



Experimental protocol for metabolic acidosis induction by intravenous administration of hydrochloric acid in sheep

[*Protocolo de indução experimental de acidose metabólica por administração intravenosa de ácido clorídrico em ovinos*]

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ABSTRACT

The aim of this study was to assess the magnitude and duration of blood and urine changes and the side effects of hyperchloremic acidosis induced by the intravenous administration of hydrochloric acid in sheep. Five healthy, crossbred adult ewes, with a mean body weight of 44 ± 2.9 kg were used. The hydrochloric acid solution was administered intravenously at a rate of 25 mL/kg/h for 4 hours continuously. Venous blood and urine samples were collected and pH values, blood carbon dioxide partial pressure, bicarbonate, base excess, strong ion difference, anion gap, total concentration of nonvolatile buffers, creatinine, plasma L-lactate, plasma and urine sodium, potassium, and chloride were determined. The experimental protocol induced severe hyperchloremic acidosis at the end of the infusion, with a decreased plasma strong ion difference. The fractional excretion of sodium and chloride remained increased during 4 hours after the infusion. Aciduria was observed at approximately 24 hours. Twenty-four hours after the infusion, the animals showed mild and compensated metabolic acidosis. This protocol was effective in inducing severe and long-lasting hyperchloremic acidosis and did not cause serious side effects. Therefore, this protocol can be used safely in adult sheep for studies on the treatment of this condition.

Keywords: acid-base balance, hyperchloremic acidosis, ruminant, strong ion acidosis

RESUMO

O objetivo deste estudo foi avaliar a magnitude e a duração das alterações sanguíneas e urinárias, bem como os efeitos colaterais da acidose hiperclorêmica induzida por administração intravenosa de ácido clorídrico, em ovinos. Foram utilizadas cinco ovelhas mestiças, adultas, sadias, com peso médio de $44 \pm 2,9$ kg. A solução de ácido clorídrico foi administrada por via intravenosa, na velocidade de 25 mL/kg/h, totalizando quatro horas de administração contínua. Amostras de sangue venoso e de urina foram colhidas, e determinaram-se os valores de pH, pressão parcial de dióxido de carbono, bicarbonato, excesso de bases, diferença dos íons fortes, ânion-gap, creatinina, lactato L, sódio, potássio e cloro. O protocolo de indução experimental foi capaz de induzir acidose hiperclorêmica grave ao término da infusão, com diminuição da diferença dos íons fortes plasmáticos. Houve aumento da excreção fracionada de sódio e cloro por até quatro horas após o término da infusão. A acidúria foi observada por cerca de 24 horas. Após 24 horas do início da infusão, os animais apresentaram acidose metabólica leve e compensada. Esse protocolo foi eficaz na indução da acidose hiperclorêmica grave e duradoura e não causou efeitos colaterais. Conclui-se que o protocolo pode ser usado com segurança em ovelhas adultas, para estudos sobre tratamento dessa condição.

Palavras-chave: equilíbrio ácido-base, acidose hiperclorêmica, ruminante, acidose por íons fortes

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INTRODUCTION

Metabolic acidosis is an acid-base imbalance associated with several diseases in ruminants and can be divided into organic acidosis caused by the accumulation of organic anions, such as lactic acid (acidosis with an increased anion gap), and hyperchloremic acidosis caused by electrolyte imbalances (with a normal anion gap) (Morais and Constable, 2012).

Hyperchloremic acidosis may be caused by diarrhea, renal tubular acidosis, administering electrolyte solutions containing a chloride concentration equal to or greater than that of sodium, and by the intake of anionic diets (Berchtold, 2009; Jones *et al.*, 2009). Because this disorder is consequential to a primary electrolyte imbalance, the pathophysiologic mechanism can only be understood when using the nontraditional or physicochemical approach to acid-base equilibrium, proposed by Stewart (Stewart, 1983), for the interpretation of results. The strong ion difference (SID) in plasma is the metabolic component of acid-base balance and must be accepted as essential information to understand this imbalance (DiBartola, 2012).

