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Clinical observations and acid-base imbalances in sheep during chronic copper poisoning

Avaliação clínica e hemogasométrica de ovinos com intoxicação cúprica acumulativa

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Abstract

Twelve male sheep were intoxicated with copper and four served as controls. When hemoglobinuria was first diagnosed, the poisoned sheep were randomly distributed into two groups: 4 untreated and 8 tetratiomolybdate-treated. Blood samples and clinical evaluation were performed daily, from the onset of poisoning until the 30th day. Analysis of packed cell volume, plasma free hemoglobin, and blood gas were made. Elevated heart rates and rectal temperature, and reduced respiratory and ruminal movement rates were recorded in the intoxicated group. The poisoned sheep developed mild alkalosis caused by bicarbonate retention, while a short-periodic increase of pCO_2 occurred to compensate the ongoing alkalosis. Elevated degree of anemia was directly proportional to heart rate, while high degree of alkalosis was inversely proportional to respiratory rate. Further, there was an elevated positive relationship between plasma free hemoglobin and rectal temperature, and an increase in rectal temperature accompanied a reduced ruminal movement.

Key words: Sheep, cpper toxicosis, hemolysis, packed cell volume, alkalosis, plasma free hemoglobin

Resumo

Foram utilizados 16 cordeiros, sendo 12 submetidos à intoxicação cúprica e quatro animais controle. Quando foi verificada a presença de hemoglobinúria, os animais intoxicados foram aleatoriamente distribuídos em dois grupos, quatro animais não tratados e oito animais tratados com tetratiomolybidato de amônia. Foi realizado exame clínico e coleta de sangue diariamente desde o início da intoxicação até 30 dias após. Foram analisados o volume globular, concentração de hemoglobina plasmática e avaliação hemogasométrica. Nos animais intoxicados, foi observado elevação da freqüência cardíaca e da temperatura retal e redução da freqüência respiratória e dos movimentos ruminais. Os ovinos intoxicados desenvolveram alcalose moderada causada por retenção de bicarbonato seguido de um aumento pontual da pCO_2 para compensar a alcalose em curso. Quanto maior o grau de anemia foi maior

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a freqüência cardíaca, enquanto que quanto maior o grau de alcalose menor a freqüência respiratória. Houve uma relação positiva entre a elevação da hemoglobina plasmática livre e a temperatura retal, e quanto maior a temperatura retal menor a freqüência de movimentos ruminais.
Palavras-chave: Ovinos, intoxicação cúprica, hemólise, volume globular, alcalose, hemoglobina

Introduction

Copper is an essential microelement to domestic animals but when ingested in large quantities can become toxic to ruminants (MINERVINO et al., 2009a; MIRANDA et al., 2010), principally sheep (HUMANN-ZIEHANK et al., 2001; ORTOLANI; ANTONELLI; SARKIS, 2004; HEADLEY et al., 2008). Lambs maintained on high concentrate diets are particularly susceptible to chronic copper poisoning. There is evidence suggesting that the incidence of this poisoning increase as more intensive methods of sheep production are adopted (UNDERWOOD; SUTTLE, 1999).

Ruminants receiving diets rich in copper but with normal concentrations of molybdenum and sulfur, for long periods, can accumulate increasing amounts of copper, predominantly, in the liver (MINERVINO et al., 2009b; MIRANDA et al., 2010). During this phase, clinical sings are absent making the diagnosis of liver copper accumulation difficult (LOPEZ-ALONSO et al., 2006; MINERVINO et al., 2008; ORTOLANI; MACHADO; SUCUPIRA, 2003). When maximum hepatic levels are reached, the unbounded copper is released into the bloodstream (MINERVINO et al., 2009a; RADOSTITIS et al., 2007). The unbound copper is up taken by erythrocytes and combines with the reduced form of glutathione oxidizing this compound. Glutathione plays a crucial role in preventive oxidative damage to the membrane of erythrocytes. Thus, hemolysis occurs, some 24h later, when reduced glutathione levels are lowered (HOWELL; GOONERATNE, 1987; RADOSTITIS et al., 2007).

