

D-Lactate Production and Excretion in Diarrheic Calves

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The origin of D-lactate, the most important acid contributing to metabolic acidosis in the diarrheic calf, is unknown. We hypothesized that because D-lactate is produced only by microbes, gastrointestinal fermentation is the source. The objective of this study was to determine whether D-lactate production occurs in the rumen, colon, or both, and to measure D- and L-lactate concentrations in urine. Fecal, rumen, blood, and urine samples were obtained from 16 diarrheic and 11 healthy calves. Serum electrolyte concentrations were measured in both groups, and blood gas analyses were performed for diarrheic calves. All samples were analyzed for D- and L-lactate by high performance liquid chromatography (HPLC). Diarrheic calves were generally hyperkalemic with high serum anion gap, depressed serum bicarbonate, and low blood pH. L-lactate was markedly higher in rumen contents (22.7 mmol/L [median]) and feces (8.6 mmol/L) of diarrheic calves than healthy calves (0.5 mmol/L and 5.1 mmol/L, respectively), but not different in serum or urine. Rumen, fecal, serum, and urine D-lactate concentrations were all significantly higher ($P < .05$) in diarrheic calves (17.0, 25.4, 13.9, and 19.2 mmol/L, respectively) than in healthy calves (0.5, 9.1, 1.4, and 0.5 mmol/L, respectively). Higher D-lactate concentrations in the rumen and feces of diarrheic calves suggests these sites as the source of D-lactate in blood and urine.

Key words: Acid-base; Diarrhea; Metabolic acidosis.

Despite advances in the prevention of neonatal calf diarrhea, including better management practices and the development of effective vaccines against enterotoxigenic *Escherichia coli*, diarrheic calves continue to be admitted to veterinary clinics in large numbers.¹ The major systemic effects of diarrhea are hypovolemia, acidemia, and electrolyte imbalances. These problems are not unique to calves, but calves may suffer from a more severe acidemia than other species.¹

The etiology of diarrhea-associated acidemia has routinely been assumed to be a mixture of loss of bicarbonate in the feces and accumulation of organic acids in the blood.² L-lactate contributes to acidemia in calves, and until recently was assumed to be the major organic acid present in plasma.³ However, D-lactate accounts for approximately 64% of the total increase in organic acids in plasma of acidemic, diarrheic calves.⁴ Since D-lactate is of microbial origin⁵ and not produced by mammalian cells, we postulate that D-lactic acidosis results from fermentation of lactose in the intestine, particularly the large intestine. This mechanism is likely similar to that documented for D-lactic acidosis in short bowel syndrome in humans where massive small intestinal resection leads to the presence of maldigested food in the large intestine.⁶ This substrate is fermented by indigenous bacteria resulting in production of short chain fatty acids (acetate, propionate, butyrate), and consequently decreased lumen pH.⁷ Acid-stable microbes, particularly *Lactobacilli*, proliferate and produce D- and L-lactate, which are subsequently absorbed. Since D-lactate is poorly metabolized by mammals compared with L-lactate, it accumulates in body fluids and causes acidosis.⁸

D-lactic acidosis is also the major component of acidemia

in calves diagnosed as ruminal drinkers.^{9,10} Pooling of milk in the rumen, either as the result of excessive intake or malfunction of the esophageal groove, leads to ruminal fermentation of lactose and D-lactic acidosis. Esophageal groove function may be compromised in sick or diarrheic calves.⁹

The purpose of this study was to further document the contribution of D- and L-lactic acidosis in diarrheic calves, to quantify their relative contribution, and to determine if the rumen or the intestine was the major site of production of D-lactate. Additionally, the urinary excretion of D- and L-lactate was investigated. Elucidating the origin of D-lactate could allow the development of novel treatment strategies that target acidosis at the source, possibly by probiotic or prebiotic therapy.

Materials and Methods

Subjects

Rumen, fecal, blood, and urine samples were obtained from 16 mixed breed calves (Red Angus, Charolais, Aberdeen-Angus, Simmental, Limousin, Holstein-Friesian, Hereford, and Gelbvieh) less than 35 days of age selected from those admitted to the Western College of Veterinary Medicine, University of Saskatchewan, for treatment of diarrhea. Calves were included in the study on the basis of having diarrhea, defined as 3 or more profuse or watery stools per day. Samples were taken within 1 hour of admission. Samples (rumen, fecal, blood, and urine) were also obtained from 11 healthy calves less than 21 days old, which were housed at Goodale Farms, University of Saskatchewan. All calves normally had an oral intake of pooled milk before the study; however, some diarrheic calves received oral rehydration solution (ORS) before admission. This study was approved by the Animal Care Committee of the University of Saskatchewan and was carried out in accordance with guidelines specified by the Canadian Council of Animal Care.

