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Original article

Acid-base disorders in calves with chronic diarrhea

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Abstract

The aim of this study was to analyze disorders of acid-base balance in calves with chronic diarrhea caused by mixed, viral, bacterial and *Cryptosporydium parvum* infection. We compared results obtained with the classic model (Henderson-Hasselbalch) and strong ion approach (the Steward model). The study included 36 calves aged between 14 and 21 days. The calves were allocated to three groups: I – (control) non-diarrheic calves, group II – animals with compensated acid-base imbalance and group III calves with compensated acid-base disorders and hypoalbuminemia. Plasma concentrations of Na^+, K^+, Cl^-, Cl^{2+}, Mg^{2+}, P, albumin and lactate were measured. In the classic model, acid-base balance was determined on the basis of blood pH, pCO_2, HCO_3^-, BE and anion gap. In the strong ion model, strong ion difference (SID), effective strong anion difference, total plasma concentration of nonvolatile buffers (A_{Tot}) and strong ion gap (SIG) were measured.

The control calves and the animals from groups II and III did not differ significantly in terms of their blood pH. The plasma concentration of HCO_3^-, BE and partial pressure of CO2 in animals from the two groups with chronic diarrhea were significantly higher than those found in the controls. The highest BE (6.03 mmol/l) was documented in calves from group II. The animals from this group presented compensation resulted from activation of metabolic mechanisms. The calves with hypoalbuminemia (group III) showed lower plasma concentrations of albumin (15.37 g/L), Cl^- (74.94 mmol/L), Mg^{2+} (0.53 mmol/L), P (1.41 mmol/L) and higher value of anion gap (39.03 mmol/L). This group III presented significantly higher SID_3 (71.89 mmol/L), SID_7 (72.92 mmol/L) and SIG (43.53 mmol/L) values than animals from the remaining groups ($P<0.01$), whereas A_{Tot} (6.82 mmol/L) were significantly lower. The main finding of the correlation study was the excellent relationship between the AG_{corr} and SID_3, SID_7, SIG. In conclusion, chronic diarrhea leads to numerous water-electrolyte disorders. Characterization of acid-base disturbance in these cases suggests that classic model have some limitations. This model can not be recommended for use whenever serum albumin or phosphate concentrations are markedly abnormal.

Key words: calves, diarrhea, acid-base balance, strong ions difference, hypoalbuminemia

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Introduction

Alimentary and respiratory disorders represent nearly 80% of all the conditions diagnosed in calves, and are principal cause of economic loses. The risk of the diarrheal diseases is the highest during postnatal period, weaning and transfer to a calf house. In uncomplicated cases, diarrhea usually lasts no longer than one week, and complete recovery is observed after another six days. The etiology of calf diarrhea is heterogeneous and includes dietary, bacterial, fungal, viral, parasitic and/or allergic factors. While the diarrhea of neonatal calves is usually associated with bacterial (*E. coli*) or rotaviruses and coronaviruses infections, *Cryptosporidium* spp. seems to be the most common cause of diarrhea in older, 3-week-old animals (Coutinho et al. 2008, Foster and Smith 2009, Ok et al. 2009). Clinical signs of diarrhea include loose watery stools, lack of appetite and abdominal pain. Persistent diarrhea may result in dehydration, weakness and loss of suckling reflex (Gomez et al. 2013). Significant loss of fluids leads to hypovolemia and cardiovascular disorders, accompanied by a decrease in body temperature (Guzelbektes et al. 2007). Clinical status of an animal may further deteriorate due to acid-base disorders and electrolyte imbalances, usually leading to metabolic acidosis, often with associated neurological symptoms (Bachmann et al. 2009, Berchtold 2009). More severe or untreated diarrhea may result in death due septicemia, acidosis, hyperkalemia, prolonged malnutrition, hypoglycemia and hypothermia (Sen and Constable 2013).