Unbounded copper also oxidizes the hemoglobin of erythrocytes, thereby increasing methemoglobinemia and the formation of Heinz body. The methemoglobin, observed during hemolysis, reduces the capacity for oxygen transport of the remaining erythrocytes. The unbounded copper interacts with sulphydryl groups of the membranes of red blood cells to form disulfides, which are accompanied by the formation of superoxide radicals. The alterations that occur to the red blood cells and/or Heinz body formation might be the cause of extensive hemolysis that is frequently observed in copper-poisoned sheep (HOWELL; GOONERATNE, 1987). Despite the large amount of oxidative studies realized in the copper-poisoned sheep, there has been no evaluation of the consequences of anemia, methemoglobinemia, the formation of superoxide radicals, or any other additional effect, on the overall acid-base balance of the poisoned sheep.

Hemolysis and hypercupremia might result in overload to the renal tubular epithelium with hemoglobin, copper, and iron, resulting in cell death and consequent renal insufficiency. In most cases, death can result from the combined associations of renal insufficiency, liver dysfunction, and anemia (MINERVINO et al., 2009a; RADOSTITIS et al., 2007).

Information relative to the alteration of vital functions, e.g., respiratory, cardiac, rectal temperature, and rumen functions, are scarce and frequently contradictory. While the relationships between changes to any of these vital functions and other biochemical dysfunctions, e.g., plasma free hemoglobin, the degree of anemia and blood pH, are lacking. Consequently, the object of this study was to evaluate the evolutionary changes of specific vital functions (respiratory, cardiac and ruminal rates, and rectal temperature), and their relationship with other biochemical dysfunction in copper-poisoned sheep. Further, the alterations to the blood acid-base balance were also studied.

Materials and Method

Protocol

Sixteen 6-month-old, cross-breed (Suffolk x Crioulo), male lambs were used. These animals were randomly assigned into 2 groups: one control (n=4) and one copper-load group (n=12), which received 3 mg of copper (as $CuSO_4.5H_2O$) per kg of BW daily during the first week of the experiment, with the addition of 3 mg of Cu weekly until the animals presented clinical signs of copper toxicosis. Thereafter, the poisoned sheep were randomly assigned into two groups of four untreated and eight tetratiomolybdate-treated (TTM) sheep. This treatment begun when sheep presented macroscopic hemoglobinuria and was performed by daily endovenous injection of TTM (3.4 mg/kg/BW), diluted in saline solution at 35ºC, during five consecutive days (HUMPHRIES et al., 1986).

The animals were evaluated daily since the onset of the hemolytic crisis until the 30th day. Blood samples with anticoagulant (ethylenediaminetetraacetic acid) for hematological analysis were taken daily and stored at 4 ºC until laboratory analysis, which occurred not more than 4 hours thereafter. The blood for gas analysis was collected anaerobically into a plastic syringe that contained heparin, and analyzed within 15 min according to recommendation (SUCUPIRA; ORTOLANI, 2003). The packed cell volume (PCV) was determined by a microhematocrit method, while the plasma free hemoglobin values were determined as described by Kaneko, Harvey e Bruss (1997).

The blood gas determinations were performed using an automated blood gas analyzer (Blood gas analyzer, model AVL 330, ROCHE©, Basel, Schweiz). The results obtained were corrected to the temperature of the animals and the blood hemoglobin concentration. Clinical evaluations were done prior to each blood collection; at this moment, the respiratory, heart, and rumen motility rates, as well as the rectal temperature were recorded.

Statistical analysis

The data of all copper-loaded sheep, independent of the administration or not of TTM, were aggregated into a single group and compared to the animals used as controls. All data, except that of the plasma free hemoglobin, presented a parametric distribution. The differences between and within the treatments were compared by analysis of variance using the Duncan's multiple range test to compare the means of data with normal distribution. The plasma free hemoglobin concentration was assessed by using the Mann-Whitney test. The correlations between the variables were evaluated by the Pearson's correlation coefficient (SAMPAIO, 2002).

