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Original article

Hypertonic saline solution (NaCl 7.2%) enhances renal excretion of acids in cattle with acute ruminal lactic acidosis

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Abstract

The present study aimed to evaluate the efficiency of hypertonic saline solution (HSS) as a novel treatment of acute ruminal lactic acidosis (ARLA) in cattle, focusing on urinary excretion of acids. Twelve cannulated steers were submitted to experimentally induced ARLA by administering sucrose into the rumen. Twenty hours later, the cattle were randomly divided into two equal groups. The first group was treated with 7.5% HSS (5 mL/kg) over 15 min, and isotonic saline solution (ISS; 20 mL/kg) for the subsequent 165 minutes. The control group was administered ISS instead of HSS. Rumen and urine samples were collected at different times during the experiment from the baseline to 64 h post-induction. The induction caused a medium-to-moderate ruminal acidosis, and a moderate degree of systemic acidosis and dehydration. Steers treated with HSS increased by 50% its glomerular filtration rate (1.61 mL/min) compared to ISS group (1.06 mL/min; $p < 0.03$). The overall volume of urine excreted by HSS group was higher than that in ISS group (1.62 L vs 0.7 L; $p < 0.02$). This increase in total volume of urine provided by HSS favored a greater excretion of H⁺ ions in urine, which was 3.39-fold higher in HSS group ($64.3 \cdot 10^{-7}$ vs $18.9 \cdot 10^{-7}$ Mol) as well as lactate (241.7 vs 181.8 mMol) and P urinary excretion (3.8 vs 1.1 mMol) that reduced the urine pH (5.3 vs 5.7). Only the HSS group decreased significantly blood total lactic acid concentration (20.3 %) throughout the treatment. A positive relationship was found between the excretion of urinary phosphorus and urinary pH ($r^2 = 0.562$). The results showed that this novel treatment with HSS enhanced renal excretion of acids and may be recommended as an additional treatment for cattle with lactic acidosis.

Key words: steers, ruminal lactic acidosis, hypertonic saline solution

Introduction

Acute ruminal lactic acidosis (ARLA) is a metabolic disorder caused by the sudden ingestion of a high-carbohydrate diet, especially when the ruminal environment is not fully adapted (Owens et al. 1998, Oliveira et al. 2016). It mainly affects confined beef cattle, but can also affect dairy cows and other ruminants inadequately subjected to diets rich in hyperglycemic foods, representing an important disease in ruminants production (Owens et al. 1998, Barrêto Júnior et al. 2008, Oliveira et al. 2015a, Stefańska et al. 2017).

The treatment of ARLA involves correcting the systemic metabolic acidosis and dehydration. Acidemia is corrected with buffer solutions, such as sodium bicarbonate and lactate. Due to the migration of water into the acidotic ruminants causing marked dehydration, the success of the treatment depends on the correction of dehydration (Huber 1971, Ortolani et al. 2010, Rodrigues et al. 2012, Oliveira et al. 2015b).

Hypertonic saline solution (HSS; NaCl 7.2-7.5%) has proved to be an interesting therapeutic tool for experimental hemorrhagic shock since 1917 (Friedman et al. 2008) and has been indicated for the treatment of several types of shock and in several states of dehydration (Oliveira et al. 2002). Thus, this hypertonic solution containing 7.2% sodium chloride (2,400 mOsm/L) functions by rapidly replenishing the plasma volume by means of a "loan" of water from the intracellular space and gastrointestinal tract. This treatment has several hemodynamic benefits, including a rapid expansion of the plasma volume, temporary vasodilatation, an improvement in the blood pressure, and an increase in the glomerular filtration rate, thus resulting in greater urine excretion (Friedman et al. 2008, Rodrigues et al. 2012). Although there are studies supporting the use of HSS in the correction of severe dehydration states due to diarrhea, and hemorrhagic and endotoxic shock (Constable 1999), no study has evaluated the possibility of using HSS in the treatment of acidosis in cattle, especially with respect to urine excretion and elimination of acid ions in this fluid, which would aid in the correction of metabolic acidosis.

Thus, the present study aims to compare the efficacy of HSS and isotonic saline solution (ISS) in acute rumen lactic acidosis, emphasizing the ability of the rumen to absorb fluid and the subsequent volume recovery, systemic acidosis, global production of urine, glomerular filtration rate, and the excretion of H⁺, lactate, and phosphate ions in this fluid.

Materials and Methods

All procedures and animal handling followed the ethical principles in animal experimentation, adopted by the present study was approved by the Ethics Committee on Animal Research of the Faculty of Veterinary Medicine and Zootechnics, University of São Paulo.