Sample Collection

After the admission of a diarrheic calf to the clinic (typically within 30 minutes), 10 mL of blood were drawn from the jugular vein and allowed to clot at room temperature for 20 minutes, centrifuged, and serum drawn off and frozen at -20°C . A second blood sample was collected into a heparinized syringe for immediate blood gas analysis. Approximately 20 g of feces were collected after perineal massage, mixed with 15 mL of thimerosal^a (1:10,000) as a bacteriostatic agent,

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Submitted November 3, 2003; Revised January 12, 2004; Accepted March 1, 2004.

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0891-6640/04/1805-0022/\$3.00/0

Table 1. Organic acid concentrations in biological matrices of healthy (n = 11) and diarrheic (n = 16) calves.

Acid	Rumen		Feces		Serum		Urine	
	Median (semi-interquartile) ^a Range		Median (semi-interquartile) Range		Median (semi-interquartile) Range		Median (semi-interquartile) Range	
	Healthy (mmol/L)	Diarrheic (mmol/L)	Healthy (mmol/L)	Diarrheic (mmol/L)	Healthy (mmol/L)	Diarrheic (mmol/L)	Healthy (mmol/L)	Diarrheic (mmol/L)
Acetic	15.1 (7.9) 7.9–33.8	10.3 (17.9) 2.1–76.7	77.4 (15.6) 30.7–99.1	22.5 ^c (10.4) 0.5–68.7	0.0 (0.0) 0.0–3.0	0.1 ^b (1.5) 0.0–7.1	—	—
Pyruvic	0.11 (0.15) 0.00–0.45	0.11 (0.04) 0.03–0.23	0.16 (0.16) 0.16–0.16	0.89 ^c (1.91) 0.02–12.04	0.06 (0.01) 0.04–0.11	0.04 (0.02) 0.02–0.11	—	—
L-lactic	0.5 (0.0) 0.5–7.3	22.7 ^c (16.0) 0.5–63.9	5.1 (1.9) 0.5–9.2	8.6 ^b (5.6) 1.3–46.8	1.7 (0.2) 1.2–2.9	1.6 (0.3) 0.9–3.5	0.5 (0.3) 0.5–0.9	0.5 (0.1) 0.0–2.4
D-lactic	0.5 (0.4) 0.0–1.6	17.0 ^c (10.9) 0.5–53.9	9.1 (3.7) 4.5–23.0	25.4 ^b (11.2) 3.7–51.3	1.4 (0.4) 0.8–3.8	13.9 ^c (5.1) 1.2–22.7	0.5 (0.6) 0.5–7.4	19.2 ^c (16.9) 0.5–162.0

^a Semi-interquartile range = (75th percentile–25th percentile)/2.

^b Significant difference between healthy and diarrheic calves at $P < .05$.

^c Significant difference between healthy and diarrheic calves at $P < .01$.

and frozen at -20°C . Rumen juice was collected by the tip and tubing from a human enema kit.^b The tube was introduced PO into the rumen, and approximately 20 g of rumen juice was suctioned out. Rumen fluid samples were mixed with 15 mL of thimerosal (1:10,000) and frozen at -20°C . Approximately 10 mL of urine were collected from each calf as soon as possible after presentation and stored at -20°C until analysis. Some calves required intravenous rehydration before urine was passed.

Sample Analysis

After thawing, serum samples were prepared for high performance liquid chromatographic (HPLC) analysis for lactate enantiomers and other select organic acids as previously described.¹¹ Briefly, serum was mixed with internal standard and ultrafiltered through Ultrafree MC microcentrifugal filtration units^c at $5,000 \times g$ for 30 minutes. The filtrate was injected into the HPLC. Both a stereospecific [D(-)- and L-(+)-lactate] and nonstereospecific (acetate, pyruvate, and racemic lactate) HPLC assay were performed.¹¹

The second blood sample was analyzed by an automated blood gas analyzer.^d Simultaneous determination of plasma sodium, potassium, and chloride ions was carried out by a spectrophotometric auto-analyzer.^e

Fecal samples were thawed at 4°C and shaken for 20 minutes on an automatic shaker. One gram of feces was added to 9 mL double distilled water, homogenized for 1 minute, and centrifuged at $20,000 \times g$ for 30 minutes. The supernatant was filtered through an Acrodisc PF (0.8/0.2 μm) filter^f and ultrafiltered as described for serum. Preparation of rumen samples for HPLC analysis was the same as for feces, except initial dilution was 1:5.

