

SERUM COPPER AND COPPER RELATED LIVER ENZYMES IN SHEEP FROM RIO GRANDE DO SUL, BRAZIL

COBRE SÉRICO E ENZIMAS RELACIONADAS AO SEU METABOLISMO EM OVINOS DO RIO GRANDE DO SUL

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SUMMARY

The aim of this work was to determine serum copper levels, aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) activity in purebred show sheep fed concentrate and field sheep raised on natural pastures of Rio Grande do Sul, the southernmost State of Brazil. Blood samples of 120 show sheep and 110 field raised animals were collected independently of age, sex and breed. Mean serum levels of copper found on purebred and field sheep were 66.14 ± 9.76 and 62.01 ± 16.36 $\mu\text{g}/\text{dl}$ ($P < 0.0357$), respectively. AST activity was higher in show sheep than in field animals (85.01 ± 40.02 vs. 65.58 ± 16.19 IU, $P < 0.0001$). GGT activity levels found were 41.02 ± 10.61 and 41.91 ± 9.49 IU, with no significant differences between groups. It is concluded that serum copper levels of purebred show sheep group were within international reference values for the species, whereas in the grass grazed group, the level of this mineral was below the lower limit in several individuals. Enhanced AST serum activity in blood samples of show sheep examined appear to be related to copper liver levels detected in these animals.

KEYWORDS: Aspartate Aminotransferase. Chronic Cooper Poisoning. Gamma-Glutamyl Transferase. Sheep.

RESUMO

O objetivo desse trabalho foi identificar as concentrações séricas de cobre e enzimas aspartatoaminotransferase (AST) e gamaglutamiltransferase (GGT), tanto em ovinos preparados para exposição e alimentados com concentrados como criados a campo, a fim de contribuir para a formação de valores de referência e auxiliar no prognóstico da saúde desses animais. Colheram-se 120 amostras de sangue de animais de exposição e 110 de animais de campo, independente de sexo, raça e idade. Os níveis séricos médios de cobre, AST e GGT dos animais de exposição e a campo foram iguais a $66,14 \pm 9,76$ e $62,01 \pm 16,36$ $\mu\text{g}/\text{kg}$ ($P < 0,0357$); $85,01 \pm 40,02$ e $65,58 \pm 16,19$ UI ($P < 0,0001$); $41,02 \pm 10,61$ e $41,91 \pm 9,49$ UI ($P > 0,05$), respectivamente. Em conclusão, a concentração de cobre em ovinos preparados para exposição mostraram-se dentro dos valores de referência e as de alguns animais mantidos a campo abaixo do intervalo fisiológico. Os níveis séricos de AST aumentados no grupo exposição parecem estar relacionados com o acúmulo hepático de cobre.

PALAVRAS CHAVE: Aspartatoaminotransferase, Gamaglutamiltransferase. Intoxicação Crônica por Cobre. Ovino.

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INTRODUCTION

After the wool crisis in the 70's, sheep raising for meat has increased sharply in Rio Grande do Sul (RS); thus becoming more common the diseases of metabolic and nutritional origin, especially the chronic intoxication by copper (CCI). According to Soli (1980), sheep are more vulnerable to changes in production methods compared to other species. This sensitivity is attributed to the less efficient mechanism by which copper is excreted by the biliary system of the species (FIGHERA, 2001).

Cases of CCI have been described in Rio Grande do Sul, almost always in sheep stabled and fed concentrates (RIBEIRO, 1985; RIET-CORREA et al., 1989). The first reference to such poisoning in Brazil was reported in 1966 in sheep being prepared for an Exposition in Rio Grande do Sul. The authors then, pointed out diets with high copper levels, as well as stress and environment changes as triggering factors (GALVÃO & WILLIAMS, 1966).

More recently, Möllerke & Ribeiro (2005) researched and studied for two consecutive years copper content of diets fed to sheep from an agricultural fair in RS. The average values ranged from 11.3 to 16.3 mg/kg. According to Wells et al. (2000), these values are above the limit tolerated by sheep, which would be 10 mg/kg.

