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Studies on Acid-Base Status in Calves with Ammonium Chloride Induced Metabolic Acidosis

[1]Nawal Mohamed Elkhair and [2]Helmut Hartmann

[1]Department of Physiology,
Faculty of Veterinary Medicine, University of Khartoum Sudan
[2]Institute of Veterinary Physiology,
Faculty of Veterinary Medicine, Free University of Berlin, Germany

Abstract: The changes in the acid-base status of the blood and urine in relation to the age after acid-load were studied in dairy calves. Nineteen healthy calves (German Blacked-coloured and cross breeds, age: 4-104 days) were infused with 5M NH_4Cl (dose 1.0ml/kg, dilution 1:10) through a permanent intravenous catheter for a period of ≤2.5hrs to induce experimentally metabolic acidosis. Blood and urine samples were collected at 0, 2, 4, 6, 8 and 24 hrs to determine blood and urine pH, serum and urine concentration of Na^+, K^+ and Cl^-. Serum and urine- [SID_3] was calculated by using the equation: serum or urine- [SID_3] = [Na^+] + [K^+] - [Cl^-]. The initial mean values of blood and urine pH were 7.35-7.4 and 6.6-6.8, respectively. Serum and urine- [SID_3] were 44-48 mmol/l and 15-50 mmol/l, respectively. Serum- [SID_3] was higher in the younger calves compared to the older calves. By 2-6 hrs after the infusion of NH_4Cl solution, the blood pH and serum- [SID_3] significantly ($P<0.05$) decreased. Urine pH was lower in the youngest calves (1st week of life) than in the older ones (1-2 months). Urine pH decreased significantly in response to the NH_4Cl load after 2 hrs of the beginning of the infusion. The negative values of urine- [SID_3] indicated that the concentration of urine- [Cl^-] was higher in urine than that of [Na^+] and [K^+]. The nutritional status of the calves may have an influence on the blood and urine acid-base parameters. This observation should be considered in the management programme to avoid acid-base disturbances related to the age and nutritional status of calves.

Key words: Calves · Metabolic acidosis · Acid-base status · pH · Strong ion difference

INTRODUCTION

Acid-base disorders have been commonly observed in association with respiratory, cardiovascular, gastrointestinal and renal dysfunctions in calves [1-5]. Metabolic acidosis is an acid-base disturbance caused by a decrease in the plasma HCO_3^- and the higher production of acid in the colon [6]; sometimes it is followed by a secondary respiratory compensation [2, 4]. Metabolic acidosis is a well recognised potentially life-threatening consequence of diarrhoea in calves [3, 7] and it is a complicating factor in a number of diseases that affect cattle, including ketoacidosis [8] and lactic acidosis [9]. In calves, a close relationship has been reported between calf diarrhoea and acid-base disorders characterised by acidaemia, hypocapnia and electrolyte imbalances [10-14]. Furthermore, severe diarrhoea was observed in association with renal damage which was characterised by strong alterations in glomerular capillary and tubular vessels of the calves' kidney [15]. Renal electrolyte disturbances are usually associated with diarrhoea and dehydration in calves [16].

NH_4Cl is used as a therapeutic drug for urine acidification in humans [17] and animals [18]. NH_4Cl is also used to enhance the effectiveness of antibiotics in urine and as a diagnostic drug for renal tubular acidosis or liver function testing [19]. Administration of NH_4Cl has been widely used as model of metabolic

Corresponding Author: Nawal Mohamed Elkhair, Department of Physiology Faculty of Veterinary Medicine, University of Khartoum, P.O. Box 32, Post code 13314, Khartoum North, Sudan.

acidosis in both humans and animals [20, 21]. In the body, NH_4Cl is taken up by the liver with formation of urea and net release of HCl, which is ultimately responsible for lowering the body's acid-buffering capacity and induction of acidosis [22].

