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What is This?
Predictive values of aspartate aminotransferase and gamma-glutamyl transferase for the hepatic accumulation of copper in cattle and buffalo

Antonio H. H. Minervino, Raimundo A. Barrêto Júnior, Genilson F. Queiroz, Selwyn A. Headley, Enrico L. Ortolani

Abstract. Ten cattle and 10 buffalo were divided into 2 groups (control \([n = 8]\) and experimental \([n = 12]\)) that received daily administration of copper. Three hepatic biopsies and blood samples were performed on days 0, 45, and 105. The concentration of hepatic copper was determined by spectrophotometric atomic absorption, and the activities of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were analyzed. Regression analyses were done to verify the possible existing relationship between enzymatic activity and concentration of hepatic copper. Sensitivity, specificity, accuracy, and positive and negative predictive values were determined. The serum activities of AST and GGT had coefficients of determination that were excellent predictive indicators of hepatic copper accumulation in cattle, while only GGT serum activity was predictive of hepatic copper accumulation in buffalo. Elevated serum GGT activity may be indicative of increased concentrations of hepatic copper even in cattle and buffalo that appear to be clinically healthy. Thus, prophylactic measures can be implemented to prevent the onset of a hemolytic crisis that is characteristic of copper intoxication.

Key words: Aspartate aminotransferase; buffalo; cattle; copper poisoning; diagnosis; gamma-glutamyl transferase.

Copper (Cu) toxicosis is considered an important disease associated with the death of sheep in Brazil, resulting in great economic losses to the sheep industry.\(^1\) Although the frequency of this intoxication is lower in other species of ruminants, Cu intoxication in cattle has recently evolved as an emerging disease, with descriptions of cases of acute and chronic intoxication in several countries, including Brazil.\(^2\)\(^\text{–}\)\(^6\)\(^,\)\(^12\)\(^,\)\(^16\) Recent research\(^1\) has shown that there is an increase in the number of cases in cattle and has described 14 outbreaks within 6 months. However, in buffalo, there is only 1 description of this intoxication, during which young animals that received reconstituted Cu-enriched powdered milk for a few months demonstrated signs indicative of chronic Cu poisoning and died suddenly.\(^18\)

In cases of chronic Cu poisoning, clinical manifestations are only observed during the hemolytic crisis, or a few days before, so it is very difficult to establish an early diagnosis of this disease based only on clinical signs.\(^6\)\(^,\)\(^11\) Consequently, several researchers have tried to establish parameters of clinical diagnosis based on laboratory analyses.\(^9\)\(^,\)\(^11\)\(^,\)\(^13\) Serum Cu and other traditional biochemical variables have limited diagnostic value in terms of Cu accumulation,\(^9\)\(^,\)\(^13\) and the best results were observed in sheep in association with alterations of hepatic enzymes.\(^7\)

Some researchers\(^13\) have concluded that the most reliable factor in diagnosing early accumulation of Cu is determination of the serum activities of aspartate aminotransferase.
(AST) and gamma-glutamyl transferase (GGT), not the determination of serum Cu concentration, acid phosphatase, and sorbitol dehydrogenase serum activities. Consequently, it is necessary to determine the usefulness of measuring hepatic enzyme activities in cases of Cu accumulation, in which animals are close to an intoxication episode. Therefore, the current study evaluated the alterations in the serum activities of 2 hepatic enzymes to determine if these values can be used as early reliable diagnostic indicators of Cu poisoning in cattle and buffalo.