Experimental models reproducing hyperchloremic acidosis have been studied in ponies (Gossett *et al.*, 1990), horses (Romão *et al.*, 2017), and calves (Abeysekara *et al.*, 2007; Gentile *et al.*, 2008; Schwedhelm *et al.*, 2013) and consisted of the intravenous administration of hydrochloric acid (HCl) to induce metabolic acidosis. These authors used different protocols for induction; thus, different results were observed, particularly regarding the severity of the imbalance. Studies of metabolic acidosis induction have application, particularly for determining the effectiveness of therapeutic choices for this imbalance.

No information is available on the induction of hyperchloremic acidosis in ruminant species because the studies have focused on newborn calves. Moreover, in all of these studies, the animals were only monitored for a few hours after the induction, which is less time than that required for the spontaneous correction of the imbalance. The renal excretion of electrolytes, recognized as the essential mechanism for correction, was also not shown by these

researchers, excepting the study in horses (Romão *et al.*, 2017).

The aim of this study was to evaluate a protocol for the induction of hyperchloremic metabolic acidosis by the intravenous administration of HCl (100mmol/L) in healthy adult ewes and verify the magnitude and duration of blood and urine changes and the present side effects.

MATERIAL AND METHODS

The project was approved by the Animal Care and Use Committee of Universidade Estadual de Londrina (CEUA/UEL- process n. 4798.2012.64). Five crossbred healthy ewes, 3 to 6 years old, not pregnant nor lactating, with 44 ± 2.9 kg of BW, were used. These animals belonged to the flock of the Veterinary Teaching Hospital of the Universidade Estadual de Londrina and were maintained in a Coast-cross (*Cynodon dactylon*) paddock and supplemented with sorghum silage once daily.

Metabolic acidosis was induced with a solution containing 100mmol/L of HCl and 152.5mmol/L NaCl, with a pH of 1.70 and a calculated osmolarity of 505mOsm/L (152.5mmol/L Na⁺ and 252.5mmol/L Cl⁻). The solution was prepared by adding 8.3mL of HCl (hydrochloric acid 37%; Cinética Reagents and Solutions) to 991.7mL of a commercial isotonic saline solution (0.9% sodium chloride; JP Indústria Farmacêutica).

The solution was infused intravenously through an 18G catheter attached to the right jugular vein. The volume administered was equivalent to approximately 10% of the ewe's BW, and the rate of infusion was maintained at 25mL/kg/h. A total of 4L was infused in 4 hours of continuous administration. During the infusion, the ewes were restrained in a standing position and afterwards remained in a stall with free access to water, and Coast-cross hay, receiving sorghum silage once a day.

Blood samples were obtained from the jugular catheter at the following times: 0h (beginning), 2h (middle), 4h (end of infusion), 6h, 8h, 10h, 12h, and 24 hours after the start of the infusion. Urine samples were collected 0h, 2h, 4h, 8h, and 12 hours after the start of the infusion. Preferably, the urine samples were obtained by

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spontaneous urination. When spontaneous urination did not occur, micturition was induced by momentary asphyxia (short obstruction of the nostrils and preventing the mouth from opening).

Physical examinations, including measurements of rectal temperature, heart rate, respiratory rate and ruminal movements, and evaluation of mucosal color, attitude, behavior and appetite were performed before starting the infusion, every two hours for 12 hours, and on the following day (24 hours). The animals were observed for behavioral changes or other clinical signs for three consecutive days.

Blood samples for biochemical evaluations were taken using tubes containing sodium fluoride (Fluoreto + K₃ EDTA, Labor Import). The fluoride plasma was obtained by centrifugation (1,100g for 10min) immediately after collection. Plasma intended for L-lactate and creatinine analysis was maintained frozen (-20°C) until processing, within 5 months. The concentration of plasma L-lactate was determined by enzymatic colorimetric method using a specific commercial reagent (Lactic Acid, Dimension Clinical Chemistry System, Dade Behring; Siemens), while the creatinine in plasma was measured by kinetic method (Creatine, Dimension Clinical Chemistry System, Dade Behring; Siemens).

Samples intended for blood gas analyses were taken in anaerobic conditions using 3mL heparinized plastic syringes and processed immediately after collection. Venous blood pH, partial pressure of carbon dioxide (pCO₂), bicarbonate (HCO₃⁻), base excess (BE), sodium (Na⁺), potassium (K⁺), and chloride (Cl⁻) were measured in a blood gas analyzer (Omni C; Roche).

