.41 \pm 9.76 \mu\text{mol/L}$). In Pernambuco, Marques et al. (2011) found Fe serum concentrations of 35.380 $\mu\text{mol/L}$ in sheep and 25.06 $\mu\text{mol/L}$ in goats. Some authors consider serum Fe below 29 $\mu\text{mol/L}$ to be indicative of marginal deficiency (Tokarnia et al. 1988, Jones et al. 1984). The low serum concentrations of Fe found in this research could be related to blood loss caused by *Haemonchus contortus*, which is the main gastrointestinal parasite that affects goats in the region (Silva et al. 2008). Iron deficiency is very rare in sheep and goats raised under extensive grazing, occurring almost exclusively in cases of parasitism (Pugh 2005, Smith & Sherman 2009). This feature was confirmed by the high copper concentrations found in the liver.

The liver concentrations of Fe were significantly lower ($P < 0.0001$) in goats ($189.37 \pm 6.51 \text{mg/kg}$) than in sheep ($313.70 \pm 12.89 \text{mg/kg}$) and were significantly lower ($P < 0.0001$) during the rainy season ($189.01 \pm 6.75 \text{mg/kg}$) than in the dry season ($312.73 \pm 12.11 \text{mg/kg}$). Males showed a higher ($P < 0.0001$) Fe concentration in the liver ($284.69 \pm 13.55 \text{mg/kg}$) than females ($242.60 \pm 10.91 \text{mg/kg}$). These concentrations

are similar to those reported in various regions of Brazil, ranging from 181 to 380 mg/kg (Tokarnia et al. 1988), and higher than the mean value (138.8 mg/kg) reported by Jones et al. (1984). The high Fe concentrations in the liver may be due to high Fe content in the pastures or water or by the ingestion of mineral supplements containing excessive concentrations of this mineral. Also the ingestion of soil may be responsible for high iron intake. High Fe concentration in the pastures has been associated with secondary copper deficiency (Ramirez et al. 1998, Moraes et al. 1998, Marques et al. 2003), suggesting that the low copper concentrations in the liver of sheep and goats in northeastern Brazil maybe at least partially secondary to high Fe ingestion.

No significant differences in the concentrations of Mo ($P > 0.1879$) were found between goats ($0.14 \pm 0.04 \mu\text{mol/L}$) and sheep ($0.29 \pm 0.06 \mu\text{mol/L}$). The serum concentrations of Mo were significantly higher ($P < 0.0048$) in the dry season ($0.41 \pm 0.01 \mu\text{mol/L}$) than in the rainy season ($0.13 \pm 0.01 \mu\text{mol/L}$). No significant variations were found between sexes ($P > 0.5946$). Hepatic Mo concentrations in goats (6.09 ± 0.23) and sheep (6.22 ± 0.15) were not influenced by the season ($P > 0.8065$), species ($P < 0.6099$) or sex ($P > 0.8105$). The metabolism of Mo in small ruminants has been rarely studied, and the few values found in the literature vary from 0.28 to 0.8 $\mu\text{mol/L}$ in serum and from 3.62 to 8.10 mg/kg in the liver (Van Ryssen & Stielau 1981, Marques et al. 2011). The Mo values found here in the serum and liver of goats and sheep were within normal ranges, suggesting that the copper deficiency is not associated with high Mo intake.

The serum concentrations of Zn in goats ($8.30 \pm 1.91 \mu\text{mol/L}$) and sheep ($8.63 \pm 2.22 \mu\text{mol/L}$) showed no significant differences ($P > 0.4269$). However the concentrations in the dry season were significantly higher ($P < 0.0104$) in the dry season ($8.64 \pm 2.22 \mu\text{mol/L}$) than in the rainy season ($7.91 \pm 2.05 \mu\text{mol/L}$). No significant differences were observed between sexes ($P > 0.0546$). No differences were observed in liver Zn concentration between species ($P > 0.0938$) or sex ($P > 0.0600$). However, during the dry period, the liver concentrations of Zn ($125.22 \pm 2.99 \text{mg/kg}$) were significantly lower ($P < 0.0500$) than during the rainy season ($133.93 \pm 3.46 \text{mg/kg}$). Normal serum and liver concentrations of Zn in small ruminants vary

Table 1. Serum and liver concentrations of Cu, Fe, Mo and Zn in different seasons in male and female goats and sheep slaughtered in the semiarid region of Paraíba

Variables	Factors					
	Period		Species		Sex	
	Serum (μmol/L)					
	Drought	Rain	Goats	Sheep	Male	Female
Cu	10.97±3.61	11.61±2.98	11.82±3.28	10.97±3.61	10.45±3.23	11.55±3.68
Fe	25.30±9.76 ^{A*}	16.57±5.00 ^B	16.38±4.51 ^B	25.41±9.76 ^A	24.62±8.84	23.07±10.06
Mo	0.41±0.01 ^A	0.13±0.01 ^B	0.14±0.04	0.29±0.06	0.30±0.09	0.25±0.03
Zn	8.64±2.22 ^A	7.91±2.05 ^B	8.30±1.91	8.63±2.22	8.99±2.05	8.32±2.20
	Liver (mg/kg)**					
Drought		Rain	Goats	Sheep	Male	Female
Cu	152.12±13.16	166.58±11.94	160.37±11.77	152.12±13.16	170.30±19.45	151.33±8.82
Fe	312.73±12.11 ^A	189.01±6.75 ^B	189.37±6.51 ^B	313.70±12.89 ^A	284.69±13.55 ^A	242.60±10.9 ^B
Mo	6.22±0.15	6.16±0.23	6.09±0.23	6.22±0.15	5.92±0.20	6.35±0.17
Zn	125.22±2.99 ^B	133.93±3.46 ^A	132.80±3.39	130.70±2.99	128.68±4.07	129.46±2.73

* Different uppercase letters on the same line within each factor (seasonal period, species and sex) differ at 5% probability, ** mg/kg = mg by kg dry matter.

from 12 to 18.5 µmol/L (Sanz Lorenzo et al. 1996, Suttle 2010) and from 101 to 200 mg/kg (Tokarnia et al. 1988) respectively. The serum concentrations of Zn in this study were below the normal ranges, and the liver values were within normal ranges but significantly lower in the dry season than in the rainy season. In the semiarid region of the state of Pernambuco, Marques et al. (2011) reported marginal Zn concentrations in goats and sheep, and the authors considered the drought to be an important factor that determines low serum and liver Zn concentrations. These results, taken together, suggest the need for Zn supplementation in grazing small ruminants in the Brazilian semiarid region, mainly during the dry season.

In conclusion, the low or marginal serum and liver concentrations of Cu and Zn in small ruminants in the semiarid region of Paraíba indicate that these minerals should be included in mineral supplements. Copper deficiency may be primary or secondary to Fe ingestion.

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