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Treatment of copper deficient buffaloes through the application of parenteral copper

[*Tratamento de búfalos deficientes em cobre, por intermédio da aplicação de cobre parenteral*]

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ABSTRACT

The objective of this study was to investigate the effectiveness of the treatment of copper deficiency, by parenteral route, in buffalo calves raised on the Marajó Island (PA). Fourteen animals were divided into two groups. Group 1 received three doses of 120 mg of copper glycinate (20mgCu/mL), intramuscularly, on days 1, 45 and 90; in the group 2 (control) the animals were Not supplemented with copper. In both groups liver biopsies were performed on days 1 and 135. The diagnosis of copper deficiency was established through history, clinical signs, and by the analysis of hepatic copper content. In group 1, the mean hepatic copper content increased from 17.4±5.84mg/kgDM (before administration) to 311.2±133.5mg/kgDM (after 135 days) (P<0.05). In the control group, the mean of hepatic copper contents decreased from 17.1±3.4 to 9.11±1.1mg/kgDM at the end of the experiment (P<0.05). Based on the results, it was demonstrated that the administration of copper, by parenteral route, was efficient in the treatment of copper deficiency in buffalo calves and could become a very promising option for the reality of buffalo producers on the Island of Marajó.

Keywords: biopsy, liver, Marajó Island, mineral

RESUMO

A eficácia do tratamento da deficiência de cobre, por via parenteral, em bezerros bubalinos criados na Ilha de Marajó (PA), foi investigada em 14 animais, distribuídos em dois grupos. O grupo 1 recebeu três doses de 120mg de glicinato de cobre (20mgCu/mL), por via intramuscular, nos dias um, 45 e 90, e o grupo 2 (controle) foi formado por animais que não receberam o cobre, seja suplementado por via oral ou por via parenteral. Em ambos os grupos, foram realizadas biópsias hepáticas nos dias um e 135. O diagnóstico da deficiência de cobre foi estabelecido pelo histórico, pelos sinais clínicos e também pelos teores de cobre hepático. No grupo 1, a média do teor de cobre hepático aumentou de 17,4±5,84mg/kgMS (antes da administração) para 311,2±133,5mg/kgMS (após 135 dias) (P<0,05). Ao final do experimento (135 dias), nos animais do grupo controle, as médias dos teores de cobre diminuíram de 17,1±3,4 para 9,11±1,1mg/kgMS, (P<0,05). Diante dos dados, ficou demonstrado que a administração de cobre, por via parenteral, foi eficiente no tratamento da deficiência de cobre nos bezerros búfalos e pode se tornar uma opção muito promissora para a realidade dos criadores de búfalos da Ilha de Marajó.

Palavras-chave: deficiência, minerais, biópsia, fígado

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INTRODUCTION

The Marajó Island is an archipelago located at the delta of the Amazon River, with an area of approximately 40 to 50 thousand km², divided into 16 municipalities. It has, according to Köppen's classification, an AMI-type climate, with an average annual precipitation of 2500 mm (Cardoso and Pereira, 2002). The rainy period occurs from January to June (Teixeira Neto *et al*., 1991). During the wet season, large parts of the pastures are flooded, which forces ranchers to concentrate their animals in the highest parts of the island. In contrast, during the dry period, there is a marked reduction in forage availability and quality. In both periods of the year, herds suffer from nutritional limitations that affect their productivity (Teixeira Neto *et al*., 1991). According to Falesi (1972), the soils of the Marajó Island are extremely poor in nutrients (*i.e.* low fertility).

Even with these adversities the Marajó archipelago has a herd of approximately 670 thousand of ruminants, of which approximately 300,000 are buffaloes (Pesquisa…, 2010). In general, these animals are raised in a system that is characterized by high technical misinformation on the part of producers, low diffusion of rural technologies, poor productivity indicators, high rates of infectious diseases and low or even negative profitability (Arima and Uhl, 1996). According to Barbosa *et al.* (2005) among the problems that affect buffaloes in the state of Pará, mineral deficiencies stand out for causing severe losses in productivity. The latter authors state that adequate mineral supplementation represents a limiting factor for raise ruminants in the region. In Marajó Island, the mineral deficiency is even more serious, because clinical deficiencies of phosphorus and copper are frequently diagnosed in buffaloes; these deficiencies often occur in isolation or in association.