Results

All copper-loaded sheep developed the typical manifestations (e.g., hemoglobinuria, depression, lethargy, anorexia, jaundice, and pallor) of chronic copper poisoning (HEADLEY et al., 2008, BOZYNSKI et al., 2009; MINERVINO et al., 2009ab; ORUC; CENGIZ; BESKAYA, 2009). Three out of 4 TTM-untreated sheep died within 3 days; the other animal died on day 10. One out of 8 TTM-treated lambs died on the 9th day. Clinical features, PCV, plasma free hemoglobin, and blood acid-base data revealed discrete fluctuation, and were maintained within normal reference values, in all control animals. Since similar results was obtain for treated and non-treated sheep for the parameters evaluated, and because most untreated sheep died few days after hemolytic crisis, which made group comparison difficult, we only considered 2 groups for statistical analyses: control group, without copper supplementation; and intoxicated group, copper-loaded sheep including treated and untreated animals.

Clinical features

The results relative to the alterations of heart rate, respiratory rate, and rectal temperature, respectively,

are presented at figures 1, 2 and 3. Elevated ($p < 0.05$) heart rates were observed in copper-loaded sheep from day zero to the 15th day. Alternatively, lower ($p < 0.05$) respiratory rates occurred in the poisoned lambs from day zero to the 3rd day. Additionally, elevations ($p < 0.04$) of rectal temperature and reduced frequency of ruminal movements were observed in all intoxicated sheep; these alterations began with the onset of hemoglobinuria and were evident until the 5th day.

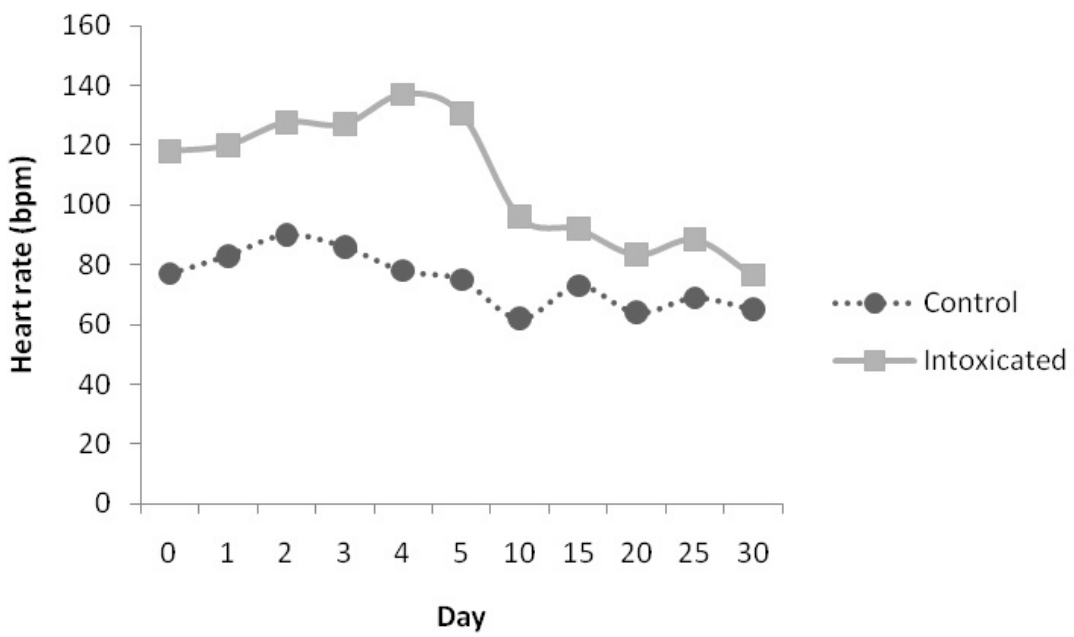

Figure 1. Comparative mean values of the heart rate of sheep from intoxicated and control groups. Day zero was when the hemolytic crisis occurred in the copper poisoning animals.