Urine pH was determined with an electronic potentiometer (pHmeter Tec-2, Tecnal) from freshly collected samples and was done immediately after collection. The urine was then frozen (- 20°C) until the time of processing, within 5 months. Urinary concentrations of Na⁺, K⁺ and Cl⁻ were measured by ion-selective electrodes (Quik Lyte Na⁺, K⁺, Cl⁻, Dimension

Clinical Chemistry System, Dade Behring; Siemens).

The following variables were calculated using the corresponding formulas:

- Anion Gap (AG) (Berchtold, 2009):

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

- Strong Ion Difference (SID) (Constable *et al.*, 2005)

$$SID = (Na^+ + K^+) - (Cl^-)$$

- Fractional clearance of electrolytes (Garry *et al.*, 1990), where (a) is the excreted substance

$$FC(a) = \frac{[\text{urinary concentration of (a)} \times \text{plasma creatinine}]}{[\text{plasma concentration of (a)} \times \text{urine creatinine}]} \times 100$$

Data were expressed as means \pm standard deviations (SD), and a *P* value < 0.05 was considered significant. One-way repeated measures ANOVA was used to test the effect of the HCl solution on the electrolyte and acid-base balances. When the *F* statistic was significant, multiple pairwise comparisons were conducted using Tukey's test. A statistical software program was used for all analyses (SigmaStat for Windows 3.1).

RESULTS

Decreases in blood pH (*P* < 0.001), HCO₃⁻ (*P* < 0.001), and BE (*P* < 0.001) were evident during the infusion of HCl solution, and the lowest values were observed at the end of the infusion. In the following hours these values increased gradually (Table 1). The decrease in pCO₂ (*P* < 0.001) was observed from the middle of the infusion time onward, remaining low during the observational period.

Plasma concentrations of Na⁺ (*P* < 0.001) and Cl⁻ (*P* < 0.001) increased in the middle of the infusion, intensified at the end, and remained high, returning to baseline values on the following day (Table 2). The K⁺ levels, however, did not change (*P* = 0.113). The SID value decreased during the infusion (*P* = 0.004) and remained low for 4 hours after the end of the infusion.

Table 1. Mean \pm SD values for venous blood pH, carbon dioxide partial pressure (pCO₂), bicarbonate (HCO₃⁻) and base excess (BE) in healthy ewes that received an intravenous infusion of an HCl solution (100mmol/L) at a volume equivalent to 10% of its body weight: before (0h), in the middle (2h), and at the end of the infusion (4h) and 6, 8, 10, 12 and 24 hours from the start of the infusion

Hour	pH ^f	pCO ₂ ^f (mmHg)	HCO ₃ ^{-f} (mmol/L)	BE ^f (mmol/L)
0	7.417 ^a \pm 0.06	39.78 ^a \pm 4.9	24.96 ^a \pm 1.71	0.42 ^a \pm 2.33
2	7.260 ^d \pm 0.02	31.44 ^b \pm 1.97	13.82 ^d \pm 1.2	-13.22 ^d \pm 1.39
4	7.176 ^e \pm 0.05	30.04 ^b \pm 2.38	10.86 ^e \pm 0.39	-17.60 ^e \pm 1.12
6	7.297 ^{cd} \pm 0.02	28.60 ^b \pm 3.16	13.64 ^d \pm 1.20	-12.82 ^d \pm 1.21
8	7.324 ^{bcd} \pm 0.02	28.84 ^b \pm 2.74	14.62 ^{cd} \pm 1.22	-11.40 ^{cd} \pm 1.24
10	7.320 ^{bcd} \pm 0.02	29.18 ^b \pm 2.23	14.68 ^{cd} \pm 0.30	-11.42 ^{cd} \pm 0.35
12	7.351 ^{abc} \pm 0.04	30.32 ^b \pm 2.64	16.36 ^c \pm 0.79	-9.22 ^c \pm 1.21
24	7.387 ^{ab} \pm 0.04	33.30 ^b \pm 3.76	19.62 ^b \pm 2.37	-5.40 ^b \pm 2.79

^{a-e} Within a variable, the different superscripts indicate that the values differ significantly between times (^fP < 0.001; repeated measures ANOVA).

