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Contribution of L^+ and D^- lactic acid to metabolic acidosis during neonatal calf diarrhea

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Summary

Neonatal calf diarrhea is often complicated by lactic acidosis. There are two sources of endogenously produced lactic acid (LA), namely L- and D- LA. Both forms of this metabolite are preferentially generated in the muscles and alimentary tract respectively. Because there are discrepancy about participation of the both forms of LA in development of acidosis in young calves, the aim of this study was to establish the degree of these two optical enantiomers contribution to acidosis in affected animals. To determine particular involvement of each LA izoform in the acid-base disturbances, the HPLC method with chiral column, which permits both accurately separate and quantify the alalysed enatiomers, was used. Moreover, to characterize the origin and importance of this kind of metabolic acidosis, the anion gap was calculated. Calves (n = 29) fed with milk replacer (composition: concentrate of soybean protein, dry sweet whey, vegetal lipids, vitamins mixture, bioactive compounds, calcium formate, probiotics) were divided into three groups: I – healthy calves (control), II – affected calves with mild course of diarrhea and III – calves with severe forms of diarrhea. In the control group of calves, blood pH averaged 7.44 ± 0.02. In the other groups, concomitantly with progress of clinical signs of diarrhea, the pH value systematically decreased. Both diarrhoeic groups of calves demonstrated higher value of anion gap in comparison to healthy animals (14.06 ± 2.25 mEq/l). However, only in third group of calves AG elevated significantly ($p \leq 0.05$) to 27.03 ± 1.26 mEq/l. Among two izoforms of LA present in serum, D(–) enantiomer dominated only in diarrhoeic groups of animals. During mild as well as severe course of diarrhea, D-LA concentration markedly increased to 1.82 ± 0.54 mM/l and 4.74 ± 1.89 mM/l respectively. In calves with severe form of diarrhea there was high positive correlation between D–LA serum level and anion gap (r = 0.722)

Keywords: neonatal diarrhea, lactic acid, anion gap

Metabolic acidosis, apart from dehydration and electrolyte disturbances, is a frequent complication of neonatal calf diarrhea (2, 8, 10, 11, 16). During the course of diarrhea very important for prevision of the resumption of this disturbance is to show the direction and the range of deviation in an anion gap (AG). A severe form of this acid-base disturbance may cause death due to altering the intra- and extracellular myocyte potassium ratio and causing heart failure (5, 16). Initially, the intestinal loss of bicarbonate (HCO_3^-), H_2O and electrolyte is the main pathogenic mechanism of acidosis. The augmentation of L-lactate observed in such circumstances stems from poor tissue perfusion because of dehydration and endotoxemia with subsequent anaerobic glycolysis (16). Another way of „calf scours" – proceeding with accumulation of lactic acid in the colon – is frequently found in animals fed with soybean protein contained in milk replacers (5, 10, 11, 17). The soybean protein intolerance, occurring in many calves, results in villus atrophy and crypt hyperplasia and finally leads to maldigestion and malabsorption. In such conditions, the intestinal bacterial fermentation of a large supply of undigested, fermentable carbohydrates leads to the production not only short-chain fatty (SCFAs), such as acetic, propionic or butyric acid but, above all, lactic acid (LA) (5). Moreover, milk replacer-fed calves very often demonstrate ruminal acidosis, which occurs as a consequence of the failure of the esophageal groove reflex (ruminal drinking). Instead of being delivered directly to abomasum, milk spills into the reticulo-ruminal cavity, where LA are produced by bacterial fermentation (6, 10, 11). In both diarrhetic and „ruminal drinking" calves, overproduction of LA in the gastrointestinal tract results in absorption of these unsaturated, non-volatile acids into the systemic circulation. Since L+ enantiomer of LA is readily metabolized to pyruvate in the liver and kidney, it tends not to be present in the

blood in high levels. D-LA is metabolized relatively slowly and can be accumulated in the blood to the cardio- and neurotoxic level.

It should be mentioned that till now the analysis of plasma LA concentration both by non-chiral high performance liquid chromatography and spectrophotometrically does not discriminate between two different forms of this compound. Thus, it was impossible to determine alternative sources of plasma acidification (15). Using a rapid, sensitive and stereospecific HPLC assay, suitable for the measurement of LA enantiomers, this study aimed to investigate the particularly of L^+- and D^--lactic acid contribution to the development of metabolic acidosis during neonatal diarrheic calves. Moreover, to characterize the origin and range of metabolic acidosis, the anion gap was calculated after the quantification of the acid-base balance.