It is well known that the most efficient way to prevent and treat mineral deficiencies in ruminants is through the supply of the deficient element(s) associated with sodium chloride (Tokarnia *et al*., 2010). However, this form of mineral supplementation, on Marajó Island, is not usually adopted because the breeding system is extensive and also for financial and cultural reasons, the animals from different producers are raised collectively; there is also a lack of adequate troughs and the flooding of pastures, for a long period, makes it difficult or even impossible to routinely supply the mineral mixture to the animals. Therefore, the objective of this study was to investigate the effectiveness of cooper intramuscular injections on the treatment of copper deficiency in buffaloes raised on the Marajó Island (PA).

MATERIALS AND METHODS

The study was carried out on a property located in the municipality of Cachoeira do Arari, Ilha do Marajó, Pará (0º56'33"S; 48º39'57"W). In this region, copper deficiency may occur in clinical and subclinical forms. In the case of the studied herd, a diagnosis of clinical copper deficiency was performed based on herd history and clinical signs. The experiment, as well as all the procedures with the animals, was approved by CEUA/UFPA, under CEUA protocol 8117280421 (ID001663).

Fourteen lactating buffalo calves of both sexes, aged between two and eight months were divided into two age-balanced homogenous groups. Group 1 consisted of seven animals that received 120mg of copper, parenterally, on days 1, 45 and 90. The product administered by deep intramuscular route was copper glycinate, which contained 20mg of Cu per mL. Group 2 (control) consisted of seven animals that were not treated with parenteral copper. The 14 calves were properly identified, with ear tags, and kept, along with their respective dams, in native pastures without mineral supplementation.

To assess the levels of copper in the liver, hepatic biopsies were performed on days 1 (before parenteral administration of copper) and on day 135. The collection of liver samples in the 14 animals was performed by biopsy in the upper third of the 11th intercostal space on the right side, according to Oliveira (2014). To perform the biopsies, the animals were initially submitted to a 12h fasting. Sedation was performed, intramuscularly, with xylazine hydrochloride (at 2%) at a dose of 1mL for each 100kg of body liveweight. After sedation, animals were properly restrained with ropes and kept in left lateral decubitus. The region of the upper third of the 11th intercostal space was washed with clean water then applied a neutral detergent to carry

out a wide trichotomy. Then, local anesthesia of the intramuscular and subcutaneous infiltration type was performed, using 40mL of 2% lidocaine hydrochloride. Subsequently, a second washing of the clipped area was performed, with water and neutral detergent, and proper disinfection using 10% iodized alcohol. Then, an incision of approximately 10 cm was made in the skin of the 11th intercostal space, followed by blunt dissection of the subcutaneous and muscle tissue, to expose the caudal edge of the caudate lobe of the liver. Then, a Doyan atraumatic forceps was used to pull the caudal edge and at the same time to perform hemostasis after the cut and removal of approximately 5 grams of liver sample (Figure 1).

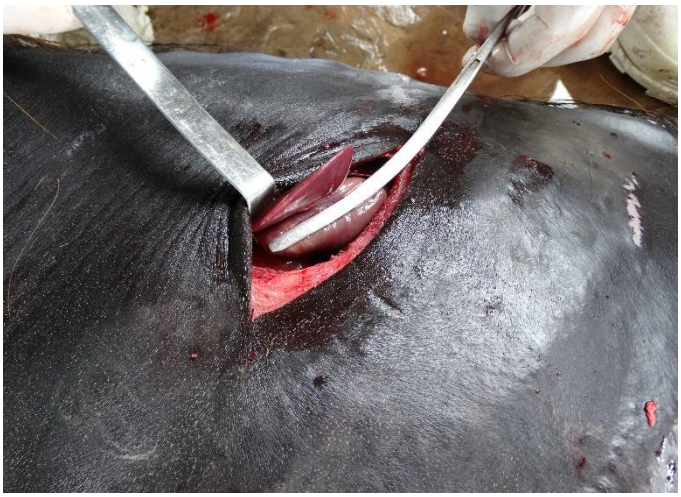

Figure 1. Caudal edge of the caudate lobe of the liver, clamped at the time of the biopsy with a Doyan model atraumatic forceps.

Before suturing the surgical wound, the muscles and the remaining subcutaneous tissue were sutured together, and the skin was repositioned in its original position and sutured in a simple interrupted pattern with nylon # 2. The animals were treated with a single dose of oxytetracycline (1mL per 10kg of BW - IM), flunixin meglumine (2mg per kg of BW, IM). Once a day, the closed wound was sprayed with a spray solution mixture containing silver sulfadiazine (0.1g), aluminum (5.0g) and cypermethrin (0.4g) per 100g (Figure 2).

