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Acid-base status of ammonia-poisoned steers

ABSTRACT

Although several studies on ammonia poisoning have been carried out, there is a lack of information on acid-base balance status in ammonia-poisoned cattle. Twelve crossbred steers received intraruminally 0.5 g of urea per kg of body weight in order to induce a clinical picture of ammonia poisoning. Blood samples were collected throughout the trials in order to determine the blood ammonia, lactate, and perform blood gas analysis. All cattle presented a classical clinical picture of ammonia poisoning, with a blood ammonia concentration rising progressively from the beginning until reaching higher values at 180 min (27 ± 3 to $1719 \pm 101 \mu\text{mol L}^{-1}$), with a similar pattern occurring with blood L-lactate levels (1.7 ± 0.3 to $26.0 \pm 1.7 \text{ mmol L}^{-1}$). The higher the blood ammonia concentration the higher the blood L-lactate levels ($r = 0.86$). All animals developed metabolic acidosis, as blood pH lowered to 7.24 ± 0.03 . The steers tried to compensate the metabolic acidosis mainly through the use of blood buffers and respiratory adjustments by lowering the pCO_2 levels in the blood to $32.8 \pm 2.0 \text{ mmHg}$.

Key words: blood gas analysis, bovine, toxicity, urea

Estado ácido-básico de garrotes intoxicados por amônia

RESUMO

Apesar dos diversos estudos sobre a intoxicação por amônia, ainda existe uma lacuna de informação sobre o estado do equilíbrio ácido-básico em bovinos intoxicados por amônia. Doze garrotes mestiços receberam, intraruminalmente, 0,5 g de ureia por kg de peso vivo com a finalidade de induzir quadro clínico de intoxicação por amônia. Amostras de sangue venoso foram coletadas para determinação de amônia e lactato-L e realização de hemogasometria. Todos os animais apresentaram quadro clínico clássico de intoxicação por amônia. A concentração de amônia sanguínea elevou-se progressivamente desde o início do experimento até atingir seus valores mais elevados, após 180 min da administração da ureia (27 ± 3 to $1719 \pm 101 \mu\text{mol.L}^{-1}$), e os teores de lactato-L apresentaram padrão similar ($1,7 \pm 0,3$ to $26,0 \pm 1,7 \text{ mmol.L}^{-1}$). Quanto mais elevada foi a concentração de amônia sanguínea maior foi a concentração de lactato-L no sangue ($r=0,86$). Todos os animais desenvolveram acidose metabólica sendo que o pH sanguíneo diminuiu a valores médios de $7,24 \pm 0,03$. Os garrotes tentaram compensar a acidose metabólica através principalmente do uso de tampões presentes no sangue e compensação respiratória por meio da diminuição dos teores de pCO_2 para valores médios de $32,8 \pm 2,0 \text{ mmHg}$.

Palavras-chave: hemogasometria, bovinos, intoxicação, ureia

INTRODUCTION

The occurrence of outbreaks of ammonia poisoning is getting more often as the use of urea is increasing among cattlemen as a protein replacer to cattle raised under extensive management on subtropical or tropical environment, mainly during the dry season (Kitamura et al., 2003). In Brazil, more than 20 million beef and dairy cattle are supplemented with urea yearly (Antonelli et al., 2004).

Although the supplementation of urea to ruminants is generally safe, acute ammonia poisoning due to urea ingestion resulting in the death of animals are often reported by veterinarians and cattlemen (Ortolani et al., 2000). Ammonia poisoning may occur mainly when ruminants not adapted to urea consume large quantities of it, or when feeds are inappropriately mixed with urea. Usually the clinical picture is acute with a quick development and most often the clinical signs are characterized by restlessness, dullness, weakness, muscle tremors, profuse salivation, rumen atony, bloat, dyspnea, incoordination, vocalization, lung edema, tonic-clonic convulsions, and finally death by heart failure that may occur within 30 minutes (Froslie, 1977; Antonelli et al., 2004; Radostits et al., 2007; Kitamura et al., 2010a).

Besides the clinical signs, some laboratory findings, such as blood ammonia concentration, are helpful to make an accurate diagnosis. Early diagnosis has an expressive role in the success of the treatment. Establishing the acid-base status will not assure the success of the treatment but might help when handling with severe cases of ammonia poisoning that would require a correction of the acid-base balance in addition to conventional treatment (Ortolani et al., 2000). But there is no consensus between researchers about the effects on acid-base status in ammonia-poisoned animals as different authors described the effects as a metabolic alkalosis, a metabolic acidosis, and a respiratory acidosis (Rash et al., 1968; Roller et al., 1982).