In veterinary medicine, intravenous administration of 5M NH_4Cl (1.0 ml/kg) has been used to induce metabolic acidosis in calves within 180 min [21] and in camels [23]. Administration of 1.5% NH_4Cl for 7 days has been used successfully to induce metabolic acidosis in mice [24]. Several studies have shown that oral administration of NH_4Cl (100 mg/kg) every 12 hrs for 8 days caused a significant decrease in plasma pH, PCO_2, standard HCO_3^- and BE in dogs [18]. The treatment also increased urine acid excretion. In human medicine, Osther *et al*. [20] have studied renal responses to acid load by oral administration of various doses of NH_4Cl (0.1, 0.2 and 3 g/kg BW); they concluded that the treatment caused a significant decrease in systemic and urine pH. In camels, intravenous infusion of 5M NH_4Cl caused a significant decrease in urine pH and an increase in the fraction excretion of Na^+ and Cl^- [23]. Furthermore, NH_4Cl is also known to cause kidney hypertrophy by inducing an imbalance between protein synthesis and degradation [25].

The kidneys play a central role in acid-base homeostasis by adjusting urine electrolyte excretion to maintain constant systemic pH. Evaluation of the renal functions has been accomplished with the analysis of blood and urine to compare the concentration of electrolytes in blood and urine. Previous investigators have used fractional excretion of electrolytes ($FE_{electrolyte}$) to evaluate renal functions in adult cattle [26] and in healthy and diarrhoeic calves [5, 27]. On the other hand, $FE_{electrolyte}$ has been applied to cattle before calving in order to reduce their susceptibility to metabolic diseases mainly milk fever [28, 29]. Furthermore, measurement of urine pH has been used as a practical and inexpensive method for monitoring the effectiveness of dietary cation-anion difference (DCAD) in lactating cows [30]. Recently, application of urine strong ion difference (SID) has been used to assess the effectiveness of nutritional status in animals [31]. This concept is based on the hypothesis that different amounts of SID in the diet may cause corresponding changes in urinary SID and thus urine pH [32]. The importance of urinary SID concept is to understand the output of the therapeutic solutions in humans and the changes in the diet content in animals (mainly DCAD). It has been concluded that DCAD had an important influence on urinary SID [31]. The consideration of urinary SID is not easy due to the difference between the negative charge of the plasma ($[A^-]$, $[HCO_3^-]$ and $[H_2PO_4^-]$) and the lower amounts of weak acids in the urine [33]. Equations have been developed to explain the relationship between urine pH and urine SID, urinary NH_4^+ and PCO_2 [31]; the authors concluded that the changes in these parameters independently and directly led to cause changes in urine pH in cattle fed diets containing acidogenic salts.

To our knowledge only one study has been reported in literature on urinary SID in cattle [31]. Therefore, the main objectives of the present study were:

- To evaluate the use of the intravenous infusion of 5M NH_4Cl solution (pH = 5.05, osmolality = 893 mOsmol/kg, $[SID_3]$ = -500 mmol/l) to induce metabolic acidosis in calves.
- To validate the use of urine SID application in calves.
- To evaluate the renal responses in calves after experimental acid-load with 5M HN_4Cl.
- To provide additional information for certain blood and urine acid-base parameters in calves in relation to their age.

MATERIALS AND METHODS

Animals: Nineteen healthy calves (German Blacked-coloured and cross breeds, age: 4-104 days) were used. The calves were assigned to 6 groups according to their age, .i.e. 1st week: n = 3, 2nd week: n = 4, 3rd week: n = 4, 4th week: n = 4 and 1-2 months: n = 4. They were housed individually in indoor pens and standard calf management procedures were used (Clinic of Small Animals, Faculty of Veterinary Medicine, Free University of Berlin, Germany). The calves were fed milk which was provided three times daily (7:30 am, 13:30 and 22:30 pm). Hay and grain were added to the diet when the calves were 2 weeks old. The calves had free access to fresh water.

Induction of Experimental Acidosis: Metabolic acidosis was induced by using 5M NH_4Cl (pH=5.05, osmolality= 893 mOsmol/kg, $[SID_3]$ = -500 mmol/l) at a dose of 1.0 ml/kg body mass. The final solution was prepared by diluting the calculated dose of 5M NH_4Cl by 1:10 with distilled water. The diluted solution was infused via a permanent intravenous catheter for a period of 2-2.5hrs.