The present study was carried out in accordance with appropriate ethical standards of animal care, as approved by the Bioethics Committee of the College of Veterinary Medicine, University of São Paulo (São Paulo, Brazil; protocol 746/2005). Twenty male animals were used in the current study: 10 mixed-breed cattle and 10 Murrah breed of buffalo. All animals were 8 months old, approximately 170 kg in body weight, and were divided into 2 groups. The treatment group ($n = 12$; 6 cattle and 6 buffalo) received daily increasing doses of Cu via ruminal fistula. The initial dose was 2 mg/kg body weight (BW) of Cu (in an aqueous solution) administered during the first week; this was increased by 2 mg weekly until the 14th week, when the final dose was 28 mg/kg BW. The control group ($n = 8$; 4 cattle and 4 buffalo) received only normal rations and water by ruminal fistula. Three serial hepatic biopsies were obtained in all animals to determine the concentration of Cu on the following days: day 0 (baseline), day 45 after administration of Cu, and day 105 (at the end of the experiment).

To determine the serum activities of AST and GGT, blood was obtained from the jugular vein using vacuum collecting tubes a few hours before the hepatic biopsies. Serum activities of AST, GGT, and creatine kinase (CK) were determined using a biochemical automatic analyzer with a commercial kit. Enzymatic activity was determined at 30°C, as recommended.

A total of 60 correlation points (30 for each species) between hepatic Cu concentration and serum enzyme activities were obtained. Individual regression analyses and respective coefficients of determination were done for each species to verify the relationship between the variables that were being evaluated. The data were analyzed for linear regression by the $F$-test. Sensitivity, specificity, accuracy, and positive and negative predictive values were calculated to obtain the best discrimination value to determine the relationship between hepatic enzymatic activity and Cu accumulation.

The correlations between the GGT serum activities and hepatic Cu concentrations observed in cattle and buffalo are illustrated in Figures 1 and 2, respectively. For cattle, an elevated coefficient of determination ($R^2 = 0.5838$) and a first-degree regression equation were observed. In buffalo, the coefficient of determination was poor ($R^2 = 0.2625$), and the regression equation was linear.

Considering 1,000 ppm to be indicative of excessive Cu accumulation within the liver and the elevation of serum GGT activity for cattle and buffalo are shown in Figures 3 and 4, respectively. For cattle, these positive and negative predictive values were highest (100%) when the activity of GGT was 24.6 U/l. Since no values were observed in either the first quadrant (false-positive result) or fourth quadrant (false-negative result), the sensitivity, specificity, and accuracy were 100%. For buffalo, the discrimination value for GGT serum activity was 20 U/l, which was similar to that observed in cattle.

The correlations between the serum activities of AST and the hepatic accumulation of Cu in cattle and buffalo are demonstrated in Figures 5 and 6, respectively. In cattle, an elevated coefficient of determination ($R^2 = 0.6338$) and a first-degree regression equation were observed. In buffalo, the coefficient of determination was poor ($R^2 = 0.2625$), and the regression equation was linear.
The discrimination values for the elevated serum activity of AST in cattle and buffalo indicative of hepatic Cu accumulation are presented in Figures 7 and 8, respectively. When hepatic concentrations (1,000 ppm) indicative of chronic Cu poisoning were used to evaluate serum AST activity, the discrimination value was obtained at 90 U/l. With these limits, the positive (75%) and negative (100%) predictive values, sensitivity (100%), specificity (91.7%), and accuracy (93.3%) were determined. For buffalo, the best discrimination value for serum AST activity was 180 U/l. This resulted in lower positive (46%) and negative (86%) predictive values, sensitivity (50%), specificity (83%), and accuracy (76.7%).

Although the number of animals used during the present study was relatively low, the results obtained were consistent and indicate that determination of serum GGT activity is a better indicator of hepatic Cu accumulation than serum AST activity. However, both enzymes can be used to predict elevated hepatic Cu concentrations before the onset of the hemolytic crisis associated with Cu toxicosis. Similar results were previously obtained when these enzymes were evaluated in sheep. In the current study, it was observed that the serum activity of GGT increased earlier and was more consistent than that of AST (data not shown). During abnormal accumulations of hepatic Cu, discrete areas of necrosis occur within the biliary excretory system that result in proliferation of biliary epithelial cells that begin to synthesize GGT continually, which could explain why serum GGT activity is the better indicator of Cu toxicosis. Alternatively, significant leakage of AST into the circulatory system only occurs when there are extensive lesions of hepatocytes that disrupt membrane integrity, and this leakage is normally indicative of an imminent hemolytic crisis.