Animals and feed

Twelve 12-months-old healthy steers, weighing 253±12 kg were used. The animals were mixed breed from taurine beef cattle. Two months before the start of the experimental period, all the animals received ivermectin-based endectocides and clostridial vaccines. The ruminants subsequently underwent a surgery for the placement of latex rumen cannula. After the surgical procedure, there was a period of adaptation to the facilities and the new alimentary management.

The animals were kept in a tie-stall system and were administered a total feed composed of 65% of the dry matter (DM) of Coast-cross grass hay (*Cynodon dactylon*) and 35% of the DM of a commercial concentrated feed with 14% crude protein. The amount of DM in the total feed offered to the animals was calculated in relation to 2.7% of the individual live weight and was corrected during the test.

The ruminants had free access to water and received 35 g of a mineral supplement (Fosbovi 20®, Tortuga Cia Zootécnica Agrária, Mairinque, SP, Brazil) added over the total ration. The total ration (hay + concentrate) used was composed by 87.1% DM, 25.9% crude fiber, 9.5% crude protein, 1.9% ethereal extract, 9.8% mineral matter, and 53.0% of non-nitrogenous extract.

Experimental design

All the animals were submitted to experimentally induced ARLA according to the rumen sucrose administration protocol (Ortolani 1995) with reduction of 15% of total sucrose amount. In average 3.2 kg of sucrose were used in each animal diluted in 5 L of water. Twenty-four hours after induction with sucrose, lactic acidosis was considered established, and medical treatment of the animals was initiated; the animals were divided into two groups: the control group treated with isotonic saline solution (ISS) and the treatment group treated with HSS.

Urine and ruminal contents were collected during the test, at the following time points: basal (M0), immediately before the induction of ARLA; 20 hours after the induction of ARLA (M20h), the critical moment of the ARLA; 30 minutes after the initiation of treatment (M30'); 60 minutes after the initiation of

treatment (M60'); 120 minutes after the initiation of treatment (M120'); 180 minutes after the initiation of treatment (M180'); 24 hours after M20h (M44h); and 48 hours after M20h (M68h).

Treatment protocol

At M20h, five liters of the ruminal contents were removed and the same volume of water was added. The animals were subsequently administered 5 mL/kg of live weight of HSS (NaCl 7.2%) via intravascular jugular cannula infusion (HSS group) and the same volume of ISS (NaCl 0.9%) was administered for the ISS control group; the fluids were administered over 15 minutes, followed by a 20 mL/kg P.V. infusion of isotonic saline solution over three hours in both the groups. To complete the total infusion of HSS or ISS during the initial 15 minutes, a pressurizing pouch was used. After this period, to correct systemic acidosis, the animals were administered isotonic sodium bicarbonate solution (NaHCO_3 , 1.3%), based on the blood gas results. Only steers that still had low blood pH received additional treatment, but not all. The data collection ended before bicarbonate administration.

Collection and processing of the ruminal content and urine samples

The ruminal content samples were collected as mentioned above, using a plastic hose inserted into the rumen via the ruminal fistula. Approximately 300 mL of ruminal contents were collected consistently from the ventral sac of the organ, using a vacuum pump.

To determine the total volume of urine eliminated by each animal during the experiment, shortly before the start of the treatments (M20h), plastic probes were coupled with bovine foreskins, which in turn were attached to plastic containers with a capacity of 2 L. The probes were kept in place throughout the course of the treatment, and the total volumes of urine eliminated during the different time periods were collected and measured in a 1 L-glass beaker.

The urine samples obtained during each experimental period were stored in a sterile universal container and kept under refrigeration until they were processed. The urine aliquots were centrifuged at $1400 \times g$ for 15 minutes and maintained at -20°C for further analysis. The urine samples at M30', M60', M120', and M180' were collected directly from the probe while the urine samples at M0, M20h, M44h, and M68h were collected directly from the foreskin by massage.

Laboratory analysis

The pH values of the ruminal content and urine

samples were checked immediately after sample collection. If the animals urinated more than once during one time period, an aliquot of the same was collected and the urinary pH was measured immediately thereafter. In such cases, the mean values were considered.

To determine the total lactic acid concentration, a colorimetric technique was used (Pryce 1969). Osmolality was measured using a freezing point osmometer (The Advanced Micro-Osmometer 3300, Advanced™ Instruments, Norwood, USA). To determine the concentration of creatinine and phosphorus in the urine, an automatic biochemical analyzer (Rx Daytona, Randox Laboratories Ltd., Crumlin, UK) was used along with the commercial kits of the same brand.

Excretion calculations

The estimated molar amount of H^+ ions excreted in the urine was calculated throughout the course of the treatment according to the following formula: $[\text{H}^+] \text{ (mol)} = (1/10 \text{ pHM}) \times \text{VM (L)}$; where $[\text{H}^+] =$ molar quantity of H^+ ions excreted during the time period; pHM = urinary pH value for the time period; VM = urine volume during the time period (in liters). The glomerular filtration rate (GFR) was calculated according to the classical formula considering the whole treatment period (M30' to M180') (Roeder et al. 1997).