Sample Collection and Laboratory Analysis: Venous blood samples were collected by using heparinised plastic syringes (1.0 ml, Klinika Medical GmbH, Germany) before and after 2, 4, 6, 8 and 24 hrs starting the infusion. The syringes were immediately sealed with a rubber cap and stored on crushed ice. The blood samples were analysed within 15 min after sampling for the determination of venous blood pH and blood gases using a blood gas analyser CCX (Nova biomedical GmbH-Adam-Opel., Rödermark, Germany). The values were corrected for the calves' rectal temperature. Serum samples were used for the determination of the concentration of sodium (Na^+), potassium (K^+) and chloride (Cl^-) (Ion-selective electrode) using a biochemical analyser (Roche Hitachi Modular, Fa. Roche Diagnostics, Berlin, Germany).

The urine samples were collected in sterile containers via free catch or by perineal or preputial stimulation of the calves every 2 hrs at the same time of the blood collection and they were stored for further analysis. Fresh urine samples were used to determine urine pH by using a pH meter (InoLab, Scientific Technical Workshops, Weilheim, Germany). The pH-meter was calibrated by using a two-point calibration with pH 5.0-10.0. The rest of urine samples were used for the determination of the concentration of sodium (Na^+), potassium (K^+) and chloride (Cl^-) (ion-selective electrode measurement) by using a biochemical analyser (Roche Hitachi Modular, Fa. Roche Diagnostics, Berlin, Germany). The values of the electrolytes were used for the calculation of serum and urine strong ion difference ($[SID_3]$) using the equation $= [Na^+] + ([K^+] - [Cl^-])$ [35].

Statistical Analysis: Statistical analysis was performed using SPSS for Windows version 17.0. The values of serum and urine acid-base parameters after the infusion (time: 2-24 hrs) were compared to the initial values (time: 0 hrs). The paired T-test was used to estimate the significant difference in the serum and urine acid-base parameters at each time point after the infusion. ANOVA tests (Levine's Test and Post Hoc Test) were used to assess the possible significant differences between the age groups. Linear regression procedure was used to explain the relationship between urine pH and urine- $[SID_3]$. The mean difference was considered significant at $P \leq 0.05$.

RESULTS

Blood pH and Serum- $[SID_3]$ of the Calves during Experimental Metabolic Acidosis: Table 1 indicates that the initial mean values of pH were 7.38-7.4. The general pattern of response showed that 2 hrs after the beginning of the infusion, there was a sharp significant ($P<0.001$-0.05) decrease in the mean values of blood pH in all calves. The most pronounced significant ($P<0.001$-0.05) decrease in blood pH was observed after 4 hrs. After 6-8 hrs, the blood pH remained significantly ($P<0.001$-0.05) lower compared to the initial values. After 24 hrs, the blood pH increased gradually towards normal in all groups to values ranging from 7.36 to 7.39.

Table 1 shows that the initial mean values of serum- $[SID_3]$ were 44-47 mmol. Intravenous infusion of 5M NH_4Cl solution ($[SID_3] = -500$ mmol/l) induced a rapid decrease in serum- $[SID_3]$ in all groups from the initial

Table 1: Effect of the infusion of ammonium chloride solution (5M NH_4Cl, diluted 1:10) on blood pH and serum- $[SID_3]$ (mmol/l) in healthy calves of various ages (mean±SD)