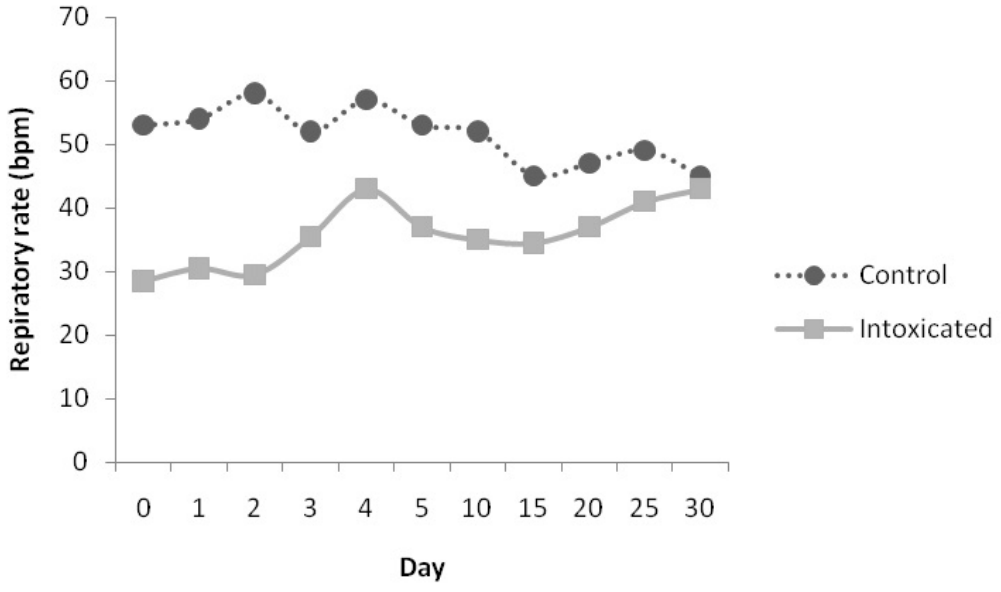

Figure 2. Comparative mean values of the respiratory rates of sheep from intoxicated and control groups.

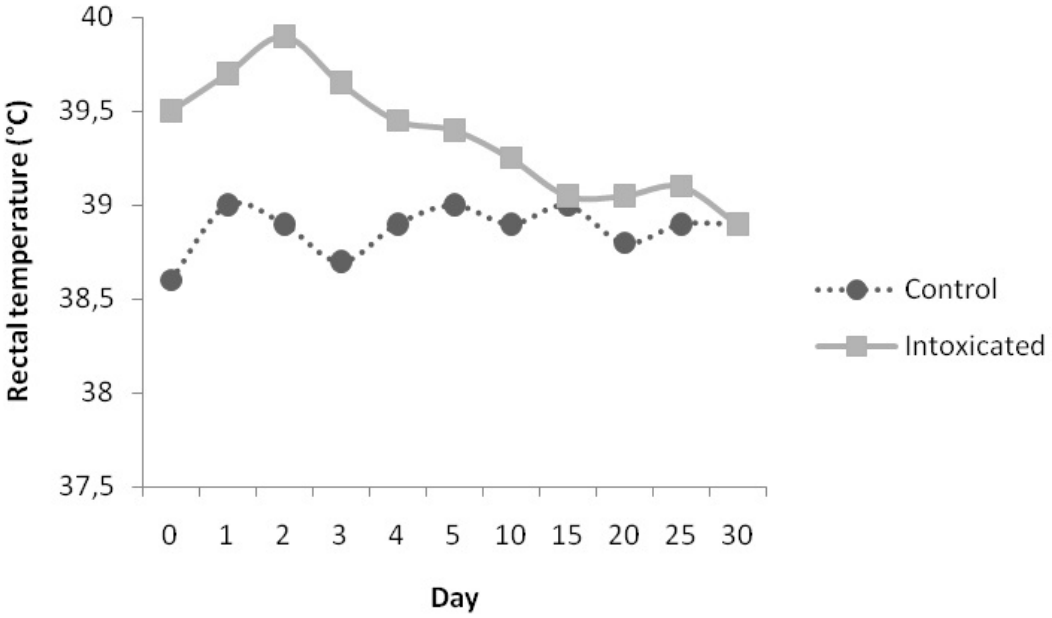

Figure 3. Comparative mean values of the rectal temperature of sheep from intoxicated and control groups.

PCV and plasma free hemoglobin

A marked decrease in the PCV values ($p < 0.001$) was observed in blood samples from poisoned animals obtained from day zero to the 20th day. This decrease was from 32% to 11% in the fourth day, with a gradual and slow recuperation of PCV values starting at day five. The plasma free hemoglobin concentrations were more than 30 times higher ($p < 0.03$) in poisoned animals on day zero (mean of 51 mg/L for control group and 1632 mg/L for poisoned sheep), and remain elevated from the beginning of the hemolytic crisis until the 6th day, when compared with non-intoxicated sheep.