Material and methods

Simmental (20) and Holstein Friesian (9) calves, 19 males and 10 females, aged between two and ten days were used in this experiment. All the conducted animal experiments were constantly supervised by the Local Ethics Committee in Lublin n°2 (nr 17/24). Based on clinical investigations, including appearance and maintenance fever, degree of dehydration, inappetence, feces consistence and frequency of defecation, calves were divided into three groups: I – healthy calves (control) (n = 10); II – affected calves with mild course of diarrhea (n = 10); and III – calves with severe forms of diarrhea (n = 9). All animals were fed three times daily with „Sanolac rot", a milk-replacer, composed of dry sweet whey, soya protein concentrate, vegetal lipids, vitamins mixture, bioactive compounds, calcium formate, probiotics (Sano-Futter GmBH, Loiching, Germany) (tab. 1). None of the calves were treated with antibiotics and were submitted fluid therapy.

Blood samples (2 × 5 ml) were taken from the jugular vein immediately following the submission of the calves. An aliquot of blood was placed into a heparinised tube for blood gas and electrolyte analysis and another aliquot was placed into EDTA containing tubes for (±)- lactic acids enantiomers determination.

Blood analysis. Gasometry and serum minerals concentration (Na, K, C and Cl) was performed by the ABL80 Flex (Radiometer, Copenhagen) blood gas analyser. The anion gap (AG) was calculated using the following formula $AG = (Na^+ + K^+) – (Cl^- + HCO_3^-)$ (1, 3, 20).

The stereospecific analysis of DL-(±)- lactic acid enantiomers. Serum samples were deproteinised by specific ultrafiltration. 100 µl of serum was added to an Ultrafree centrifugal filter unit (Whatman VectaSpin Micro Centrifuge Tube Filters, 5 Kd, Whatman International Ltd., England), along with 50 µl internal standard (malonic acid) solution and made up to 200 µl with double distilled water. The mixture was then spun down at 5500 *g* for 30 min. Afterwards, aliquots of 20 µl from the filtrate were injected into a high performance liquid chromatography system (HPLC, Beckman, Gold System, USA). The analysis employed a stainless steel 3 µm ODS packed analytical column (50 × 4.6 mm I.D., Chiral Pak MA+) coated with N,N-dioctyl-L-alanine as chiral selector (Chiral Technologies, Exton, PA, USA). The mobile phase consisting of 2 mM copper sulphate containing 1% acetonitrile was pumped at 0.4 ml/min in the isocratic mode (125 SM Beckman, USA) at the room temperature. The UV detection was performed at 236 nm. This wavelength represented the UV maxima of a solution of lactic acid in the stereospecific mobile phase (16). The concentration of each racemic forms of LA was calculated according to the formula:

$$C_p = C_s/A_s \times A_p$$

C_p – sample concentration of LA; C_s – standard concentration; A_s – standard peak area; A_p – sample LA peak area.

Tab. 1. Ingredients and composition of milk replacer Sanolac Rot

Composition of milk-replacer (%)		Content in 1 kg of Sanolac Rot	
Total protein	20.0	Vit. A	50 000 j.m.
Lipid	18.0	Vit. D3	5000 j.m.
Lysine	1.6	Vit. E	100 mg
Methionine	0.8	Vit. B1	4 mg
Threonine	0.95	Vit. B2	4 mg
Tryptophan	0.55	Vit. B6	2 mg
Calcium	0.9	Vit. B12	20 µg
Phosphor	0.7	Nicotinic acid	20 mg
Natrium	1.0	Ca-D-panthotenian	10 mg
Magnesium	0.2	Folic acid	1 mg
Fibre	1.0	Choline chloride	250 mg
		Biotin	200 µg
		Vit. C	100 mg
		Ferrous	100 mg
		Str. faec.	0.8 mld

Tab. 2. The values of plasma pH obtained in healthy and diarrhoeic calves (means ± SD)

	Healthy calves (n = 10)	Calves with mild course of diarrhea (n = 10)	Calves with severe course of diarrhea (n = 9)
Plasma pH	7.44 ± 0.02	7.39 ± 0.02	7.24 ± 0.04

Results and discussion

Blood pH averaged 7.44 ± 0.02 (tab. 2) in the control group of calves. In the other group, concurrently with the progression of clinical signs of diarrhea the pH value systematically decreased. In the calves with a severe course of diarrhea pH dropped to 7.24 ± 0.04.