Statistical analysis

All data distributions were tested using the Kolmogorov-Smirnov test. The data that had a normal distribution were initially evaluated using the analysis of variance (F test) and if significant, were compared using the Student's t-test (for comparison between the two groups) and the paired t-test (for comparison between the different time periods within the same group). Data with non-normal distributions were evaluated using the Mann Whitney test and expressed in terms of medians for comparison between the groups. Regression analysis and their respective determination coefficients (r^2) were used to verify the relationships between the variable pairs. The level of significance was set at $p < 0.05$.

Results

Table 1 shows the data from rumen and urine pH and serum creatinine. The pH values of the ruminal contents were similar in both the groups; the pH decreased to 4.4 at M20h and returned to the baseline values at M44h. The lactic acid concentration peaked at M20h (87.4 and 89.4 mmol/L for the ISS and HSS

Table 1. Mean values and standard deviations (SD) of the cattle urine pH from the isotonic saline solution (ISS) and hypertonic saline solution (HSS) groups during the study.

Time points	Urine pH (Mean ± SD)				Rumen content pH (Mean ± SD)				Serum creatinine (Mean ± SD)			
	ISS		HSS		ISS		HSS		ISS		HSS	
M0	5.8	0.3	6.1	1.1	6.73 ^A	0.19	6.77 ^A	0.13	133	25	130	26
M20H	5.6	0.2	5.4	0.1	4.42 ^B	0.23	4.41 ^B	0.12	132	26	124	30
M30	5.5	0.1	5.5	0.1	4.44 ^B	0.25	4.49 ^B	0.15	127	43	105	16
M60	5.7	0.1	5.4	0.1	4.54 ^B	0.28	4.50 ^B	0.18	124	24	119	27
M120	5.7 ^a	0.1	5.3 ^b	0.1	4.61 ^B	0.35	4.51 ^B	0.11	128	19	118	29
M180	5.6 ^a	0.1	5.4 ^b	0.1	4.64 ^B	0.32	4.87 ^B	0.63	113	25	105	18
M44H	5.8	0.2	5.7	0.2	6.49 ^A	0.67	6.54 ^A	0.40	118	18	108	14
M68H	5.7	0.3	5.5	0.3	6.47 ^A	0.17	6.63 ^A	0.27	120	17	123	25
P*	0.660		0.200		0.001		0.001		0.811		0.490	

Different lower-case letters in the same line indicate a significant difference between groups. ($p < 0.05$).

* Comparison between time points within each group. Different capital letter in the same column denotes difference between time points.

Table 2. Median total excreted urine volume and mean values and standard deviations of the glomerular filtration rate (mL/min) of the isotonic saline solution (ISS) and hypertonic saline solution (HSS) groups during the treatment period.

Groups	Glomerular filtration rate (mL/min)		Urine volume excreted (mL)
	Mean	SD	Median (range)
ISS	1.06 ^B	0.31	712 ^A (0–2450 mL)
HSS	1.61 ^A	0.35	1620 ^B (1110–6485 mL)
P	0.035		0.025

Different capital letters in the same column present a significant difference ($p < 0.05$).

Table 3. Median molar amount of H⁺ (mol), lactate (mmol), and phosphate (mmol) ions removed throughout the treatment (M30' to M180') in the isotonic saline solution (ISS) and hypertonic saline solution (HSS) groups

Groups	Molar amount of H ⁺ ions excreted in the urine (mol)	Molar amount of lactate excreted in urine (mmol)	Molar amount of phosphorus excreted in urine (mmol)
ISS	18.97 × 10 ⁻⁷ ^B	181.8 ^B	1.07 ^B
HSS	64.30 × 10 ⁻⁷ ^A	241.7 ^A	3.80 ^A
P	0.028	0.025	0.010

Different upper-case letters in the same column represent a significant difference on the Mann-Whitney test ($p < 0.05$).

groups, respectively) and maintained high values at M60', M120', and M180' ($p < 0.001$) before returning to the baseline values at M44h. Only the HSS group decreased significantly blood total lactic acid concentration (20.3%) throughout the treatment ($p < 0.05$).

Ruminal osmolality was measured for both the groups at M20h (336 and 343 mOsm/L for ISS and HSS, respectively). A subsequent decrease in these values was noted at M30', which was more prominent in

the HSS group due to a significant difference ($p < 0.05$) compared to other time periods (M60', M120', and M180'), which was not observed in the ISS group. The serum creatinine remained during the entire study within reference values for the species and no differences between groups or time points was found for this metabolite.