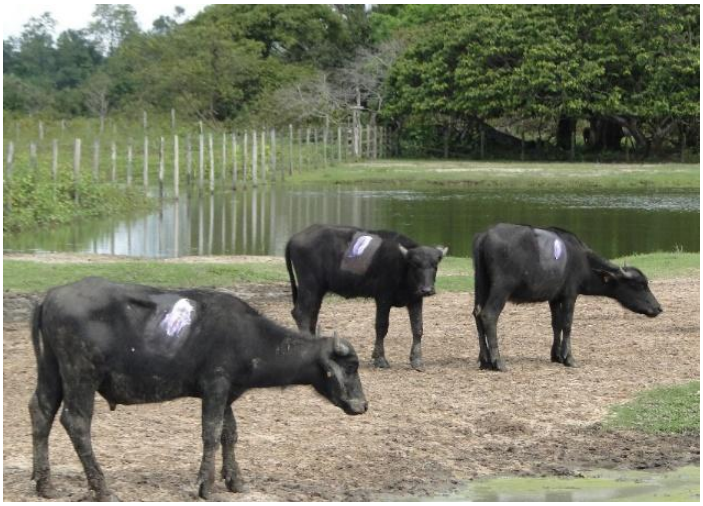

Figure 2. Buffalo calves after liver biopsy surgery.

The liver samples were stored in plastic bags, identified, and placed in an isothermal box with ice. Subsequently, they were frozen at -20ºC until chemical analysis of copper.

For determination of copper concentrations, frozen liver samples were cut into thin blades using clean stainless-steel blades; then, they were placed in 50mL Falcon tubes and submitted to the lyophilization process for 12 hours in an automated LIOTOP® device (model L101). After this processing, the dehydrated samples were macerated in gral and pistil. Subsequently, 0.25 to 0.26 grams of the macerate were weighed and placed in a Teflon digestion tube (Xpress model). Then 3mL of 65% nitric acid P.A., 1mL of 30% hydrochloric acid and 1mL of 30% P.A. hydrogen peroxide were added. The samples were left to rest for 2 hours for pre-digestion and, later, were placed for final digestion for a period of 50 minutes, in a closed system by microwave radiation (MARSXpress, CEM Corp. Matthews, NC, USA). After digestion of the samples, the copper was analyzed using the technique of optical emission spectrometry with induced plasma (ICP OES), in the ICP-OES equipment (Vista-MPX CCD simultaneous, axial from VARIAN) in an automatic sampling system (SPS - 5). The control of the operational conditions of the ICP-OES was carried out with the ICPExpert Vista software. Analytical blanks were prepared by the same procedures, but without the addition of liver samples.

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 independent samples *t* test to compare the means of two independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different. The means were described with the respective standard errors.

RESULTS AND DISCUSSION

The results of the hepatic Cu contents, expressed on a dry basis, of the animals treated with parenteral copper and of the control group are shown in Table 1.

Table 1. Hepatic copper contents (mg/kg of hepatic tissue dry matter (DM)) of the animals that received injectable Cu and the control group

Animals Cu-treated	Cu concentration (1st biopsy)	Cu concentration (2nd biopsy)	Control animals	Cu concentration (1st biopsy)	Cu concentration (2nd biopsy)
1	12.6	444.9	26	12.2	9.00
2	24.9	451.0	27	19.6	10.2
3*	xx	xx	28	19.7	9.70
4	19.1	287.6	29	19.2	7.33
5	12.9	154.2	30	15.6	14.6
6	30.9	218.1	32	15.0	9.30
7	5.23	128.2	33	20.8	16.4
Means	17.4 ± 5.84^a	311.2 ± 133.5^b		17.1 ± 3.38^a	9.11 ± 1.09^b

Means followed by different lowercase letters within each treatment group indicate significant differences ($P<0.05$).
*Data from animal 3 were discarded as they were considered completely out of biological values.

Due to the scarcity of data in the literature mentioning normal Cu contents in the liver of buffaloes, the results of hepatic Cu contents in this study were compared with those described for cattle (Underwood, 1977), where it was established that Cu contents between 0 to 50

mg/kg of hepatic DM are from clinically deficient animals, between 50 to 100 mg/kg DM from sub deficient animals and values above 100 mg/kg DM from animals not deficient in copper.