Two analytical models are used to describe the acid-base disturbances: the classic model (Henderson-Hasselbalch equation) and strong ion model (Stewart model). Although both models describe the respiratory component of acid-based balance on the basis of changes in plasma carbon dioxide pressure, they differ in terms of metabolic components analyzed. The classic model is based on changes in plasma bicarbonate concentration (HCO_3^-), anion gap and base excess. In general terms, decreased bicarbonate concentration is considered a marker of acidosis, and elevated values of this parameter correspond to alkalosis (Constable 2000). The Stewart model is based on three independent determinants of acid-base balance: pCO_2, strong ion difference (SID) and total nonvolatile weak acid concentration. The SID is calculated as the difference between the sum of strong cation concentrations $[(Na^+)+ (K^+)+ (Cl^{2+}) + (Mg^{2+})]$ and the sum of strong anion concentrations $[(Cl^-) + (lactate^-)]$. The difference between SID and SIDEff is referred to as the strong ion gap (SIG) and reflects the balance of anion and cation concentrations, similar to the anion gap in the classic model (Constable 2002, Trefz et al. 2012). The positive values of SIG reflect excess of unmeasured anions (acidosis) and negative ones correspond to the excess of unmeasured cations (Constable 2000, Constable 2002, Gomez et al. 2013).

In previous studies, the acid-base balance of calves was determined using the Stewart approach (Gomez et al. 2013, Trefz et al. 2013) after administration of oral rehydration therapy (Leal et al. 2012, Kirchner et al. 2014), intravenous fluid therapy or alkalizing agents (Berchtold et al. 2005, Berchtold 2009, Leal et al. 2012) in case of acute diarrhea. To the best of our knowledge none of the previous studies analyze the acid-base balance disorders in calves with chronic diarrhea. Therefore, the aim of this study was to analyze the disorders of acid-base balance in calves with chronic diarrhea caused by mixed, viral, bacterial and *C. parvum* infection, and to compare the classic model and strong ion approach for their ability to detect, characterize, and quantify complex metabolic acid-base abnormalities found in such animals.

Materials and Methods

Experimental design

The protocol of the study was approved by the 2nd Local Ethics Committee for Experiments on Animals in Wroclaw, Poland (decision no. 23/2011). The study included 36 calves (52% females and 48% males) aged between 14 and 21 days. Mean age of the animals examined was 17 days. The calves were allocated to three groups, based on the presence of chronic diarrhea lasting for 5-6 days, acid-base disorders and biochemical parameters of the blood. Group I (control group) included clinically normal non-diarrheic calves with appropriate acid-base balance (n=12), group II – animals with compensated acid-base imbalance (n=12), and group III – calves with compensated acid-base disorders and hypoalbuminemia (n=12). None of the controls showed clinical abnormalities throughout the study period.

Clinical and microbiological examination

All the calves were subjected to clinical examination and their stool samples were tested for the presence of most important enteropathogens. Rotaviruses, coronaviruses and *C. parvum* were detected by means of ELISA (BioX Diagnostic, France), and enterotoxigenic strains of *E. coli* (ETEC) using methods PCR described below.

Table 1. Sequence of primers for toxins and fimbrias of *E. coli* used in this study (Casey and Bosworth 2009).

Virulence factor	Name of target gene	Primers sequence (5'-3')	Length of product (bp)
STb	*estB*	TGCCTATGCATCTACACAAT GCAGTACCATCTCTA	113
STa	*estA*	ACTGAATCACTTGACTCTT TTAATAACATCCAGCACAGG	158
F5 (K99)	*fanA*	AATACTTGTTCAGGGAGAAA AACTTTGTGGTTAACTTCCT	230
LTb	*eltB*	GGCGTTACTATCCTCTCTAT TGGTCTCGGTCAGATATGT	272
F18	*fedA*	TGGTAACGTATCAGCAACTA ACTTACAGTGCTATTCGACG	313
987P (F6)	*fasA*	AAGTTACTGCCAGTCTATC GTAACTCCACCGTTTGTATC	409
F4 (K88)	*faeG*	GTTGGTACAGGTCTTAATGG GAATCTGTCCGACJAATATCA	505
F41	*fedA*	AGTATCTGGTTCAGTGATGG CCACFATAAGAGGTTGAAGC	612

Table 2. Primers used in the multiplex PCR for F17 fimbrial genes of *E. coli* (Bertin et al. 1996).

Name of primer (target)	Primers sequence (5'-3')	Length of product (bp)
P7 (for subfamily I adhesins: F17a-G, F111)	CGGAGCTAATACTGCATCAACC	615
P8 (for subfamily II adhesins: F17b-G, F17c-G/GafD)	CGTGGGAAATTATCTATCAACG	615
P9 (for subfamilies I and II adhesin genes)	TGTTGATATTCCGTTAACCGTAC	

E. coli isolation

Rectal swabs were cultured on MacConkey agar (Oxoid Ltd, UK). After an overnight incubation at 37°C, suspected *E. coli* colonies were subjected to biochemical identification. Isolated strains were kept at -80°C in Microbank Storage Boxes (Pro-Lab Diagnostic, Canada).