Blood acid-base balance

The results of the alterations to the blood acid-base balance, pH, bicarbonate concentration, and base excess, respectively, are given at figures 4, 5 and 6. Significant ($p < 0.045$) elevations of the blood pH and bicarbonate concentrations occurred from the onset of the hemolytic crisis and continued until the 4th day. Similar results were obtained with base-excess (BE) concentration: the intoxicated sheep presented elevated levels of BE within the first three days of hemolysis ($p < 0.05$). No alterations to the pO_2 values occurred throughout the experiment. However, elevated pCO_2 values were present between the 3rd and 4th day.

Relationship between specific variables

The reduced PCV values were directly related to elevations of the heart rate ($r = -0.676$; $p < 0.01$). The elevated values of blood pH were directly related to reduced respiratory rates ($r = -0.664$; $p < 0.01$). Further, there was a high positive relationship between the values of plasma free hemoglobin and rectal temperature ($r = 0.788$, $p < 0.01$).

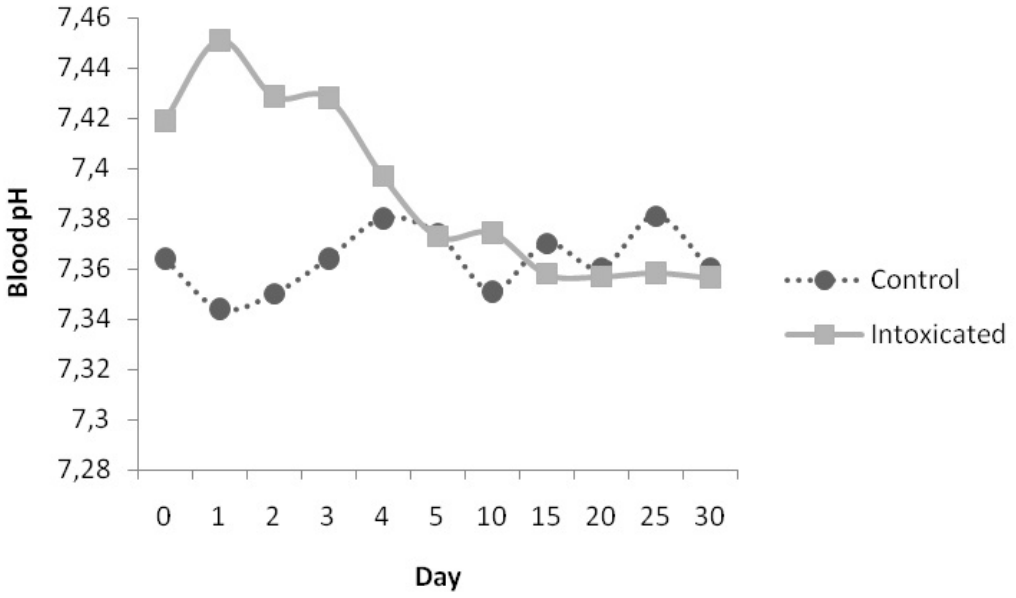

Figure 4. Comparative mean values of blood pH of sheep from intoxicated and control groups.