The urine pH presented difference ($p < 0.05$) between the groups at M120' (pH being 5.7 and 5.3 for

ISS and HSS, respectively) and at M180' (pH being 5.6 and 5.4 for ISS and HSS, respectively). There was no difference in the urinary pH over the course of the experiment for the ISS group; however, urinary pH decreased ($p < 0.05$) at M120' and M180' in the HSS group.

Table 2 shows the total volume of urine excreted and the glomerular filtration rate for the ISS and HSS groups over the course of the treatment period (M30' to M180').

Table 3 shows the median values of the total amount of H^+ , lactate, and phosphorus ions excreted in the urine throughout the treatment period (M20h to M180').

A significant correlation was present between the urinary pH values and the total lactic acid concentration in the bovine rumen ($r^2 = 0.9127$), and between the urinary pH values and the urinary phosphorus concentrations ($r^2 = 0.5629$).

Discussion

The experimental model was able to induce a medium-to-moderate ruminal acidosis, attaining a pH between 4.2 and 4.8, a ruminal lactic acid concentration of 55 to 100 mmol/L, and a moderate systemic metabolic acidosis between M20h and M180' with an average pH of 7.24. The beneficial effects of HSS on plasma volume expansion, correction of dehydration through the passage of water from the rumen, and inter-cellular spaces achieved in this study was demonstrated in a previous contribution (Rodrigues et al. 2012). A rapid intravenous infusion of a large volume of HSS did not cause any side effects or discomfort for the animals.

The mean ruminal pH at the critical moments of the present experiment was approximately 4.5; in such an environment, a large part of the acid was dissociated. The administration of water into the rumen did not alter the pH of the contents; it only diluted it.

The infusion of HSS caused an increased total excretion of lactic acid in the urine, probably because the excreted urine volume was higher in these animals. Dehydrated animals usually conserve water by excreting less fluid in the urine (Radostits et al. 2000, Barrêto Júnior et al. 2008). The mechanisms used to reduce urine excretion involve decreased blood flow through the renal arteries, with a concomitant reduction in glomerular filtration rate, and increased tubular reabsorption of water (Ortolani et al. 2008, Klein 2013).

The kidneys are the main routes of excretion of H^+ ions in a healthy ruminant; although H^+ ions are excret-

ed in the urine of a dehydrated animal with systemic acidosis, an increase in the urinary volume would increase the global excretion of H^+ ions and relieve the metabolic acidosis (Roeder et al. 1997). It was found that the amount of H^+ excreted by the HSS group was approximately 3.4 times higher than that excreted by the ISS group, proving the hypothesis raised. The higher excretion of H^+ ions resulted not only from the increase in the urinary volume, but also from the lower urinary pH of the HSS group; this lower pH was due to the higher concentration of phosphate ions in the urine of these animals; there was a positive relation ($r^2 = 0.562$) between the excretion of phosphate ions in the urine and the urinary pH.

The data from serum and urine creatinine are subjected to a wide fluctuation in a clinical study due to solution infusion and the frequency of urination and urine volume, which varies among the animals. The glomerular filtration rate, that involves both urinary and serum creatinine and the urine volume, is a more robust variable to evaluate the efficacy of the tested solutions (Kaneko et al. 2008). The glomerular filtration rate of HSS-treated sows increased by approximately 50% as compared to the ISS group. This might have been the result of a better recovery of hypovolemia caused by increased passage of water from the rumen and extracellular spaces into the blood by expanding the plasma volume and more effectively unblocking the mechanism of reduction in the blood circulation of the renal parenchyma (Roeder et al. 1997, Constable 1999). Intravenous infusion of HSS cause an immediate vasodilation of the renal artery, which in turn causes an increase in the overall volume of urine excreted by HSS-treated animals (Velasco et al. 1980, Ortolani et al. 2008).

HSS treatment also allowed the animals to excrete about 3.5 times more lactate ions in their urine than those undergoing ISS therapy. D-lactate represents more than 90% of the total lactate excreted in the urine of cattle with similar acidosis (Ortolani et al. 2008) and a very significant portion of the L-lactate filtered by the glomeruli is reabsorbed by the renal tubules and subsequently converted to pyruvate (Dunlop and Hammond 2006). D-lactate is recognized for its deleterious effects on the body, especially its strong influence on the central nervous system, and is responsible for symptoms such as depression in the animal's consciousness and lethargy, observed in ARLA (Ewaschuk et al. 2005). It is therefore possible that HSS treatment plays an important role in the clearance of D-lactate, an isomer that causes depression in the state of consciousness and lethargy.

Conclusions

Treatment with HSS did not cause any side effects. This therapy provided a greater excretion of urinary volume and consequently, a greater elimination of H⁺, lactate, and phosphate ions. The glomerular filtration rate was significantly increased. The overall results indicate that treatment of ARLA with HSS is advantageous and appropriate, compared to ISS alone.

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