PCR test

Microbial DNA was obtained by resuspending strains incubated on Mueller-Hinton broth (Oxoid Ltd, UK) in 500 µl of ultra pure water and boiling at 100°C for 10 min. A multiplex PCR which detects genes virulence factor of ETEC was preformed using the methods described by Casey and Bosworth (2009) (Table 1). A second multiplex PCR, designed to be specific to the 2 subfamilies of the F17 adhesins genes, was performed using 3 primers (Table 2) described by Bertin et al. (1996). Both multipex PCR were conducted in a total volume of 25 µl with 5 µl DNA templates, 2.5 µl of 10X polymerase buffer, 5 µl 25 mM $MgCl_2$, 1U Dream Taq DNA polymerase (Fermentas, Lithuania) and primers at concentration of 0.5 µmol each. All the primers were synthesized by Oligo (Warsaw, Poland). The procedure of PCR amplification included 1 cycle at 95°C (10 min), 30 cycles at 90°C (30 s each), 55°C (45 s) and 72°C (90 s), and 1 cycle at 72°C (10 min); all the reactions were performed in a thermal cycler (Biorad, Great Britain). The amplification products were resolved in 2% agarose gel and stained with Mindori Green (Nippon, Japan).

Acid-base balance and biochemical parameters

Heparinized jugular venous blood samples were collected anaerobically between the 5th and 6th day of diarrhea (calves from group II and III) and from healthy calves (control group) in similar day of life. The blood pH, partial pressures of oxygen (pO_2) and partial pressures of carbon dioxide (pCO_2), concentration of bicarbonates (HCO^-_3) and base excess (BE) were determined using a VetStat analyzer (Idexx, USA). Moreover, plasma concentrations of sodium, potassium, chloride, calcium, magnesium, inorganic phosphorous, albumin and lactate were measured with a Pentra 400 analyzer (Horiba ABX, France).

Table 3. Results of clinical examination of the calves.

Parameters	Item	Group I (Control)	Group II	Group III
Temeperatue	(C)	38.6 (38.0—38.8)	38.7 (38.1—39.2)	38.3 (37.9—38.6)
Behavior/posture	No changes	12 (100.0%)	4 (33.3%)	0 (0.0%)
	Weakness	0 (0.0%)	7 (58.3%)	4 (33.3%)
	Recumbence in sternal position	0 (0.0%)	1 (8.3 %)	8 (66.7%)
Dehydration	No changes	12 (100.0%)	4 (33.3%)	0 (0.0%)
	5-6%	0 (0.0%)	6 (50.0%)	7 (58.3%)
	7-9%	0 (0.0%)	2 (16.7%)	5 (41.7 %)
Body condition	Good	12 (100.0%)	6 (50.0%)	0 (0.0%)
	Moderate cachexia	0 (0.0%)	5 (41.7 %)	0 (0.0%)
	Severe cachexia	0 (0.0%)	1 (8.3 %)	12 (100.0%)

Table 4. Number and percentage of samples positive for various enteropathogens in the calves.

Group	*E. coli* F5 (K99) + STa	*E. coli* F17	Rotaviruses	Coronaviruses	*C. parvum*
I (control)	2 (16.7%)	–	–	–	1 (8.3%)
II	6 (50.0%)	3 (25.0%)	1 (8.3%)	–	3 (25.0%)
III	7 (58.3%)	5 (41.7%)	4 (33.3%)	2 (16.7%)	11 (91.7%)

Corrected serum chloride values were calculated using formula (Fencl et al. 2000):

$$[Cl^-_{Corr}] = [\text{observed } Cl^-] \times [\text{normal } Na^+] / [\text{observed } Na^+],$$

where normal sodium concentration was mean value for the control group. This value was inserted into calculation the SID equation.

In the classic model, acid-base balance was determined on the basis of blood pH, pCO_2, HCO_3^-, BE. Anion gap (AG):

$$AG = [Na^+] + [K^+] - [HCO^-_3] + [Cl^-]$$

Additionally, the anion gap corrected for albumin equation was calculated with formula (Figge et al. 1998):

$$AG_{Corr} = AG + 2.5 \times ([\text{normal albumin in g/dl}] - [\text{observed albumin in g/dl}]),$$

where normal albumin concentration was mean value for the control group.