		Time (hrs)					
Age group	parameter	0	2	4	6	8	24
1st week	pH	7.4^{a}±0.01	7.35^{b*}±0.01	7.36^{a}±0.03	7.36^{a}±0.03	7.39^{a}±0.02	7.38^{a}±0.02
	Serum- [SID3](mmol/l)	47.3^{a}±3	43.6^{b**}±1.3	43.1^{a}±1.7	44.4^{a}±1	43.4^{a}±0.7	44.7^{a}±0.7
2nd week	pH	7.39^{a}±0.02	7.36^{b*}±0.02	7.34^{b*}±0.02	7.36^{b**}±0.02	7.38^{b*}±0.02	7.38^{b**}±0.01
	Serum- [SID3](mmol/l)	46.2^{a}±3.6	42.3^{b**}±3.1	42.4^{b**}±4	42.2^{b***}±3.1	41.8^{b***}±3.2	43.6^{a}±2
3rd week	pH	7.39^{a}±0.01	7.32^{b**}±0.02	7.34^{b***}±0.02	7.35^{b***}±.0.02	7.36^{b***}±0.01	7.37^{b**}±0.02
	Serum- [SID3](mmol/l)	44.5^{a}±1.5	39.7^{b**}±2.2	39.1^{b***}±2.2	38.6^{b***}±1.2	38.2^{b***}±1.7	41.3^{a}±1.6
4th week	pH	7.37^{a}±0.04	7.34^{b***}±0.03	7.32^{b**}±0.02	7.33^{b**}±0.03	7.34^{b**}±0.02	7.36^{a}±0.02
	Serum- [SID3](mmol/l)	43.7^{a}±3.3	37.7^{b*}±3.7	38.1^{b**}±2.7	38.4^{b**}±2.7	39.3^{a}±2.7	41.2^{a}±1.6
1-2 month (s)	pH	7.4^{a}±0.01	7.36^{b***}±0.02	7.34^{b**}±0.02	7.35^{b***}±0.02	7.38^{b***}±0.01	7.38^{b**}±0.02
	Serum- [SID3](mmol/l)	44.2^{a}±2.1	40.1^{b***}±3.4	39.7^{b***}±4	39.2^{b***}±3.8	38.9^{b***}±3.2	39.9^{a}±3.2

Brackets ([ ]) denote concentration,

[a, b] Means within the same row bearing different superscripts are significantly different at $P \leq 0.05$. (*P<0.05, **P<0.01, ***P<0.001)

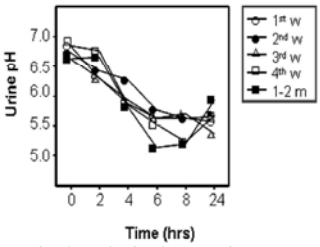

Fig. 1: Effect of the infusion of ammonium chloride (5M NH_4Cl, diluted 1:10) on urine pH in healthy calves of various ages (mean values).

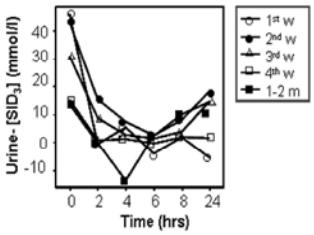

Fig. 2: Effect of the infusion of ammonium chloride (5M NH_4Cl, diluted 1:10) on urine- [SID_3] in healthy calves of various ages (mean values).

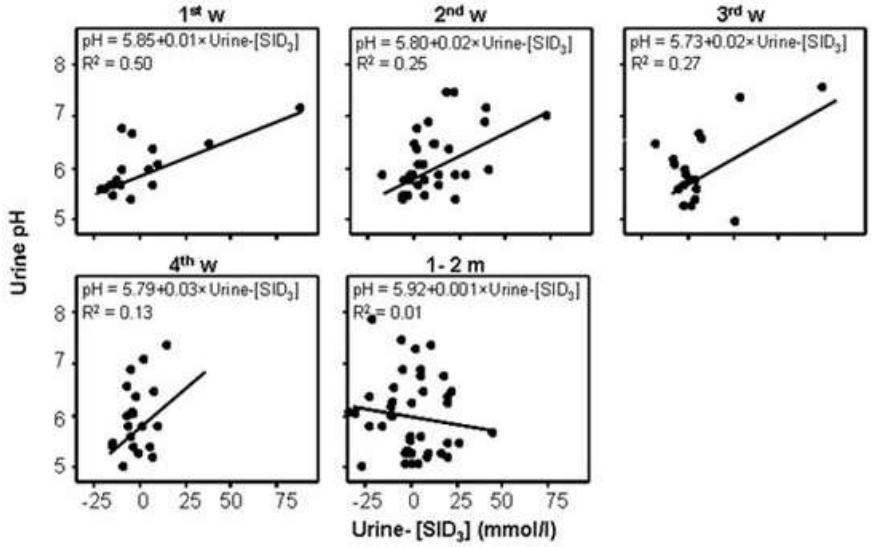

Fig. 3: Relationship between urine pH and urine- [SID_3] in healthy calves of various ages after the infusion of ammonium chloride (5M NH_4Cl, diluted 1:10) (w = week, m(s) = month(s)) (Linear regression equation: y = ax+b, R^2 = Coefficient of determination).

mean values to 44.7-39.7 mmol/l after 8 hrs. By 2 hrs after the beginning of Sthe intravenous infusion, there was a sharp significant ($P<0.001$-0.05) decrease in the mean values of serum- [SID_3] in all age groups except in the youngest calves (1st week). After 4 hrs, all the calves showed maximal significant ($P<0.001$-0.05) decrease in the serum- [SID_3] and remained low after 8 hrs. After 24 hrs, serum- [SID_3] remained significantly ($P<0.001$-0.05) lower than the initial values in the young calves in the 2nd and 3rd week and in the old calves of 1-2 m.