More recently, serum levels of the enzymes AST and GGT are being investigated in addition to copper levels, to aid the diagnosis and treatment of this disease. Möllerke & Bernhard (2002), stated that serum level of these enzymes is directly related to the time length the animals are exposed to copper. Lemos et al. (1997) monitored sheep groups fed a daily diet containing between 15 and 120 mg/kg of copper by measuring serum levels of AST and GGT. Increased levels of GGT were detected between two to three weeks before the onset of clinical signs of CCI. It was also observed that AST levels rose only during or immediately before the hemolytic crisis. It was, therefore, suggested that evaluation of serum levels of GGT would be the best parameter for previous assessment of liver damage.

Due to conflicting data in the literature about physiological copper serum levels, AST and GGT, in addition to little information about local reference values, the present work aims at determining copper serum levels, as well as the enzymes AST and GGT in purebred show sheep compared to pasture raised sheep. These data should contribute to establishing reference values, detecting possible deficiencies and assisting health prognosis of sheep.

MATERIAL AND METHODS

The sheep were divided into two groups. The first was called the Exposition group and consisted of purebred sheep raised for Exposition at the 29th edition of International Exposition of Animals, Machinery and Implements and Agricultural Products of Rio Grande do Sul (29th Expointer) that took place in Esteio, RS,

from August to September, 2006. The second group was called Field group, and consisted of sheep reared extensively in Pantano, RS, whose blood samples were collected during October, 2006.

Blood samples were collected from sheep chosen randomly, independent of breed, sex or age. From the Exposition group, 120 sheep were chosen, age varied between 1 and 2 years old. The sheep were kept in individual stalls, water was offered *ad libitum* and fed commercial ration, hay and pasture. From the field group, blood samples were collected from 110 sheep that were offered water *ad libitum* and feeding was based only on grazing of native pastures.

Blood samples of both groups were harvested by puncturing the jugular vein, using 10-mL Vacutainer tubes (Becton-Dickinson, Rutherford, NJ, USA), without anticoagulant. The samples were centrifuged at 2500 rpm for 15 minutes, and the resulting serum was stored in 1.5 mL eppendorf tubes, duly identified and frozen at -20°C for further analysis, which was performed within two weeks time.

Serum concentrations of AST and GGT were determined using a commercial kit (Labtest, Minas Gerais, Brazil), and serum copper by atomic absorption spectrophotometry (GBC 932 AA spectrophotometer, oxygen-rich air-acetylene flame analysis technique) following technique described by Fick et al. (1980).

Statistical analysis consisted of ANOVA, and means were compared by Student's t-test.

RESULTS AND DISCUSSION

Mean serum values of copper, AST and GGT are shown in Table 1. Copper serum values in both groups were mostly within the standard range from 58 to 160 µg/dL suggested by Kaneko et al. (1997) and Underwood & Suttle (1999). A careful analysis of the data on the other hand, showed that almost half of the sheep from the field group (33/72) and some from the Exposition group (24/106) had copper serum levels below the minimum recommended for the species. These results are shown graphically in Figure 1. Reference values of copper serum levels of field raised sheep in RS are not available in the literature, therefore these data are the first reference. Cases of copper deficiency or poisoning have been already reported in field raised sheep in RS. Although Copper levels of 5.3 mg/kg reported by Gaviñon & Quadros (1976) in pastures of RS appear to meet the need of the species; the same authors reported molybdenum levels of 0.1 mg/kg, which is the threshold value that interferes with copper absorption. Finally Kaneko et al. (1997) and Underwood & Suttle (1999) cite values of serum copper between 126 and 158 µg/dL as potentially hemolytic for sheep. Therefore, based on the data presented, the animals of both groups are not within this suggested risk zone.

The sheep with copper serum levels below the minimum suggested by Kaneko et al. (1997) may be suffering from lack of this mineral or the presence of high amount of copper mineral chelators in their diet. As mentioned earlier, a study conducted in the pastures

Table 1 - Mean serum values for copper, as well as AST and GGT enzymes of both male and female sheep of Exposition and grazing groups raised in central Rio Grande do Sul.

	COPPER (58-160 µg/dL)*	AST (0-90 UI)*	GGT (20-52 UI)*
Exposition	66,14 ± 9,76 ^a	85,01 ± 40,02 ^a	41,02 ± 10,61 ^a
Field	62,01 ± 16,36 ^b	65,58 ± 16,19 ^b	41,91 ± 9,49 ^a

AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase;
 Numbers followed by different letters in the column are significantly different P < 0,05.