In the strong ion model, acid-base balance was determined on the basis of strong ion difference (SID), effective strong anion difference (SID_{Eff}), total plasma concentration of nonvolatile buffers (A_{Tot}) and strong ion gap (SIG), calculated according to the following formulas (Constable 1997, 2000):

$$SID_3 = [Na^+] + [K^+] - [Cl^-_{Corr}]$$

$$SID_7 = ([Na^+] + [K^+] + [Mg^{2+}] + [Cl^{2+}]) - ([Cl^-_{Corr}] + [\text{lactate}])$$

$$A_{Tot} = ([\text{albumin in g/dl}] \times [0.123 \times pH - 0.631] + [PO_4 - \text{in mmol/l} \times \{pH - 0.469\}]$$

$$SID_{Eff} = 2.46 \times 108 \times PCO2/10\ pH + A_{Tot}$$

$$SIG = SID - SID_{Eff}$$

Statistical analysis

The results were subjected to statistical analysis with Statistica ver. 10 software. The significance of intergroup differences in the analyzed parameters was verified using ANOVA and Duncan's post-hoc test. The differences were considered significant at $P<0.05$ and $P<0.01$. Analysis of correlations between the classic model H-H and strong ion approach parameters was performed using the Speraman's correlation.

Results

Study Population

All the calves with chronic diarrhea presented with loose, yellow or gray-yellow stools, and normal or slightly decreased rectal body temperature (mean 38.5°C, range 37.9-39.2°C). Eight calves from group II showed more than 5% dehydration; moreover, moderate or severe cachexia was diagnosed in five and one animal, respectively (Table 3). The animals from group III presented with the worst general status; all of them showed severe cachexia and moderate (n=7) or severe (n=5) dehydration.

Microbiology

Two control calves turned out to be colonized with *E. coli* F5 (K99) STa and another one with *C. parvum* (Table 4). *C. parvum* was also detected in stool samples from 3 (25.0%) calves of group II. Other pathogens detected in animals from this group included *E. coli* F5 (K99) STa (n=6, 50.0%), *E. coli* F17 (n=3, 25.0%) and a rotavirus (n=1), None of the animals from group II tested positively for coronaviruses. The list of enteropathogens found in animals from group III included *C. parvum* (n=11, 91.7%), *E. coli* F5 (K99) STa (n=7, 58.3%), *E. coli* F17 (n=5, 41.7%), rotaviruses (n=4, 33.3%) and coronaviruses (n=2, 16.7%). None of the calves, irrespective of the group, were colonized with *Salmonella* spp.

Acid-base balance and biochemical parameters

The acid-base balance parameters of the study calves, estimated with the Henderson-Hasselbach equation, are presented in Table 5 and 6. Control calves, as well as animals from groups II and III did not differ significantly in terms of their blood pH, equal to 7.37, 7.38 and 7.39, respectively. Plasma concentrations of HCO_3^- BE and partial pressure CO_2 (pCO_2) of animals from the two groups with chronic diarrhea (II and III group) were significantly higher than those determined in the controls. The highest BE (6.03 mmol/l) was documented in calves from group II. The calves from group III (Table 6) showed significantly lower plasma concentrations of albumin (15.37 g/L), chloride (Cl^- 74.94 mmol/L, Cl^-_{Corr} 74.12 mmol/L), magnesium (0.53 mmol/L) and phosphorus (1.41 mmol/L), and significantly higher value of anion gap (39.03 mmol/l) and corrected anion gap (42.32 mmol/l) than the remaining animals (P<0.01).

The acid-base balance parameters of the strong ion model are presented in Table 7. The diarrheic calves with hypoalbuminemia (group III) presented significantly higher SID_3, SID_7 and SIG values than animals from the remaining groups (P<0.01), whereas A_{Tot} and SID_{Eff} were significantly lower (P<0.01).

Table 8 shows the association between selected parameters of classic model and strong ion approach. The parameters of classic model and strong ion approach did not correlate with blood pH. Data analysis shown positive correlation between pCO_2, HCO^-_3 and BE. AG and AG_{Corr} values correlated significantly (P<0.01) with SID_3, SID_7 and SIG. In addition there was a negative correlation between A_{Tot}, SID_{Eff} and AG parameters. There was no significant influence of albumin correction on anion gap for correlation of this parameters with parameters of Stewart model.