Table 2. Mean \pm SD values for plasma concentrations of sodium (Na⁺), potassium (K⁺), and chloride (Cl⁻) and the strong ion difference (SID) in healthy ewes that received an intravenous infusion of an HCl solution (100mmol/L) at a volume equivalent to 10% of its body weight: before (0h), in the middle (2h), and at the end of the infusion (4h) and 6, 8, 10, 12 and 24 hours from the start of the infusion

Hour	Na ^{+d} (mmol/L)	K ⁺ (mmol/L)	Cl ^{-d} (mmol/L)	SID ^e (mmol/L)
0	147.70 ^c \pm 2.66	3.95 ^a \pm 0.20	111.42 ^c \pm 4.68	40.23 ^a \pm 2.49
2	152.54 ^{ab} \pm 1.36	3.55 ^a \pm 0.34	121.22 ^{ab} \pm 3.45	34.87 ^{ab} \pm 3.80
4	155.24 ^a \pm 1.62	4.02 ^a \pm 0.35	127.88 ^a \pm 5.32	31.37 ^b \pm 4.52
6	153.84 ^a \pm 2.31	3.81 ^a \pm 0.25	125.32 ^a \pm 7.95	32.34 ^b \pm 7.19
8	153.46 ^a \pm 1.73	3.97 ^a \pm 0.28	126.54 ^a \pm 6.36	30.89 ^b \pm 5.60
10	153.72 ^a \pm 2.01	4.02 ^a \pm 0.35	124.06 ^a \pm 3.78	33.68 ^{ab} \pm 4.98
12	153.86 ^a \pm 1.47	3.87 ^a \pm 0.26	122.30 ^{ab} \pm 2.27	35.43 ^{ab} \pm 2.81
24	149.80 ^{bc} \pm 1.59	4.09 ^a \pm 0.80	115.34 ^{bc} \pm 1.94	38.56 ^{ab} \pm 1.22

^{a-c} Within a variable, the different superscripts indicate that the values differ significantly between times (^dP < 0.001, ^eP = 0.004; repeated measures ANOVA).

The AG did not change (P= 0.334) with the infusion, but a reduction in L-lactate concentration (P < 0.001) was evident (Table 3). Acidification of the urine (P < 0.001) was observed from the middle of the infusion time onward, and the urine pH remained low until the next day (Table 4). Na⁺ FC (P < 0.001), and Cl⁻ FC (P < 0.001) increased during the infusion and reached a maximum value at the end, decreasing gradually and returning to baseline values 8 hours after the end of the infusion. K⁺ FC did not differ, although it showed a trend for increase (P= 0.058).

All ewes showed some degree of somnolence in the last hour of HCl solution administration. No other clinical changes were observed during the infusion or the three subsequent follow-up days. Heart rate (P= 0.173), respiratory rate (P= 0.798), and rectal temperature (P= 0.187) did not change during and after the infusion of HCl solution, but the ruminal movements changed slightly (P= 0.016) (Table 5). The induced imbalances were not followed by depression or any other behavioral and attitude changes. Immediately after the infusion, the ewes were released to a stall and showed good appetite.

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Table 3. Mean \pm SD values for plasma anion gap (AG) and L-lactate concentrations in healthy ewes that received an intravenous infusion of an HCl solution (100mmol/L) at a volume equivalent to 10% of its body weight: before (0h), in the middle (2h), and at the end of the infusion (4h) and 6, 8, 10, 12 and 24 hours from the start of the infusion

Hour	AG (mmol/L)	L-lactate ^e (mmol/L)
0	15.27 ^a \pm 1.64	2.92 ^a \pm 0.41
2	21.05 ^a \pm 3.14	0.44 ^b \pm 0.33
4	20.52 ^a \pm 4.57	0.26 ^b \pm 0.089
6	18.69 ^a \pm 8.03	0.30 ^b \pm 0.071
8	16.27 ^a \pm 6.40	0.48 ^b \pm 0.20
10	19.00 ^a \pm 4.84	0.62 ^b \pm 0.83
12	19.07 ^a \pm 3.02	0.50 ^b \pm 0.21
24	18.93 ^a \pm 1.62	1.04 ^b \pm 1.15

^{a-d} Within a variable, the different superscripts indicate that the values differ significantly between times (^e P< 0.001; repeated measures ANOVA).