Another fact that must be considered when evaluating AST serum activity is that its basal activity is higher in cattle.
buffalo than in cattle. When animals that received normal quantities of Cu were compared, it was demonstrated that the serum activity of AST in buffalo was 2.4 times higher than in cattle. In a previous study with Murrah buffalo, results similar to those demonstrated in animals from the control group in the current study were described. It must be emphasized that even in animals receiving normal diet and having no alteration in GGT serum activity, animals will still have a wide range in serum AST activity test results (100–235 U/l), as was observed in buffalo. The wide range of activity in healthy animals reduces the efficacy of the serum AST determination as an indicator for clinical diagnosis of an impending Cu toxicosis. Furthermore, serum AST activity also is present in striated and cardiac muscle. Therefore, simultaneous determination of CK must be done to determine whether an increase in AST serum activity is due to liver dysfunction and/or muscle damage. In the present study, only 3% (2/60) of the serum specimens used to determine AST activity had serum CK activity above the reference interval.

Both AST and GGT serum activities had increased coefficients of determination and were almost as equally efficient in determining elevated concentrations of hepatic Cu in cattle. However, determination of AST activity was not as good at predicting elevated concentrations of hepatic Cu in buffalo, since a low coefficient of determination was verified ($R^2 = 0.26$) and false-positive and false-negative test results were observed.

Contrary to the results of previous publications, GGT serum activity was considered as a possible indicator for the diagnosis of excessive hepatic accumulation of Cu in cattle and buffalo in the current study. Considering the established discrimination value for GGT serum activity in cattle (24.6 U/l), a research study involving a Cu-overload diet (20 mg/kg BW of Cu) has shown that bulls had values indicative of excessive liver Cu accumulation. In contrast, the heifers did not have serum values of GGT activity that were indicative of elevated hepatic Cu concentration. Although AST serum activity had reduced accuracy and predictive values when compared with GGT activity, increased AST activity was relatively efficient in the diagnosis of excessive accumulation of hepatic Cu in cattle, as demonstrated by the high coefficients of determination. However, contradictory results have also been described for AST serum activity.

Under normal field conditions, liver dysfunctions, such as fatty liver, can elevate the serum activity of liver enzymes and could induce false-positive results. Nevertheless, the discrimination values obtained during the present study may have clinical significance as a superior and alternative method for the early diagnosis of Cu accumulation, since it is comparatively inexpensive, less invasive, and safer for animal health than conventional liver biopsy.

The serum activities of sorbitol dehydrogenase and acid phosphatase might have low levels of accuracy to predict Cu accumulation in sheep, while alkaline phosphatase has a weak correlation with hepatic Cu in cattle. Additionally, glutamate dehydrogenase has elevated plasma activity even after the period of Cu overload and presents better results as an indicator of hepatic Cu accumulation than do AST and GGT in sheep. However, because of their availability in Brazil and as a result of importation restrictions, these enzymatic evaluations were not performed as part of the current study. To evaluate these results, further studies are necessary with a larger number of animals using different enzymatic combinations in cattle and buffalo with high Cu intake that leads to a hemolytic crisis over a short period.

Elevated serum GGT activity may be indicative of increased concentrations of hepatic Cu even in cattle and buffalo that appear to be clinically healthy. Thus, prophylactic measures can be implemented to prevent the onset of a hemolytic crisis that is characteristic of Cu intoxication.

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![Figure 7](image1.png)

**Figure 7.** Cut-off line for the accumulation of hepatic copper and the elevation of serum aspartate aminotransferase (AST) in cattle.

![Figure 8](image2.png)

**Figure 8.** Cut-off line for the accumulation of hepatic copper and the elevation of serum aspartate aminotransferase (AST) in buffalo.
Sources and manufacturers

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