Among two isoforms of LA presented in calves serum, D(–) enantiomer dominated only in diarhetic groups of animals (fig. 1). In control conditions the equilibrium between D(–) and L(+) isoforms of lactic acid was observed. During the mild as well as severe course of diarrhea, D-LA concentration markedly in-

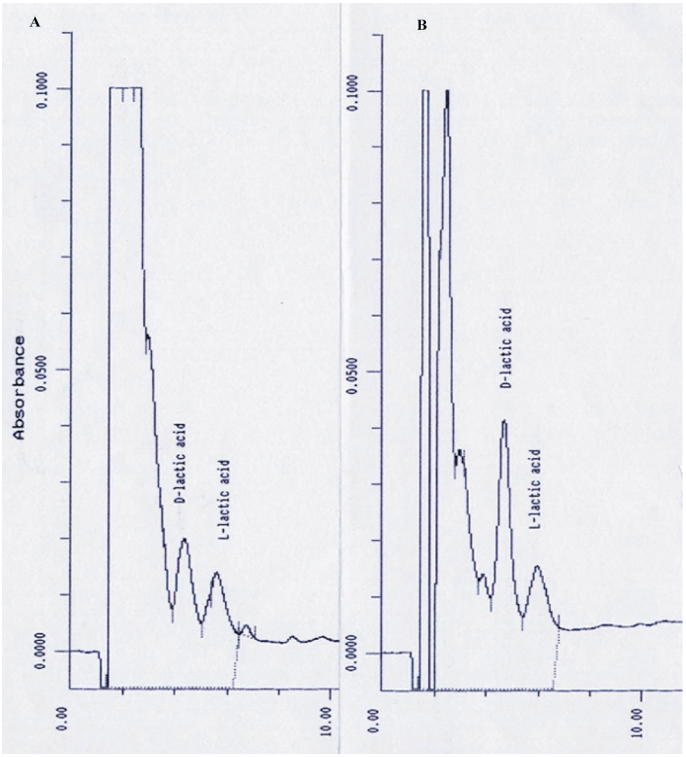

Fig. 1. HPLC chromatogram of L^+ and D^- isoforms of lactic acid in healthy calves (A) and in calves with severe course of diarrhea (B)

creased to 1.82 ± 0.54 mM/l and 4.74 ± 1.89 mM/l respectively (fig. 2). It should be stressed that L(+) enantiomer of LA remained at the same level in healthy and affected animals and did not exceed 0.72 ± 0.07 mM/l.

Both diarhetic groups of calves demonstrated a higher value of anion gap in comparison to healthy animals (14.06 ± 2.25 mEq/l) (fig. 3). However, only in the third group of calves was AG elevated significantly ($p \leq 0.05$) to 27.03 ± 1.26 mEq/l.

In calves with a severe form of diarrhea there was a high positive correlation between total lactic acid concentration and anion gap (r = 0.755). It is noteworthy that a comparable positive relationship was observed between D-LA serum level and anion gap (r = 0.722) (fig. 4). The correlation between L-LA and AG was also positive but with a very low correlation coefficient (r = 0.32).

As shown in fig. 2, the employment of the HPLC method with a chiral (Chiral Pak MA+) column, permitted the separation of L(+) and D(–) enantiomers of plasma lactic acids. Taking into account the different origin of L(+)-LA and D(–)-LA in the body, obtainment of each of the isoform concentrations is very useful in determining their source (13, 15). In their healthy state, mammalian tissues generate only minimal amounts of D-lactic acid from the methylglyoxal pathway (6). This isoform of LA might also originate from the action of gut bacteria, which are able to produce both racemic forms of LA in substantial amounts (6). Many authors indicate that the major pathogens

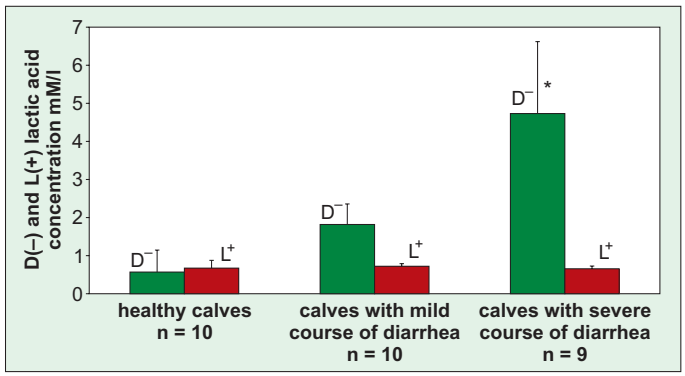

Fig. 2. Changes of plasma L-LA and D-LA concentration in healthy and affected calves
Mean ± SD; * – significant differences at $p \leq 0.05$ (vs. control group)