Table 4. Mean \pm SD values for urine pH and fractional clearance (FC) of Na⁺, K⁺ and Cl⁻ in healthy ewes that received an intravenous infusion of an HCl solution (100mmol/L) at a volume equivalent to 10% of its body weight: before (0h), in the middle (2h) and at the end of the infusion (4h) and 8, 12 and 24 hours from the start of the infusion

Hour	pH ^d	FC Na ⁺ ^d (%)	FC K ⁺ (%)	FC Cl ⁻ ^d (%)
0	8.01 ^a \pm 0.31	0.013 ^c \pm 0.03	67.48 ^a \pm 28.77	1.58 ^c \pm 0.59
2	4.96 ^b \pm 0.39	8.25 ^b \pm 5.32	147.43 ^a \pm 29.99	13.79 ^b \pm 6.34
4	5.12 ^b \pm 0.51	19.05 ^a \pm 2.98	109.66 ^a \pm 26.60	25.11 ^a \pm 3.73
8	5.16 ^b \pm 0.61	6.94 ^b \pm 3.16	110.49 ^a \pm 34.22	12.05 ^b \pm 4.28
12	4.90 ^b \pm 0.13	3.08 ^{bc} \pm 2.16	113.51 ^a \pm 48.11	7.54 ^{bc} \pm 4.46
24	5.74 ^b \pm 1.04	0.17 ^c \pm 0.23	74.06 ^a \pm 58.55	3.01 ^c \pm 2.47

^{a-c} Within a variable, the different superscripts indicate that the values differ significantly between times (^d P< 0.001; repeated measures ANOVA).

Table 5. Mean \pm SD values for heart rate (HR), respiratory rate (RR), rectal temperature (RT) and ruminal movements (RM) in healthy ewes that received an intravenous infusion of an HCl solution (100mmol/L) at a volume equivalent to 10% of its body weight: before (0h), in the middle (2h), and at the end of the infusion (4h) and 6, 8, 10, 12 and 24 hours from the start of the infusion

Hour	HR (bpm)	RR (rpm)	RT (°C)	RM (/5') ^c
0	88.8 ^a \pm 6.41	46.6 ^a \pm 15.38	38.54 ^a \pm 0.27	6.0 ^{ab} \pm 0.70
2	98.4 ^a \pm 11.86	41.6 ^a \pm 14.85	38.62 ^a \pm 0.81	4.6 ^b \pm 1.51
4	108.0 ^a \pm 11.31	48.0 ^a \pm 16.00	38.54 ^a \pm 0.66	5.6 ^{ab} \pm 1.14
6	92.8 ^a \pm 8.67	45.2 ^a \pm 16.22	39.02 ^a \pm 0.25	6.4 ^{ab} \pm 0.54
8	98.8 ^a \pm 15.33	53.2 ^a \pm 25.20	39.08 ^a \pm 0.25	5.6 ^{ab} \pm 1.51
10	96.0 ^a \pm 7.48	44.0 ^a \pm 12.88	38.78 ^a \pm 0.40	7.2 ^a \pm 0.83
12	96.8 ^a \pm 8.78	50.0 ^a \pm 17.88	38.78 ^a \pm 0.28	6.6 ^{ab} \pm 0.54
24	100.0 ^a \pm 12.64	47.6 ^a \pm 14.85	38.72 ^a \pm 0.30	6.8 ^a \pm 1.30

^{a-b} Within a variable, the different superscripts indicate that the values differ significantly between times (^c P< 0.001; repeated measures ANOVA).

DISCUSSION

The experimental protocol for the induction of metabolic acidosis used in the study was efficient for inducing severe hyperchloremic metabolic acidosis at the end of the infusion. Experimental protocols for hyperchloremic metabolic acidosis induction by intravenous HCl solution have been studied in ponies (Gossett *et al.*, 1990), horses (Romão *et al.*, 2017), and newborn calves (Abeysekara *et al.*, 2007; Gentile *et al.*, 2008; Schwedhelm *et al.*, 2013). These protocols have been effective in inducing metabolic acidosis; however, the severity of the imbalance, the infused volume and the rate of administration varied between the studies.