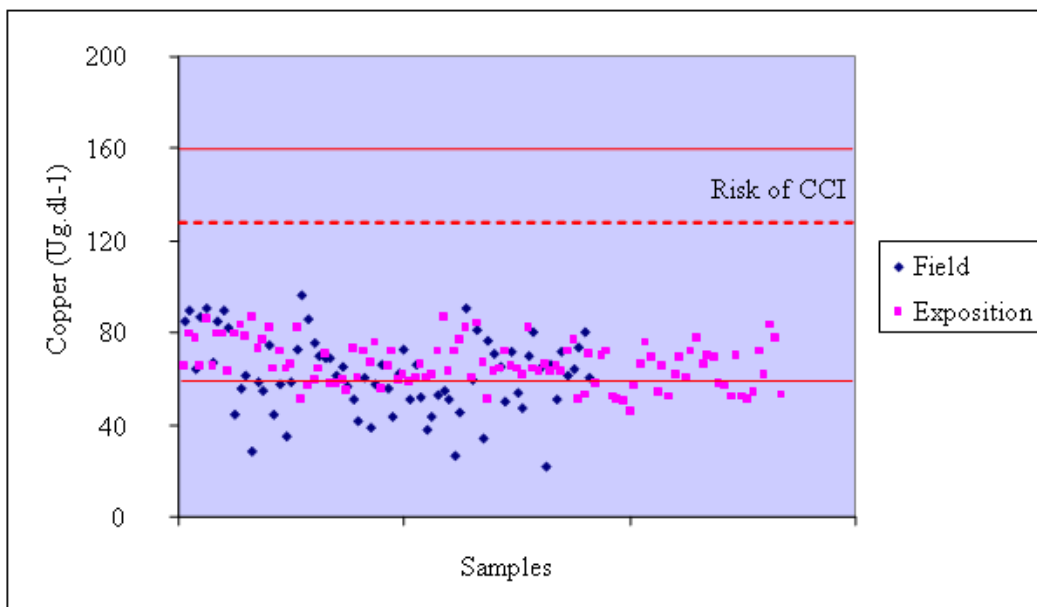


Figure 1 - Serum copper levels of female and male sheep raised in Rio Grande do Sul.

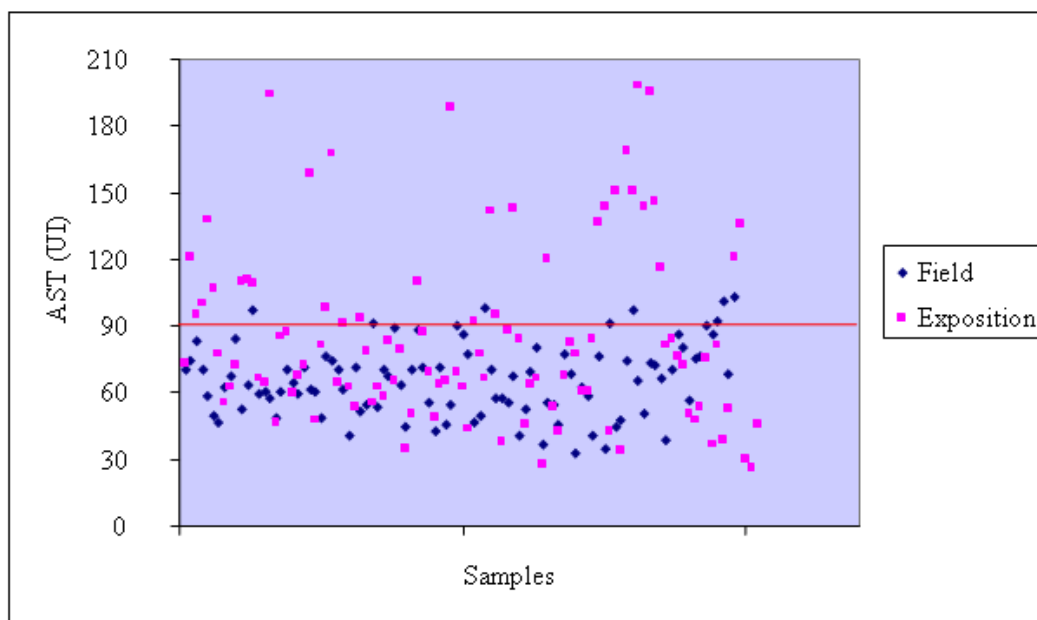


Figure 2 - Serum concentrations of the enzyme AST of female and male sheep raised in Rio Grande do Sul.