Discussion

We analyzed two types of acid-base disorders associated with chronic diarrhea: completely compensated acid-base disorder (group II) and acid-base imbalance accompanied by hypoalbuminemia (group III). The animals with these two conditions differed in terms of their clinical characteristics, acid-base balance parameters and etiology of diarrhea. The calves from the two groups were colonized with ETEC, most often *E. coli* F5 (K99). This strain is one of the most significant *E. coli* involved in the etiology of infectious calf diarrhea. In contrast, another fimbrial type of *E. coli* isolated from our material (*E. coli* F17) is not considered an important cause of diarrhea despite its high prevalence in calves (Osek 2001, Foster and Smith 2009, Herrera-Luna et al. 2009). The infection with ETECs leads to secretory diarrhea with resultant loss of water and electrolytes. Contrary to the calves from the group II, a considerable fraction of the animals with hypoalbuminemia were infected with *C. parvum*. This enteropathogen can cause chronic diarrhea and is responsible for more severe course of mixed infections. The cryptosporidial colonization of the small and large intestine results in destruction of enterocytes. Contrary to isolated bacterial or viral infections, mixed infections with *C. parvum* are associated with shortening of intestinal villi and persistent severe disorders of digestion and absorption (Coutinho et al. 2008, Ok et al. 2009).

The analysis based on the classic model of the blood pH and other acid-base balance parameters of animals from the group II and III revealed a completely compensated acid-base disorder (Kraut and

Table 5. Mean values of acid-base balance parameters (classic model) in calf blood.

Group	PH		pO_2 kPa	pCO_2 kPa	HCO^-_3 mmol/L	BE mmol/L	AG mmol/L	AG_{Corr} mmol/L
I (control)	Mean	7.37	4.27^{A}	5.92^{A}	26.44^{aA}	0.76^{A}	14.10^{A}	13.91^{A}
	SD	0.03	0.83	0.38	1.98	1.20	4.69	4.41
II	Mean	7.38	3.99^{A}	8.10^{B}	33.33^{B}	6.03^{B}	11.74^{A}	12.47^{A}
	SD	0.04	0.33	0.58	2.28	1.39	4.70	4.74
III	Mean	7.39	3.08^{B}	8.19^{B}	30.19^{b}	2.16^{A}	39.03^{B}	42.32^{B}
	SD	0.03	0.62	0.64	3.42	1.47	14.03	14.07

^{a,b,c} – significance of differences between groups at P≤0.05
^{A,B,C} – significance of differences between groups at P≤0.01

Table 6. Mean values of electrolytes, albumins, and lactate concentrations in calf blood.

Group		Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	Cl^-_{Corr} mmol/L	Ca^{2+} mmol/L	Pi mmol/L	Mg^{2+} mmol/L	Albumins g/L	Lactate mmol/L
I (control)	Mean	138.93	4.70	103.09^{A}	102.97^{A}	2.26	1.77^{A}	0.84^{A}	28.06^{A}	1.03^{aA}
	SD	2.7	0.33	3.11	2.85	0.20	0.30	0.12	1.44	0.43
II	Mean	138.50	4.95^{A}	98.39^{B}	98.72^{B}	2.37	1.72^{A}	0.83^{A}	25.46^{B}	2.03^{B}
	SD	2.71	0.28	4.29	4.58	0.28	0.19	0.08	1.44	0.63
III	Mean	139.71	4.47^{B}	74.94^{C}	$74,12^{C}$	2.18	1.41^{B}	0.53^{B}	15.37^{C}	1.66^{b}
	SD	3.19	0.23	11.87	12.38	0.22	0.17	0.08	2.26	0.50

^{a,b,c} – significance of differences between groups at P≤0.05
^{A,B,C} – significance of differences between groups at P≤0.01

Table 7. Mean values of acid-base balance parameters (strong ion approach) in calf blood.