There is another classification, proposed by Underwood and Suttle (1999), for cattle and sheep, which considers that hepatic Cu contents below 6 mg/kgDM are from clinically deficient animals, between 6 to 20mg/kgDM are from sub deficient animals and values above 20mg/kgDM are from animals not deficient in Cu.

In the present case, the means, around 17mg/kg/DM of liver tissue, obtained from the 13 animals in the first biopsy, are compatible with animals with clinical copper deficiency (Table 1). This position is reinforced by the clinical signs (*e.g.*, periocular achromotrichia and the reddish, discolored, and rough hairs on the ribs and back region) verified during the clinical examination of the animals (Figure 3) and by the history of not receiving any type of mineral supplementation.

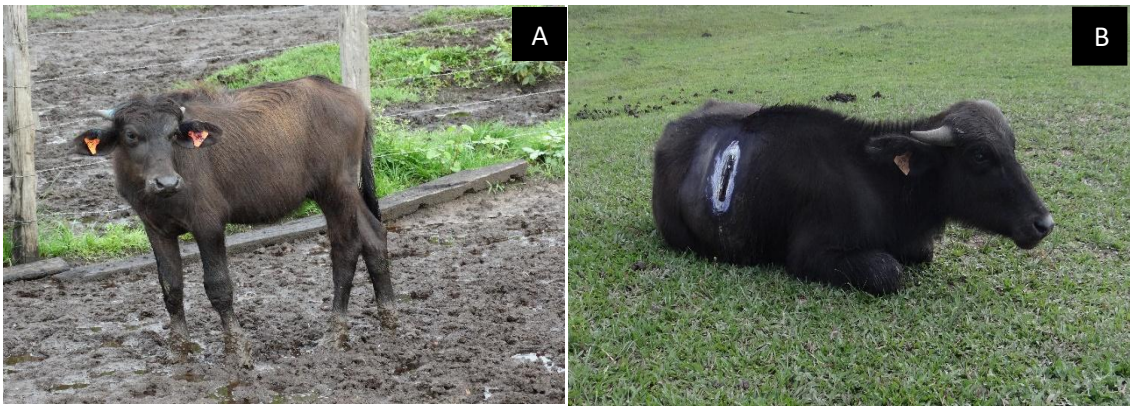

Figure 3. A - Buffalo 1 (Group 1) before liver biopsy and administration of the first dose of 120 mg of copper, showing a rough reddish hair pigmentation. B - Same animal in the 2nd biopsy, showing better body condition score and normal hair color.

In the second biopsy, performed after 135 days, the average copper content in the animals in group 1 was 311.2mg/kg of hepatic DM, quite above the 100mg/kg DM, which are considered indicators of non-copper deficient animals (Underwood, 1977; Table 1). Animals from group 2 which did not receive parenteral copper, values remained low (9.11mg/kg DM) and typical of animals clinically deficient in copper (Table 1).

The results found in the first biopsy of animals in groups 1 and 2 and in the second biopsy performed in the control group are consistent with those reported by Cardoso *et al*. (1992), Pereira and Cardoso (2009), Pinheiro *et al*. (2011) and Oliveira (2014), which found average Cu contents in the liver of 5.7, 19.5, 5 .57 and 7.75mg/kg of DM, respectively, in buffaloes from Marajó island. According to Oliveira (2014), the low levels of copper found in the liver of buffaloes from Marajó Island, are justified by the low soil fertility, low concentration of this mineral in native forages ingested by the animals and by the absence of mineral supplementation in the properties.

The increase in hepatic copper contents was also verified by Oliveira (2014), in adult buffaloes (cows) supplemented with a mineral mixture. The author observed an increase in the copper content in the liver of animals (from 7.75±1.73mg/kg DM, before supplementation, to 205.4±80.5mg/kg DM, after an average intake of 45.2±1.39mg of copper sulfate per day). Sharma *et al*. (2008) also verified a significant increase in the hepatic concentrations of Cu, due to the supplementation of this element through the mineral mixture (171.5±5.56 to 292.5±5.21mg/kg DM).

The reduction in hepatic copper levels in animals in group 2, which were not supplemented (from 17.1±3.38 to 9.11±1.09mg/kg DM, on the 135th day), probably occurred due to the low concentrations of copper in the native forages of

the Marajó Island and by the lack of mineral supplementation.