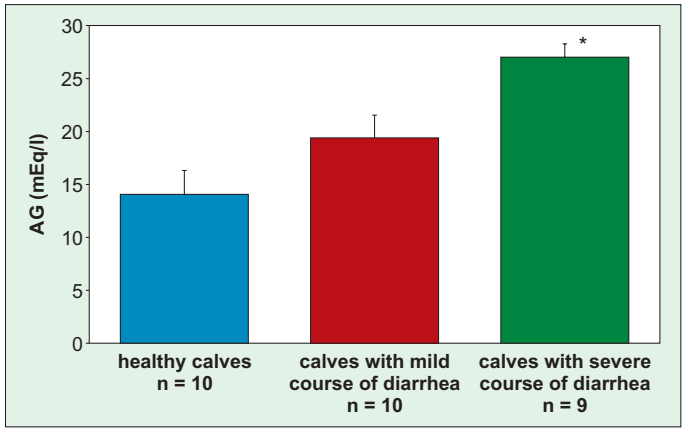

Fig. 3. The values of anion gap (AG) obtained under control and diarrheic condition in calves
Mean ± SD; * – significant differences at $p \leq 0.05$ (vs. control group)

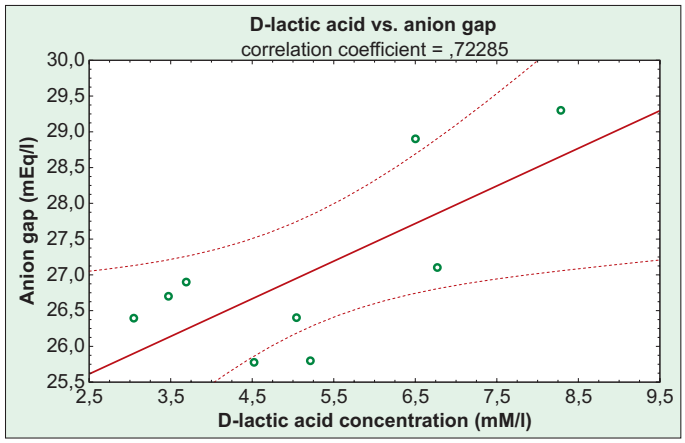

Fig. 4. Correlation between D^--LA plasma concentration and anion gap value in calves with severe course of diarrhea (r = 0.722)

involved in calf diarrhea are known to cause villous atrophy, which may lead to malabsorption of carbohydrates and their subsequent fermentation in the intestine (10, 11, 18). Similar mechanisms leading to the generation of lactic acid, especially D^- optical enantiomer, are frequently observed in milk or milk-

-replacer fed calves. Such a condition, termed „ruminal drinking", occurred when the ingested milk enters the reticulo-rumen cavity (4, 11). Intestinal or ruminal accumulation of both racemic isomers can lead to their absorption in the bloodstream, with the consequence of systemic metabolic acidosis (6). Although the L(+) isoform is rapidly metabolised to pyruvate, the non--metabolised D(–)-LA assembling in the blood results in D-lactatemia. According to Uribarri et al. (19) such a condition appears when the blood concentration of D-LA exceeds 3.0 mM/l. Additionally, in the case of dehydration, reduced renal perfusion and therefore reduced excretion of hydrogen ions exacerbates acidosis (10, 11). The obtained in diarrheic group of calves 3- and 8-fold increase in D^- -LA level without marked changes in plasma L-LA concentration, univocally indicates the progress of D-lactic acidosis in these animals. It should be taken into account that severe metabolic acidosis causes high mortality in diarrheic calves and alters the structure and function of proteins performing essential functions in the body. Moreover, the altered intracellular to extracellular myocyte potassium ratio impairs cardiovascular functions through the depression of vascular tone, myocardial contractility and metabolism (5, 12). Additionally, animals with D-lactic acidosis have neurological dysfunctions characterized by tremors, myoclonic jerks, ataxia, weakness and finally coma (5, 12).

In this study, concurrently with the intensification of diarrhea signs and plasma D-LA elevation an increase in the anion gap was observed. In this regard, the obtained results are similar with recent evidence, which indicates that a high value of AG is a reflection of an accumulation of „unmeasured" strong anions, particularly organic acids such as lactic acid (5, 7). Due to the slow metabolism of D(–)-LA, many authors indicate that only this isomer is responsible for the anion gap elevation in diarrheic animals (4, 6, 10, 11). On the other hand Hartmann et al. (9) and Naylor (14) reported that during the first week of life only L(+)-LA overproduction in diarrheic animals has been found. Despite the primary cause of AG elevation, it should be stressed that its high value is an unfavorable prognostic sign, especially in neonatal animals. Moreover, the fact must be taken into account that during diarrhea acute dehydration may cause an increase in AG due to the elevated concentration of negatively charged plasma proteins, primarily albumin (7).

Conclusion

The present study demonstrates that the HPLC-stereospecific determination of plasma lactic acid permits the precise separation of both its racemic forms. Furthermore, depending on the high concentration of negative optical isoform of LA as well as a high value of anion gap in diarrheic calves, it can be suggested that D-lactic acidosis has been the main complication of neonatal calf diarrhea.

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