The purpose of this study was to determine the effects on acid-base status during acute ammonia poisoning in cattle due to urea administration into the rumen, which will help to provide the most efficient treatment in natural cases of ammonia poisoning, leading to a faster full recovery of affected animals.

MATERIAL AND METHODS

This study followed the ethical principles of the Colégio Brasileiro de Experimentação Animal (COBEA). Twelve crossbred (Holstein-Gir) clinically healthy steers [body weight (BW) range 200-250 kg], from a single herd located in the city of Alumínio, SP were selected for this experiment. They were housed indoors in individual tie stalls and provided the following basal diet with 9.55% of dry matter of crude protein: 30% commercial concentrate and 70% coast-cross hay (*Cynodon dactylon* (L) Pers), and water was available *ad libitum*. This diet did not contain urea or any source of non-protein nitrogen. In order to administer urea, a fistula to the rumen was surgically made, and fitted and capped with a rubber cannula, during the period of adaptation to the diet.

The steers were kept fasting 15 h prior to the start of the experiment, but water was available *ad libitum*. Each animal was used only once in the trial. The cattle received intraruminally 0.5 g per kg of BW of urea plus 1.5 g per kg of BW of cornstarch. The urea and the cornstarch were administered within the ventral sac of the rumen by a plastic tube through the rubber cannula, and then the rumen content was stirred by hand to assure the complete and uniform spreading of the urea.

Venous blood samples were drawn from the jugular vein to determine blood ammonia and L-lactate in the following moments: before giving urea, at the start of the muscle tremors, at the first convulsive episode, and 30, 90, and 180 min after administering urea. All samples were analyzed in the Multiple Clinical Chemistry Autoanalyzer (Liasys®) using commercial diagnostic kits. Another sample of venous blood was drawn from the jugular vein and processed for pH, pCO₂, bicarbonate and base excess (BE) within 15 min after collection in a blood gas analyzer (Radiometer® ABL 5).

All animals were treated properly at the end of each trial as described elsewhere (Antonelli et al., 2004; Kitamura et al., 2010b), and recovering all animals within a few hours.

Data are expressed as mean ± SE. The data were assessed using ANOVA and the means compared by the Tukey-Kramer post-test to establish a comparison. The coefficient of correlation was assessed between variables, and it was established that there was a high correlation when $r > 0.60$; a medium correlation when $0.30 < r < 0.60$; and a low correlation when $r < 0.30$, with a $p < 0.0001$ value (Little & Hills, 1978). The statistical analysis was performed using the GraphPad InStat 3.06 for Windows statistical software (GraphPad Software, 1998).

RESULTS AND DISCUSSION

At the beginning of the experiments, all 12 steers were healthy, alert and active. The experimental induction was successful in all trials, resulting in definite clinical signs of ammonia poisoning that culminated with convulsive episodes. The first muscle tremors occurred with 69.7 ± 6.3 min after dosing urea, and the first convulsive episodes happened with 128 ± 16.3 min after urea administration.

The concentration of blood ammonia increased progressively, with the muscle tremors starting with 1100 ± 47 $\mu\text{mol L}^{-1}$, and as the clinical picture evolved, it reached 1624 ± 61 $\mu\text{mol L}^{-1}$ at convulsion, with highest values at 180th min (1719 ± 83 $\mu\text{mol L}^{-1}$). The detoxification of ammonia into urea by the liver elevated progressively the urea concentration from 4.1 ± 0.41 mmol L^{-1} before administering urea to 6.6 ± 0.38 mmol L^{-1} at the end of the experiment, but still within reference levels.

In the present study, the steers presented mean blood ammonia concentration of 646 ± 106 mmol L^{-1} (Table 1) and yet did not show any signs of muscular tremors, although these values were 8% higher than the amount of blood ammonia described by Bartley et al. (1976) for ammonia poisoned steers with muscular tremors. On the other hand, the onset of the muscular tremors occurred when blood reached 1100 ± 47 mmol L^{-1} (Table 1), which is 93% higher than the data

Table 1. Blood chemical variables values (mean \pm SE) for ammonia-poisoned steers**Tabela 1.** Valores de variáveis químicas do sangue (média \pm DP) para garrotes intoxicados por amônia