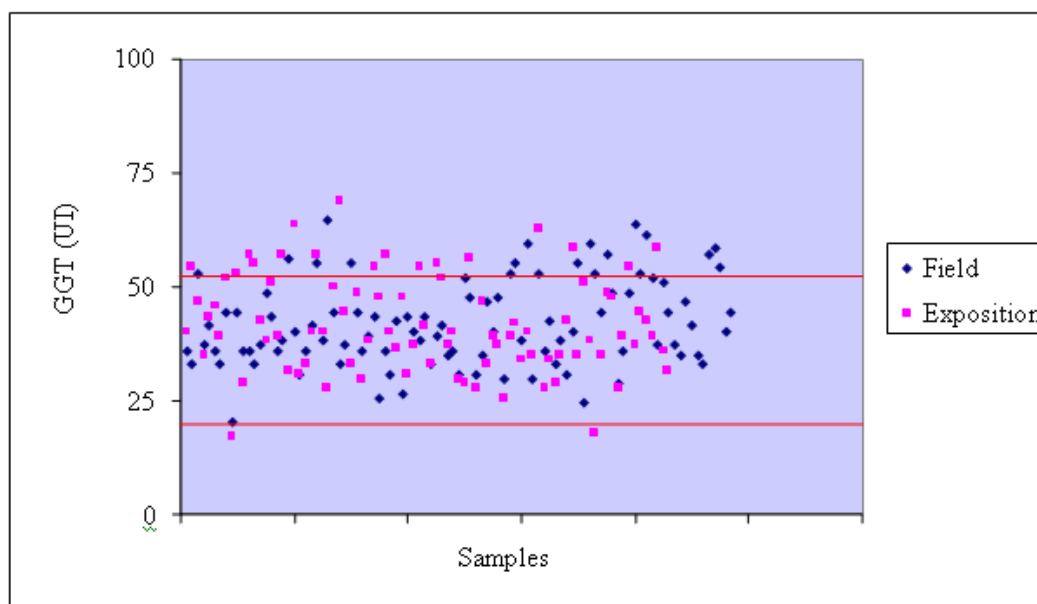


Figure 3 - Serum concentrations of the enzyme GGT of female and male sheep raised in Rio Grande do Sul.

of RS has shown that these fodders have neither excess nor deficiency of copper. It was, however, identified a lack of molybdenum in the pasture, a mineral that hinders copper absorption. The lack of molybdenum to obstruct copper absorption from the concentrate could be responsible for the higher serum copper found in Exposition sheep (66.14 $\mu\text{g/dL}$) compared to field sheep, 62.01 $\mu\text{g/dL}$ ($P < 0.001$). Field sheep consumed forage that contained an appropriate amount of copper and slight lack of molybdenum that did not interfere with serum copper levels.

Meanwhile, the Exposition sheep consumed higher amounts of copper, without a proportional increase of molybdenum intake, which resulted in increased serum copper.

Serum values of AST are shown in Table 1 and Figure 2. The majority of the values for both sheep groups (162/198) are within the reference values for the species as suggested by Kaneko et al. (1997). Few animals (5/97) from the field group, while one third (34/101) in the Exposition group, had AST levels above the limit suggested by the same authors. Mean serum values of AST of sheep raised in apple orchards and vineyards fertilized with poultry manure in RS, were 274 and 172 UI, respectively (RIBEIRO et al. 1995; RIBEIRO et al. 2007). In these cases, the animals were fed diets with copper content so high that caused their death. Möllerke & Bernhard (2002), while studying the AST levels of sheep exhibited in an international fair, found values between 274 and 339 UI, well above the values reported in this paper. Although, those sheep were also fed concentrate, which increases the copper supply. Thus, the mean serum value found for AST in the field group can be considered as physiological for grazing sheep in RS.

On the other hand, mean serum values of GGT were not significantly different between the two groups. The values found for this enzyme are within standard range and well below the limits considered at risk for chronic copper poisoning for the species, as shown in Table 1 and Figure 3.

CONCLUSIONS

From the results presented is possible to conclude that the variables copper and gamma-glutamyl transferase (GGT) were within physiological standards, and the tested sheep were not at risk of chronic copper poisoning. The differences in aspartate aminotransferase (AST) levels could be related to the more liver challenging diet that the Exposition sheep are fed prior to the event, in addition to the stress caused by transportation and during the fair itself.

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