Urine pH and Urine- [SID_3] of the Calves during Experimental Metabolic Acidosis: Fig. 1 shows that the initial mean values of urine pH for the calves were 6.6-6.8. NH_4Cl-load caused a significant ($P<0.01$) decrease in urine pH after 2-8 hrs of the beginning of infusion. These values were maintained at significantly ($P<0.01$) lower level after 24 hrs of the beginning of the infusion in the older calves.

Before the beginning of the infusion of NH_4Cl solution (SID= -500 mmol/l), the mean values of urine- [SID_3] for the calves were 15-50 mmol/l (Figs. 2). Before the infusion, the older calves (1-2 m) showed significantly ($P<0.01$) lower mean value of urine- [SID_3] compared to the young calves (1-4 weeks). By 2-6 hrs after the beginning of infusion, the urine- [SID_3] decreased significantly ($P<0.01$) only in the older calves.

Relationship Between Urine- [SID_3] and Urine pH: The relationships between the individual values of urine- [SID_3] and their corresponding values of urine pH were determined using linear regression (Fig. 3). The relationship between urine- [SID_3] and urine pH showed a rising regression line in all calves except in the older calves ($P<0.05$). The results also indicated that the low values for urine- [SID_3] corresponded to the low values for urine pH. The Coefficient of determination,

R^2 was generally higher in the younger calves (1-4 weeks) than in the older ones (1-2 months). The lowest value of R^2 (0.01) was observed in the older calves (Fig. 3).

DISCUSSION

The main finding in the present study was that intravenous infusion of 1:10 diluted 5M NH_4Cl solution has been successfully used to induce metabolic acidosis in healthy calves of different ages as indicated by the significant decrease in the blood pH and serum- [SID_3] (Table 1). The present results are in agreement with the Stewart terminology who defined metabolic acidosis as a decrease in blood pH, PCO_2 and a decrease in the serum strong ion difference (SID) [35]. This has been confirmed in the present study and the decrease in serum- [SID_3] (38-44 mmol/l) caused a marked decrease in systemic pH (7.32-7.36, Table 1). The significant decrease in the blood pH reported in the present study is in agreement with that reported previously in calves with experimentally induced metabolic acidosis using 5M NH_4Cl [21] and in young dromedary camels [23].

The results indicate that the age of the calves had a significant influence on their response to the experimentally induced metabolic acidosis. Moreover, the young calves until the 4th week of life were more acidotic (lower pH) than the older calves (Table 1). This pattern of response is in agreement with previous studies in diarrhoeic calves [3] which reported that younger calves (more than 6 days old) were more severely acidotic than older calves. In contrast, other researchers concluded that older calves tended to be more acidotic than younger calves [6]. However, Naylor, [10] reported that diarrhoeic calves (more than 8 days) were more acidotic than younger calves.

The normal serum SID_3 for healthy calves has been considered to be 43±2.4 mmol/l [36]. However, the serum SID_3 reported in the present study (44-47 mmol/l, Table 1) is higher than values reported previously [36] and lower than those reported by Grove and Michell [3].

The kidneys are well known as being the main organs in the body responsible for the regulation of the extracellular electrolyte concentration and acid-base status of mammals by altering acid excretion [37, 38]. When the blood pH falls, the kidneys usually excrete urine with a pH of less than 5.5. Therefore, the acidic urine pH (5.5) observed during the experimental period (8 and 24 hrs, Table 1) can be considered as an indicator of the renal response to the decrease in the blood pH observed (Table 1). Possibly, this is related to the increase in the activity of Na^+-H^+ antiporter and H^+-ATPase pump in response to the experimentally induced metabolic acidosis. It has been indicated that the brush border membrane of the rat renal proximal tubules responded to metabolic acidosis by increasing the activity of Na^+-H^+ antiporter [39].