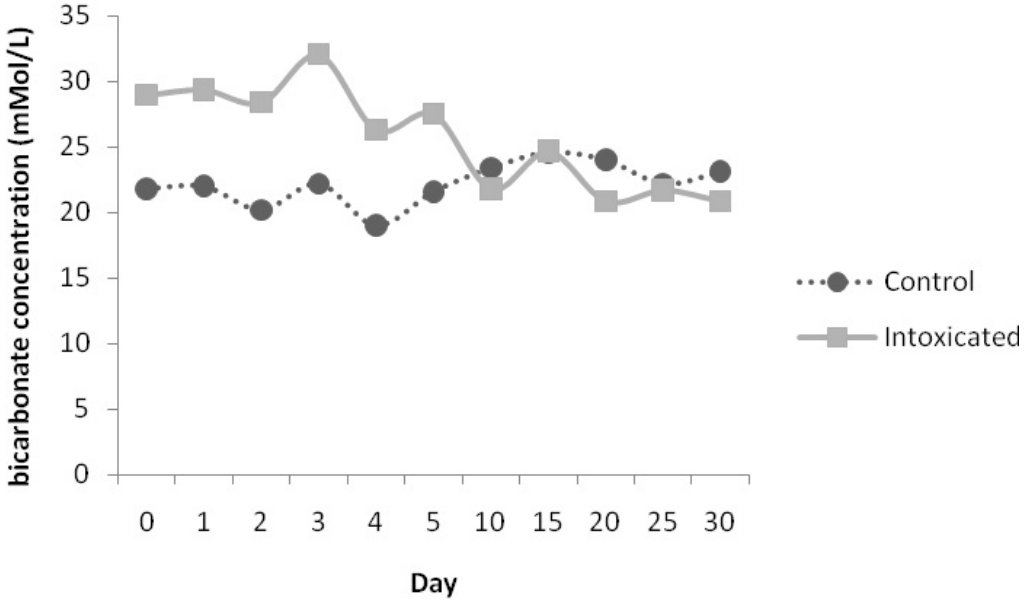

Figure 5. Comparative mean values of bicarbonate concentrations of sheep from intoxicated and control groups.

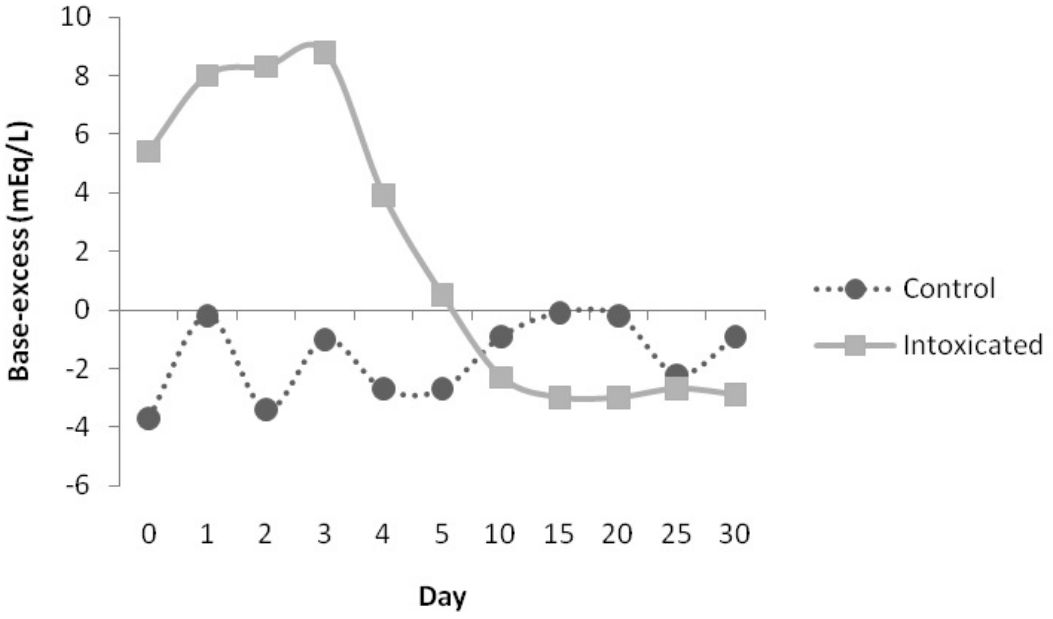

Figure 6. Comparative mean values of base-excess of sheep from intoxicated and control groups.

Discussion and Conclusion

No clinical signs were observed before the hemolytic crisis, but as soon as hemoglobinuria was observed, several organic dysfunctions were simultaneously established (BOZYNSKI et al., 2009; MINERVINO et al., 2008; MINERVINO et al., 2009b; ORUC; CENGIZ; BESKAYA, 2009). The hemolysis seen in the sheep during this experiment was severe, causing a marked reduction (60%) in the PCV.