Blood Parameter	Time (min) after urea administration					
	0	30	A ¹	90	B ²	180
Ammonia ($\mu\text{mol/L}$)	27 \pm 3 ^d	646 \pm 106 ^c	1100 \pm 47 ^b	1312 \pm 121 ^b	1624 \pm 61 ^a	1719 \pm 83 ^a
Urea (mmol/L)	4.1 \pm 0.41 ^e	5.1 \pm 0.35 ^d	5.8 \pm 0.40 ^c	6.0 \pm 0.36 ^{bc}	6.4 \pm 0.46 ^{ab}	6.6 \pm 0.38 ^a
L-lactate (mmol/L)	1.7 \pm 0.25 ^c	3.1 \pm 0.57 ^c	8.9 \pm 0.80 ^b	13.5 \pm 2.66 ^b	23.8 \pm 0.69 ^a	26.0 \pm 1.36 ^a
pH	7.41 \pm 0.01 ^a	7.43 \pm 0.01 ^a	7.46 \pm 0.01 ^a	7.42 \pm 0.03 ^a	7.27 \pm 0.01 ^b	7.24 \pm 0.02 ^b
pCO ₂ (mmHg)	46.5 \pm 0.8 ^a	45.6 \pm 0.6 ^a	38.7 \pm 1.1 ^b	33.8 \pm 1.7 ^c	32.6 \pm 1.2 ^c	32.8 \pm 1.7 ^c
Bicarbonate (mmol/L)	28.5 \pm 0.4 ^{ab}	30.2 \pm 0.5 ^a	25.8 \pm 0.9 ^{bc}	23.8 \pm 1.1 ^c	16.3 \pm 0.5 ^d	13.7 \pm 0.8 ^d
Base excess (mmol/L)	4.5 \pm 0.2 ^a	5.4 \pm 0.4 ^a	2.0 \pm 0.9 ^{ab}	-1.1 \pm 2.1 ^b	-10.5 \pm 1.1 ^c	-12.5 \pm 1.3 ^c

¹ Tremors occurred 69.7 \pm 6.3 min after urea administration; ² convulsion occurred 128 \pm 16.3 min after urea administration; different letters in the line indicate significant differences ($p < 0.05$)

presented by Bartley et al. (1976). Also this study determined that the convulsion occurred when blood ammonia reached mean values of 1624 \pm 61 mmol L⁻¹ (Table 1), which is slightly higher than the data presented by Froslic (1977) who affirmed that convulsion would occur when blood ammonia reaches between 880 and 1470 mmol L⁻¹.

The discrepancy between the present data and the other studies might be related to the blood ammonia determination technique used by those authors. They used the microdiffusion technique of Conway, which is considered a low precision and low sensitivity technique and may lead to significant loss of ammonia during the process resulting in an underestimate ammonia value (Pesce & Kaplan, 1987). On the other hand, this study used commercial diagnostic kits based on enzymatic procedures which are very accurate, the same technique used by Kitamura et al. (2010a), which obtained data similar to the present study.

Blood L-lactate increased gradually, being at tremors (8.9 \pm 0.80 mmol L⁻¹) significantly different ($p < 0.01$) from the beginning (1.7 \pm 0.25 mmol L⁻¹). At the convulsion, the values of L-lactate were 23.8 \pm 0.69 mmol L⁻¹, but not being significantly different from the 180th min with 26.0 \pm 1.36 mmol L⁻¹.

The L-lactate was a high indicative of the harmful effects of ammonia in the Krebs cycle. When there is an excessive amount of ammonia, it combines with α -cetoglutarate and there was no formation of succinate and consequently no production of ATP (Haliburton & Morgan, 1989). The lack of ATP leads the body to obtain energy by anaerobic way which results in the production of L-lactate. This fact was demonstrated by the high correlation ($r=0.86$) (Figure 1A) between the blood ammonia concentration and the blood L-lactate levels. The maximum L-lactate values (26 mmol L⁻¹) were twice as high as the values obtained in experimental cases of rumen lactic acidosis (Maruta & Ortolani, 2002), which produces a pronounced metabolic acidosis. Kitamura et al. (2010a) corroborates with these findings, with slightly lower mean values for L-lactate.