A close relationship has been reported previously between urine pH and diet composition in cattle [29, 30]. Other investigators have explained the effect of calf diarrhoea on renal function [5]. However, until now, no published data is available regarding the values of urine- [SID_3] in calves in relation to the nutritional status. Recently, studies in cattle have explained the relationship between urine pH, urine SID and DCAD [31]. Therefore, we measured values for urine pH and SID in calves to monitor their acid-base status in relation to their age after induction of metabolic acidosis.

After the experimental metabolic acidosis, the calves' kidneys responded to the changes in the acid-base status of the blood as indicated by the decreased values of blood pH and serum SID_3 (Table 1) by increasing renal excretion of H^+ ions manifested by decrease in urine pH (Fig. 1). By 24 hrs after intravenous with the NH_4Cl solution, the renal excretion of H^+ ions in urine increased dramatically as indicated by the decreased values of urine pH (5.5-6.0 = aciduria, Fig. 1). Furthermore, the analysis of the findings in the present study demonstrates that the urine pH of 6.6 for the youngest calves (1st week) was lower compared to the oldest calves of 1-2 months (pH= 6.8). This result clearly indicates the significant impact of the nutritional status of the animal on urine pH. Because the youngest calves were fed milk only while the oldest ones fed milk and hay, the urine pH of the youngest calves was more acidic than that of the oldest calves. The reported finding can be considered as a physiological renal response when the diet is shifted from milk to hay. This observation should be considered in the management programme of these animals to avoid acid-base disorders related to changes in the diet composition.

The present study showed that the age of the experimental calves can induce a significant influence in the response to the experimental metabolic acidosis. This might be attributed to immature renal function in the very young calves [23, 40], particularly acid-base transport proteins function of the kidneys. Previous researchers observed few proximal tubules in the renal cortex with few acid-base transporters mainly Na^+/H^+ antiporter and Na^+-HCO_3^- cotransporter in humans and young rats at the age of 3 days [41].

The urine- [SID_3] values obtained in the present study for calves (15-50 mmol/l) clearly indicate the influence of the intravenous infusion of a solution containing the strong cations and anions (mainly Cl^-) on the renal function. The renal response can be explained by the negative values for urine- [SID_3] observed after 4 and 8 hrs of the infusion particularly in the youngest calves (Fig. 2). Previous studies suggested that the reduction in the serum- [SID_3] can be explained by reduced concentration of strong cations (Na^+ and K^+) and/or by the increased concentration of strong anions (Cl^-) [34, 35]. Therefore, the calves showed a significant decrease in the urine pH in response to the experimental metabolic acidosis (Fig. 1), indicating an increase in excretion of these ions in urine and consequently decreased urine- [SID_3] (Fig. 2). The transient acidaemia observed (Table 1) was accompanied by aciduria (Figs. 1) and a relatively higher renal elimination of Cl^- compared to the elimination of Na^+ as indicated by the elevated urine- [SID_3] values (Fig. 2). The present results are in agreement with findings [31] which showed that the urine pH in cattle can be determined by urine- [SID].

In the view of the linear regression, the results indicated that the infusion of NH_4Cl solution has a significant impact on urine- [SID_3] and consequently urine pH (Fig. 3). At zero time, the urine- [SID_3] of 15-50 mmol/l for calves corresponded to the normal urine pH values for calves (6.0-6.5). After the infusion (4-8 hrs), the elimination of strong anions, mainly Cl- by the kidneys was higher than that of strong cations as indicated by the negative values of urine- [SID_3] (Fig. 2). As the urine- [SID_3] decreased, the urine pH decreased to ~ <5.0 (aciduria, Fig. 3).

CONCLUSIONS

The present results demonstrated the vital role of kidney in restoration of normal acid-base status of the blood after the disturbance such as metabolic acidosis. The estimation of urine- [SID_3] can be used for monitoring the influences of therapeutic solutions or dietary supplementation of cationic or anionic salts (Na^+, K^+, NH_4^+ and Cl^-) on acid-base status under physiological and pathological conditions.

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