Group		SID_3 mmol/L	SID_7 mmol/L	A_{Tot} mmol/L	SID_{Eff} mmol/L	SIG mmol/L
I (control)	Mean	40.54^{A}	42.03^{A}	11.83^{A}	38.27^{A}	3.61^{A}
	SD	2.96	3.17	0.53	1.68	2.46
II	Mean	45.07^{B}	46.23^{B}	11.30^{A}	44.23^{B}	2.44^{A}
	SD	2.82	2.44	0.85	6.88	3.93
III	Mean	71.89^{C}	72.92^{C}	6.82^{B}	36.71^{A}	43.53^{B}
	SD	16.02	16.18	0.81	3.38	8.06

^{a,b,c} – significance of differences between groups at P≤0.05,
^{A,B,C} – significance of differences between groups at P≤0.01

Table 8. Correlation coefficients between acid-base parameters of classic model and strong ion approach (Steward model) in calf blood.

Item	pH	pCO_2 kPa	HCO^-_3 mmol/L	BE mmol/L	AG mmol/L	AG_{Corr} mmol/L	SID_3 mmol/L	SID_7 mmol/L	A_{Tot} mmol/L	SID_{Eff} mmol/L
pCO_2 kPa	0,043	1								
HCO^-_3 mmol/L	0,004	0,733**	1							
BE mmol	0,190	0,497*	0,715**	1						
AG mmol/L	0,047	0,361	-0,204	-0,346	1					
AG_{Corr} mmol/L	0,066	0,395	-0,169	-0,324	0,999**	1				
SID_3mmol/L	0,046	0,561**	0,054	-0,166	0,966**	0,974**	1			
SID_7 mol/L	0,060	0,536**	0,028	-0,183	0,972**	0,979**	0,999**	1		
A_{Tot} – mmol/L	0,247	-0,594**	-0,160	0,077	-0,828**	-0,856**	-0,887**	-0,884**	1	
SID_{Eff} mmol/L	0,161	0,365	0,844**	0,700**	-0,634**	-0,617**	-0,426	-0,449*	0,395	1
SIG mmol/L	0,080	0,398	-0,160	-0,316	0,983**	0,987**	0,975**	0,980**	-0,869**	-0,616**

Correlation coefficient significant at: * P≤0.05; **P≤0.01

Madias 2007, Fidkowski and Helmstrom 2009, Gomez et al. 2013). The compensation resulted from activation of metabolic mechanisms, namely from an increase in HCO_3^- and BE concentration. This profile of changes was specific for metabolic alkalosis of a moderate degree (Radostits et al. 2007). The calves from the group III according the classic model using gasometric parameters in blood, which do not take in to account an influence of strong electrolyte and albumins, did not show markedly more severe acid-base disorders (Constable 2000, Constable et al. 2005), despite apparently worse general status.

The calves with diarrhea presented significantly lower concentrations of chloride than the controls ($P<0.01$), moreover the animals from the group III showed a more considerable decrease in concentration of this anions than calves from group II. Also previous studies documented a decrease in plasma Cl^- concentration of diarrheic calves (Seifi et al. 2006, Sobiech and Kuleta 2006, Kupczyński et al. 2012), especially in the animals with the severe diarrhea (Guzelbektes et al. 2007). However, this is not necessarily associated with concomitant decrease in sodium level. The etiology of the hypochloremia observed in our calves might be heterogeneous. It might result from alimentary disorders, abomasal atony, vomiting or lack of chloride reabsorption in distal segment of the alimentary tract (Gennari and Weise 2008). The presence of enteropathogens in stool samples from some animals from the group III may point to infection-related impairment of chloride absorption from the gut. According to Forni et al. (2006) and Lorenz (2009), the decrease in Cl^- concentration may also represent a compensatory mechanism for acidosis associated with high concentration unmeasured anions. We cannot exclude such etiology of hypochloremia in our calves, as they presented with increased AG and normal pH; these findings may point to an increase in the concentration of at least one unmeasured anion, counterbalancing the excess of cations (Kraut and Madias 2007). However, the exact nature of unmeasured anions involved in this process still remains unexplained, as similar to many previous studies (Forni et al. 2006), we did not find elevated lactate or phosphate levels. Moreover, chronic diarrhea is frequently associated with a decrease in phosphate concentration (Fidkowski and Helstrom 2009), what was as well observed is in our study. The unmeasured anions likely represent weak organic acids or ketones. Moreover, they may be quickly metabolized, with resultant decrease in anion concentration, further corrected by an increase in HCO_3^- level (Forni et al. 2006, Lorenz 2009). It is noteworthy that the hereby analyzed group of calves presented with elevated plasma concentration of HCO_3^- and increased pCO_2, which might represent a compensatory mechanism (Gomez et al. 2013), namely a response to the decrease in chloride ion concentration.