The results of blood pH showed a drastic decrease from the 90th min (7.42 \pm 0.03) to the convulsion (7.27 \pm 0.01), staying at this lower level at 180th min (7.24 \pm 0.02). The blood bicarbonate reached its highest value at the 30th min (30.2 \pm 0.5) and, similar to blood pH, begun to fall from the 90th min (22.8 \pm 1.1) until reaching its lowest values at convulsion and the 180th min with 13.7 \pm 0.8 mmol L⁻¹. The blood pCO₂ decreased from the 30th min to tremors (45.6 \pm 0.6 to 38.7 \pm 1.1 mm Hg), reaching its lowest values at convulsion (32.6 \pm 1.2 mm

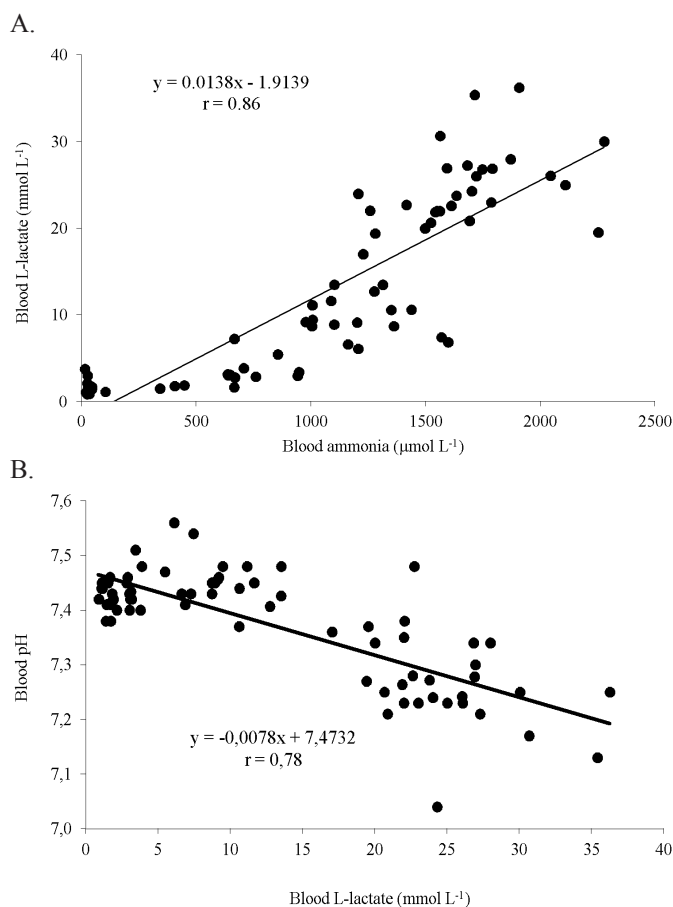


Figure 1. Correlation between blood ammonia and blood L-lactate concentrations, and between blood L-lactate concentration and blood pH in ammonia-poisoned steers

Figura 1. Correlação entre amônia sanguínea e Lactato-L sanguíneo e entre Lactato-L sanguíneo e pH do sangue em garrotes intoxicados por amônia

Hg). The base-excess value decreased from 4.5 \pm 0.2 mmol L⁻¹ in the beginning of the trials to -1.1 \pm 2.1 mmol L⁻¹ at the 90th min, reaching the lowest values at 180th min with -12.5 \pm 1.3 mmol L⁻¹. There was a high positive correlation between blood ammonia concentration and blood L-lactate ($r=0.86$) (Figure 1A), and a high negative correlation between blood L-lactate and blood pH ($r=-0.75$) (Figure 1B), blood bicarbonate ($r=-0.92$), and base excess ($r=-0.92$).

So the inhibition of the Krebs cycle by ammonia and the consequent production of L-lactate was the main cause of the metabolic acidosis confirmed by low blood pH, bicarbonate,

and base excess (Table 1). This fact occurred because lactic acid dissociates into H^+ and L-lactate, and the blood buffers tries unsuccessfully to compensate the accumulation of H^+ in the blood. In extreme situations of severe metabolic acidosis due to ammonia poisoning, the correction of the acidosis with bicarbonate is highly recommended.

This metabolic origin acidosis disagrees with Rash et al. (1968) who stated that this acidosis was mainly of respiratory origin. The ammonia released through the lungs may result in a lung edema, but its functionality was hardly affected, as seen in the pCO_2 values which decreased through the experiment (Table 1), showing that the organism was trying to compensate the metabolic acidosis through the respiratory system. According to Antonelli & Ortolani (2004), the organism also tries to compensate the metabolic acidosis with exchange of H^+ ions in the blood by intracellular potassium, leading to hyperkalemia status, which is suspected to contribute to an animal's death through cardiac arrest.

CONCLUSIONS

Based on these results, it was established that acute ammonia poisoning provokes definite changes in the blood acid-base status, and the metabolic acidosis must be controlled with the administration of buffers.

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