The accentuated increase in the levels of hepatic copper (in the animals of group 1) did not lead the animals to Cu intoxication; however, studies need to be carried out to establish the daily requirement for this mineral in buffalo species. Mass (2009) recommends parenteral administration of 100 to 200 mg of copper in calves and 400 mg in adult cattle every 4 to 6 months. In the present study, the total dose applied was 360 mg of copper per animal, in a period of 90 days, which did not lead them to have any signs of intoxication. Based on the increase in the copper content in the liver to a very high value (311mg/kg DM), as the ideal is to be in a range of 120 to 200mg/kg DM (Malafaia, personal communication), we suggest that further studies investigate the recommendation of Mass (2009) and apply 100 to 200mg of copper per calf or 400 mg of Cu in each adult, every six months, and evaluate the performance and clinical aspects of these animals.

According to results obtained by Oliveira (2014) and in the present study, copper supplementation proved to be efficient, both orally (through the mineral mixture) and parenterally.

When it comes specifically to Marajó Island, some points should be considered regarding the mineral supplementation provided in traditional troughs. The ultra-extensive system, adopted in most regions of the island, makes mineral supplementation difficult because, in large areas, animals (both cattle and buffalo) from different owners are raised together, which makes mineral supplementation via mineral mixtures available in the troughs unfeasible (Figure 4). Another condition that hinders mineral supplementation is the difficult access to troughs during rainy periods, where large areas are flooded for long periods (Figure 5), leaving the producers to utilize improvised structures that do not serve the entire herd. The animals' lack of appetite for the mineral mixture is also reported by most producers as a hurdle, which may be related to the ingestion of water rich in sodium chloride, as well as other minerals dissolved in them (Figure 6).

Given these difficulties, alternatives must be found in an attempt to mitigate the economic losses related to mineral deficiencies, especially copper. This pioneering study, using injectable copper in buffaloes, on Marajó Island, proved to be efficient in correcting the deficiency status, both by the deposition of copper in the liver and by the elimination of clinical signs that the buffalo calves had before receiving parenteral copper.

Figure 4. Buffaloes belonging to several owners and raised in a huge extensive area, which makes mineral supplementation difficult. Cachoeira do Arari, Marajó Island, PA.

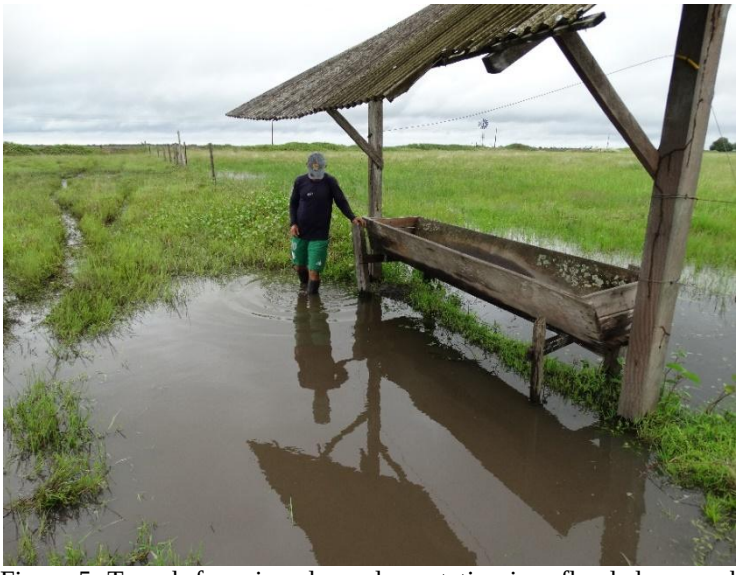

Figure 5. Trough for mineral supplementation in a flooded area, which makes it particularly difficult to supply the mineral mixture. Cachoeira do Arari, Marajó Island, PA.

Figure 6. Brackish water source that possibly reduces the intake of mineral mixtures. Note the deposit of salts on the water's edge. Cachoeira do Arari, Marajó Island, PA.

CONCLUSIONS

The administration of copper, by parenteral route, proved to be efficient in the treatment of copper deficiency in buffaloes in the Marajó Island. This form of parenteral supplementation with two or three applications per year can be very useful as it allows producers to correct the deficiency of copper, without having to carry out traditional supplementation and deal with its inconveniences, inherent to the ecosystem of Marajo Island.

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