Statistical Analysis

The concentrations of electrolytes in blood and organic acids in each biological matrix for both healthy and diarrheic calves were calculated. To compare blood gas measurements and concentrations of organic acids in healthy and diarrheic calves, the Mann-Whitney U -test ($\alpha = 0.05$) was used when data were nonparametric, and the Student's t -test assuming unequal variances was used when data were parametric. For statistical purposes, parameters that were not detectable by the method used were assigned a value of 0, and parameters that were detectable but below the quantitation limit (QL) of the method were assigned a value of half the QL (pyruvate, 0.016 mmol/L; acetate, 0.5 mmol/L; D- and L-lactate, 0.5 mmol/L). Linear regression was used to assess the relationships between acid concentrations in serum, rumen fluid, and feces.

Results

Urine samples were not collected from 2 diarrheic calves as no urine was passed in the first 12 hours after admission. There were approximately equal numbers of male and female calves in the healthy and diarrheic groups; diarrheic calves were markedly older (mean = 17.7 ± 10.8 days) than healthy calves (mean = 8.6 ± 2.3 days); all calves were less than 35 days. All diarrheic calves received non-lactate-containing intravenous fluids, and none were administered ORS at the time of sample collection.

Diarrheic calves had high serum anion gap (>25 mmol/L, 9 calves); potassium (>5 mmol/L, 5 calves); and chloride (>107 mmol/L, 7 calves) concentrations, and low serum bicarbonate (<21 mmol/L, 14 calves) and pH (<7.3 , 15 calves) compared with reference ranges.

Acetate was significantly higher in the feces, yet significantly lower in the serum of healthy calves, compared with diarrheic calves (Table 1). No difference in ruminal acetate concentration was detected between the groups. Pyruvate was markedly higher in the feces of diarrheic calves than healthy calves, with no difference in the rumen or blood (Table 1). L-lactate was significantly higher in rumen contents and feces of diarrheic calves than healthy calves, but not in serum or urine. D-lactate concentrations were significantly higher in rumen, feces, serum, and urine in diarrheic calves than in healthy calves ($P < .05$; Table 1). Although rumen D-lactate was high in diarrheic calves, it did not correlate to serum D-lactate, and the majority of calves (69%) had higher fecal D-lactate than rumen D-lactate ($P = .88$; Fig 1). In calves where fecal D-lactate exceeded rumen D-lactate (n = 11), serum D-lactate (13.2 ± 5.9 mmol/L) was greater than in those calves where rumen D-lactate exceeded fecal D-lactate (n = 5; 8.1 ± 6.2 mmol/L), but this was not statistically significant ($P = .16$). Six diarrheic calves had normal ruminal D-lactate, and all of these had high serum D-lactate (>3 mmol/L). In 5 of these 6 calves, fecal D-lactate concentration was high compared with healthy calves. Three diarrheic calves had normal fecal D-lactate, and all 3 had high rumen D-lactate, but only 1 had high serum D-lactate.

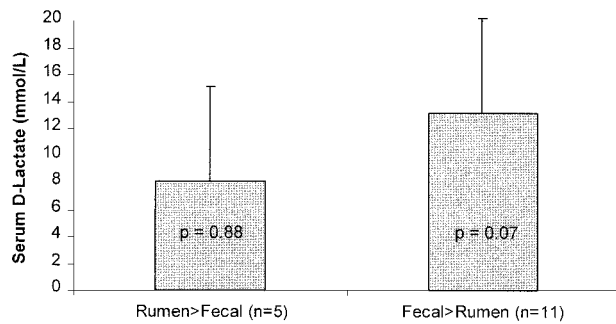


Fig 1. Relationship between fecal and rumen D-lactate concentrations and serum D-lactate concentration in diarrheic calves (n = 16). Bars indicate \pm SD; P-values represent significance of linear regression with serum D-lactate. Regression equations with serum D-lactate: rumen > fecal, $y = 3.35x - 1.99$; fecal > rumen, $y = 0.38x + 20.21$.

Discussion

D-lactic acidosis is occasionally observed in humans as a consequence of short-bowel syndrome,¹² in ruminants after grain overfeeding,¹³ and as a sequela to diarrhea in neonatal calves.^{4,14} D-lactic acidosis is metabolic acidosis accompanied by a serum D-lactate concentration of >3 mmol/L.⁷ D-lactate production and accumulation is caused by fermentation of malabsorbed carbohydrate by *Lactobacillus* species and the subsequent inability of the body to effectively metabolize D-lactate.⁷

Various viruses can survive the acidic environment of the stomach and replicate in columnar epithelial cells of the villi of the small intestine, causing cell lysis and blunting of the villi.¹⁵ Viral-induced villous atrophy leads to the disruption of transport mechanisms on the apical cell membrane of the intestinal epithelium; the loss of surface enzyme activities; and osmotic overload resulting from malabsorption of water, substrates, and ions.¹⁶ The finding of higher fecal D-lactate concentrations in diarrheic calves than in healthy calves supports the hypothesis that milk lactose or other substrates pass unhydrolyzed and unabsorbed into the colon where they are fermented. Ruminal fermentation may also be an important source of D-lactate production.