The solution infused in ponies (Gossett *et al.*, 1990) had the identical concentration used in this study (100mmol/L). The solution was infused for 60 minutes at a volume that corresponded to 2.5% of the pony's BW and caused mild metabolic acidosis (pH= 7.24; and HCO_3^- = 18.2mmol/L). The rate of infusion used by those authors (Gossett *et al.*, 1990) and in this study was identical; however, the volume of solution infused in ponies was four times smaller.

In newborn calves, Abeysekara *et al.* (2007) administered a solution of 300mmol/L HCl at a volume of approximately 8% of the calf's BW and at a rate of 20mL/kg/h, which caused severe metabolic acidosis after four hours of infusion (pH= 6.90; HCO_3^- = 7mmol/L and BE = -23.3mmol/L). Although the infusion rate and volume were similar to those used in this study, the solution infused by these authors was three times more concentrated, which resulted in a more severe imbalance.

Gentile *et al.* (2008) and Schwedhelm *et al.* (2013) infused calves with a solution containing the identical concentration used in this study. A volume equivalent to 10% of the calf's BW was infused in only 80 minutes, which caused severe metabolic acidosis at the end of the infusion. The identical solution volume was used in the ewes in this study; however, the imbalance produced in calves was more severe because the solution was administered three times faster. The slower infusion of the solution, as performed in this study, simultaneously allowed respiratory compensation and renal correction mechanisms to operate (Table 4), which probably reduced the

severity of the imbalance presented at the end of the infusion. Similar to studied ewes, respiratory compensation with a low pCO_2 value occurs at the end of infusion in calves (Abeysekara *et al.*, 2007; Schwedhelm *et al.*, 2013). However, unlike this study, the correction of metabolic acidosis by the kidneys has not been assessed.

Romão *et al.* (2017) used the same experimental protocol for the induction of metabolic acidosis used in this study, with the rate of infusion slightly lower (20mL/kg/h). The severity of the induced imbalance and the time for recovery was similar in horses and in the studied ewes.

When considering the traditional approach to acid-base balance, focused on bicarbonate and explained by the Henderson-Hasselbalch equation, hydrogen ions (H^+) administered in the solution were buffered by HCO_3^- , which caused a decrease in pH, HCO_3^- and BE (DiBartola, 2012). With the traditional approach, it is possible to interpret the severity of the imbalance that occurs; however, the mechanism of acidosis development cannot be understood. Therefore, the strong ion approach (Stewart, 1983; Constable, 2003) should be considered. In this approach, SID is the metabolic component of the acid-base balance, and its change may cause a decrease or increase in pH. A decrease in SID (independent variable) is caused by an increased concentration of Cl^- relative to Na^+ in the plasma and is necessarily followed by a reduction in pH and HCO_3^- , which are recognized as dependent variables (Morais *et al.*, 2008). The severity of the electrolyte imbalance caused by the administration of the HCl solution is shown by hyperchloremia and the consequent decrease in plasma SID during the infusion, which causes strong ion acidosis or hyperchloremic acidosis by the end of the infusion. This event has been observed in previous studies (Gentile *et al.*, 2008; Schwedhelm *et al.*, 2013, Romão *et al.*, 2017).

Hyperchloremic acidosis can occur naturally through diarrhea or renal tubular acidosis (Morais *et al.*, 2008) or can be induced by adding anionic salts containing chloride to the diet of ruminants or with the therapeutic use of electrolyte solutions containing a high concentration of Cl^- (0.9% NaCl, 7.5% NaCl and Ringer's solution). Anionic diets containing chloride can cause hyperchloremic acidosis with

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variable severity depending on the dose and frequency of ingestion (Jones *et al.*, 2009). Intravenous electrolyte solutions with effective SID equal to 0mEq/L can cause mild and transient hyperchloremic metabolic acidosis (Gossett *et al.*, 1990; Morais *et al.*, 2008; Kook and Kaske, 2008; Berchtold, 2009). Compared to the use of HCl to induce metabolic acidosis, the imbalance caused by the administration of therapeutic electrolyte solutions is less intense and shorter because high concentrations of sodium are also present in these solutions.