A prolonged tachycardia occurred throughout the onset of the clinical signs of poisoning; with an elevated heart rate (25% to 50%) in all animals. Although several systemic conditions, such as fever, uremia, toxemia, and dehydration, coexisted with the ailing sheep, and these collectively could have resulted in increased heart rate, there was a significant negative relationship between tachycardia and the degree of anemia. The primary response to tissue anoxia caused by anemia is an increase in cardiac output in response to increases in the stoke volume and heart rate, and a decrease in circulation time (RADOSTITIS et al., 2007). Two sheep were severely anemic (PCV 7 and 8%) for few days that coincided with a markedly increased heart rate (175 beats/min) and intensity of cardiac sounds. Nevertheless, these sheep survived without any administration of hematinic drug or blood transfusion.

The hemolysis observed in the sheep during this experiment resulted in a dramatic increase in the concentration of plasma free hemoglobin. In addition to produce a severe hemoglobinuric neprhosis (BOZYNSKI et al., 2009; HOWELL; GOONERATNE, 1987), resulting in renal insufficiency, excess hemoglobinemia produced a slight (up to 1 ºC), transient (3 days of duration) aseptic fever. Elevated levels of hemoglobinemia were directly proportional to elevated rectal temperature of the poisoned sheep during the first 3-4 days of hemolysis. A current hypothesis suggests that excess hemoglobinemia can act as an endogenous pyrogen inducing a chain reaction with abrupt increase in the synthesis of prostaglandins, particularly prostaglandin E_2 in the anterior hypothalamus. The elevated levels of prostaglandin

in the hypothalamus increase the thermostatic set point and activate the mechanisms associated with heat conservation and production until the blood and core temperature are elevated to corresponding levels of the hypothalamus set point (RADOSTITIS et al., 2007).

The presence of a slight fever in the sick sheep corroborated with the manifestation of several clinical signs usually described in copper poisoning, such as tachycardia, decreased rumen motility, anorexia, depression, muscle weakness, and oliguria with albuminuria (MINERVINO et al., 2008, ORUC; CENGIZ; BESKAYA, 2009). Although the febrile reaction classically produces polypnea, during this study the affected lambs exhibited oligopnea during the course of the fever.

The respiratory rate of the control sheep fell within 37-66 breaths/ min (overall mean 50), while the rate of poisoned sheep varied from 18 to 86 (overall mean 35) during the first 3 days of the hemolytic crisis. As was previously mentioned, an excess of hemoglobinemia might induce nephrosis due to severe tubular dysfunction, resulting in renal insufficiency and uremia (BOZYNSKI et al., 2009; MINERVINO et al., 2009a). During this experiment, transient or terminal uremia was diagnosed in all copper poisoned sheep within 4 days of the onset of clinical signs. Since bicarbonate passes freely through the glomerular filter, it is necessary that reabsorption be predominant at the proximal tubules (KANEKO; HARVEY; BRUSS, 1997). However, animals with renal insufficiency have increased renal tubular bicarbonate reabsorption in addition to a decreased glomerular filtration of bicarbonate, resulting in bicarbonate retention and metabolic alkalosis. Additionally, chemoreceptors of the respiratory center detect the alkalosis, while the respiratory response to metabolic alkalosis is hypoventilation and oligopnea resulting in an increase in pCO_2 to compensate the alkalosis (KANEKO; HARVEY; BRUSS, 1997).

This clinical and biochemical phenomenon was evident throughout this experiment. There was an accumulation of bicarbonate and an increase in BE, resulting in an increase of blood pH. Further, elevation in blood pH, was accompanied by reduction in respiratory rate, while a slight but significant increase of pCO_2 occurred later in the process.

Additionally, elevated rectal temperature was related to reduced ruminal movements. Eight out of 12 poisoned sheep developed ruminal atony during the first 4 days of the onset of clinical signs. This can be associated to the actions of endogenous and exogenous pyrogens which might depress the reticularruminal motor center of the medulla, thereby inhibiting the primary and secondary ruminal cyclic movements, and might result in reduced intensity of ruminal contraction (RADOSTITIS et al., 2007).

In summary, during the clinical onset of copper poisoning sheep presented an evident tachycardia generated primarily by anemia, while a moderate oligopnea caused by a transient alkalosis, partially compensated by elevated blood pCO_2. Further, elevated levels of plasma free hemoglobin gave rise to a slight and temporary aseptic fever and reduced the rate of ruminal movements or even rumen atony.

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