Parallel with low concentration chloride, the calves from the group III showed a significant decrease in albumin concentration. Hypoalbuminemia is the most important single disturbance in acid-base chemistry in critically ill patients (Kellum et al. 1995, Figge et al. 1998, Feldman et al. 2005). Albumin is the only important buffering plasma protein; its level plays a relatively major role in clinical acid-base homeostasis, and hypoalbuminemia leads to metabolic alkalosis (Feldman et al. 2005). Microbiological examination revealed that a considerable percentage of the calves with hypoalbuminemia were infected with *C. parvum*. Such etiology of chronic diarrhea was likely reflected by the severity of acid-base disorders observed in this group, especially with regards to the decrease in plasma albumin concentration. Decreased concentration of albumin is an important inflammatory marker in ruminants (Tothova et al. 2014). The presence of inflammation in our calves was also confirmed indirectly, on the basis of the presence of enteropathogens in the stool samples.

An analysis of anion gap only partly provide an answer regarding the character of the disorder, in analyzed cases of the calves from the group III. This value is calculated from the equation which takes into account the most important cations and anions (sodium, potassium, chloride and bicarbonate), but did not take into account significant plasma buffers, including albumins and phosphates. Although, the calves from the group III showed a significant increase in AG, in this case "corrected anion gap" better characterized acid-base disturbance, because of impact of hypoalbuminemia (Figge et al. 1998, Feldman et al. 2005).

The traditional approach to acid-base assessment using the Henderson-Hasselbalch equation provides a clinically useful and accurate method when plasma albumines and protein concentrations are within the reference range (Constable et al. 2005). The analysis based on the strong ion model of the calves from the group II revealed elevated values of SID_3, SID_7, accompanied by an increase in SID_{Eff}, corresponding to a compensated acid-base disorder with a shift towards the alkaline side. Markedly more severe and complex disorders were observed in the animals from the group III. Elevated SID parameters indicated a strong anion alkalosis mainly caused by low concentration of chloride. Moreover, decreased A_{Tot} indicated nonvolatile buffer ion alkalosis as a result from decrease in plasma concentrations of albumins (Figge et al. 1998, Constable et al. 2005). Elevated

SIG points the difference between the sum of all strong cation concentrations and the sum of all measured anion concentrations (Cl^-, lactate, albumins, phosphate and HCO_3^-), resulting from the presence of elevated concentrations of unmeasured strong ions, such as formate, sulfate, ketoacids or fatty acids (Forni et al. 2006). The SIG of the calves from the group III differed significantly from that of the controls, and the SIG of either the control calves or group II animals were typical for healthy individuals (Gomez et al. 2013); this laboratory evidence was consistent with the clinical status of the animals from the two groups.

There are few descriptions of correlation between parameters of classic model and Steward model. Our study revealed, that SID, SIG and AG_{Corr} or AG were tightly correlated and showed excellent agreement in the examined calves with chronic diarrhea. Accordingly with previous studies SIG and AG_{Corr} were correlated in intensive care patients, as well as in healthy volunteers (Kellum et al. 1995, Dubin et al. 2007, Fidkowski and Helstrom 2009). Moreover, Kellum et al. (1995) found tight correlation between SIG and AG_{Corr}, but not AG. On the other hand, our study showed no agreement between SID and BE, HCO_3^- and BE, what was observed by Kellum et al. (1995) in intensive care patients. Moreover, we observed correlation between SID_{Eff} and HCO_3^- or BE. This associations indicated compensation resulted from activation of metabolic mechanisms.

In conclusion, chronic diarrhea leads to numerous water-electrolyte and acid-base balance disorders. Characterization of acid-base disturbance in this cases shown that classic model have some limitations. This model can not be recommended for use whenever serum albumin or phosphate concentrations are markedly abnormal. Strong ion gap provides a more accurate estimate of the unmeasured strong anion concentration than AG, because SIG account changes in concentration of more anions (albumins, phosphate, HCO_3^-). Using this method, we found the simultaneous presence of more than one acid-base disorders. Moreover, hypoalbuminemia and compensated acid-base disorders should always be included in diagnostic procedures and treatment of calves with chronic diarrhea.

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