The solution volume administered in this study caused hemodilution and consequently, decreased L-lactate levels. This protocol did not cause an increase in organic anions as shown by the L- lactate and AG values.

Recovery from the imbalance occurred gradually after the end of the infusion: a compensatory decrease in pCO₂ was long lasting, acidemia was corrected in approximately 8 hours, and plasma bicarbonate and BE levels increased gradually. Because of the severity of the induced imbalance, the values of these variables remained low in the following day. The kidneys are responsible for the correction of acidosis by promoting the selective excretion of ions. The elimination of H⁺ is followed by the mechanism of HCO₃⁻ conservation (DiBartola, 2012). Aciduria persisted throughout the monitoring period, which demonstrates that the excretion of H⁺ continued for more than 24 hours after the start of acidosis induction.

Greater efficiency of acid excretion in the urine is achieved with the synthesis of ammonia (NH₃) in the proximal tubular cells. Ammonia combines with H⁺, and the resulting ammonium (NH₄⁺) is eliminated in the tubular fluid. The removal of H⁺ also contributes to the elimination of Cl⁻ in the distal nephron because this electrolyte is excreted as NH₄Cl (DiBartola, 2012). Thus, the kidneys play a critical role in the restoration of electrolyte and acid-base balance, and the correction of iatrogenic hyperchloremia occurs simultaneously with the correction of acidosis. The increased glomerular filtration rate caused by the excess of administered fluid (10% of BW of non-dehydrated individuals) facilitated these events, and the elimination of excess Na⁺ and Cl⁻ was

maximized at the end of the infusion, as indicated by high FC values of both electrolytes.

Intracellular buffering, which consists of the exchange of intracellular K⁺ for H⁺ in the extracellular medium, is another event that occurs during metabolic acidosis which may result in hyperkalemia (Morais *et al.*, 2008). Although hyperkalemia occurred in dogs (Swan *et al.*, 1955) and in horses (Romão *et al.*, 2017) with hyperchloremic acidosis, this was not observed in ponies (Gossett *et al.*, 1990), calves (Gentile *et al.*, 2008), or sheep. In this study, the trend for increasing K⁺ FC (P= 0.058) indicated a greater renal elimination of the electrolyte and could justify the maintenance of plasma concentrations over the range of physiological variations, thus reinforcing the suggestions of Gentile *et al.* (2008).

Studies suggest that the postural and behavioral changes in animals with metabolic acidosis are caused by organic acids such as increased plasma concentration of D-lactate, which is produced by rumen bacteria in the case of rumen lactic acidosis or intestinal bacteria in the case of calves (Berchtold *et al.*, 2009; Abeysekara *et al.*, 2007; Lorenz, 2004). In ponies, Gossett *et al.* (1990) noted that the infusion of DL-lactic acid, L-lactic acid or HCl caused depression without other changes. In calves, intravenous administration of D-lactate causes severe behavioral changes characterized by decreased suckling and palpebral reflexes, decreased menace responses, and ataxia progressing to recumbency (Abeysekara *et al.*, 2007). By contrast, the behavioral changes in calves with hyperchloremic acidosis induced by the intravenous administration of HCl were mild (Abeysekara *et al.*, 2007; Schwedhelm *et al.*, 2013) or absent (Gentile *et al.*, 2008).

In this study, it was noted that all ewes exhibited somnolence, with closed eyelids and lowered heads during the final hour of the infusion. However, from the end of the infusion onward, the ewes became alert with good appetites and normal attitudes. The overall results obviously indicated that the severity of acidosis itself does not cause depression in the unbalanced animal. Compared to acidosis induced by D-lactic acid, which produces severe behavioral changes, hyperchloremic acidosis, even severe, provokes only slight behavioral alterations.

CONCLUSIONS

This protocol was effective in inducing severe and long-lasting hyperchloremic acidosis and did not cause serious side effects. Therefore, this protocol can be used safely in adult sheep for studies on the treatment of this condition.

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