Ruminal fermentation does not typically occur in neonatal calves because the esophageal groove reflex shunts milk from the esophagus directly into the abomasum. This reflex does not occur in so-called ruminal drinkers and milk pools in the underdeveloped rumen.⁹ Appropriate bacterial populations are not adequately established in young calves, and consequently fermentation is often abnormal and D-lactic acidosis occurs.¹⁰ In this study, some diarrheic calves had very high ruminal concentrations of D-lactate (>20 mmol/L, 7 calves), perhaps indicating malfunction of the esophageal groove reflex, whereas some had low D-lactate concentration in the rumen fluid (<3 mmol/L, 5 calves).

These results indicate substantial variation in the site of production of lactate in diarrheic calves. L-lactate was generally a minor contributor to the total (D + L) lactate, comprising only 13% in serum, 33% in feces, 62% in rumen fluid, and 2% in urine. Although L-lactate was higher in the rumen of diarrheic calves than healthy calves, it either was not sufficiently high to cause systemic L-lactic acidosis, or

was not absorbed at a rate greater than its removal. For several reasons, we conclude that colonic D-lactate production contributed more to systemic D-lactic acidosis than did ruminal production. First, the majority of diarrheic calves had more D-lactate in the feces than in the rumen contents. Second, in the 5 cases in which the rumen contents had higher concentrations of D-lactate than feces, serum D-lactate was not as high as when D-lactate concentrations in the feces were greater than ruminal D-lactate concentrations. Third, fecal D-lactate concentration was more closely related to serum D-lactate than to rumen D-lactate. Since rumen D-lactate concentrations did not correlate with serum D-lactate, and fecal concentrations did, colonic fermentation and subsequent absorption is likely of greater importance than ruminal fermentation. It is possible, however, that some bacterial overgrowth occurs in the small intestine, or that ruminally produced D-lactate is not absorbed until it reaches the colon. The colon is an important absorptive site; 1 study revealed cecally infused D-lactate concentrations of 45 ± 7 mmol/L were sufficient to cause systemic D-lactic acidosis in ruminants.¹⁷ D-lactate is not absorbed from the rumen of sheep at a low pH,¹⁸ but is absorbed from the small intestine and colon,¹⁹ which express the proton-dependent monocarboxylate transporter-1. Monocarboxylate transporter-1 has only been studied in adult ruminants. Monocarboxylate transporter-1 is stereoselective, exhibiting an uptake coefficient for L-lactate twice that for D-lactate and mutual inhibitory effects with a higher affinity for the L-isomer than D-isomer.²⁰ The expression of this transporter in the neonatal calf gastrointestinal tract has not been studied; research would give further insight into how D-lactate production results in hyperlactatemia.

The metabolic pathways for the removal of D-lactate from the blood in animals are much less efficient than those for L-lactate. D-lactate is metabolized to pyruvate by the enzyme D- α -hydroxy acid dehydrogenase, which metabolizes D-lactate about one fifth as fast as L-lactate dehydrogenase metabolizes L-lactate.⁸ Both D- and L-lactate can be excreted by the kidneys. Renal reabsorption of D-lactate is much less than that of L-lactate. Oh et al¹⁸ revealed that D- and L-lactate mutually interfere with their renal absorption. Renal tubular reabsorption of lactate is reduced by increased urine volume²¹; therefore, low kidney perfusion would likely increase reabsorption, thus increasing serum D-lactate concentration. These findings are consistent with this, as most of the calves admitted to the clinic were dehydrated (as evidenced by enophthalmos) and had very high concentrations of D-lactate in the urine (up to 162 mmol/L). The source of D-lactate in diarrheic calves is likely gastrointestinal. Treatments can now be developed to target the source of acid production, rather than buffering systemic acid. Further study to elucidate the site and rate of D-lactate absorption would help clarify the role of ruminal and colonic D-lactate production.

Footnotes

^a ICN Biomedicals, Aurora, OH

^b Picker Rapidfill System, Picker International, Markham, Ontario, Canada

^c Millipore, Bedford, MA

^d Ciba-Corning 288 blood gas system, Ciba-Corning Canada Inc, Markham, Ontario, Canada

^e Abbott Spectrum System, North Chicago, IL

^f Pall Corporation, Ann Arbor, MI

Acknowledgments

This work is from the College of Pharmacy and Nutrition and the Western College of Veterinary Medicine at the University of Saskatchewan and was supported by the NSERC Discovery Grants Program, the Saskatchewan Horned Cattle Fund, and the Alberta Agriculture Research Institute. J.B.E. is supported by a NSERC Canada Doctoral Scholarship. The authors acknowledge the staff at the WCVL Large Animal Clinic and Goodale Farms, Saskatchewan, for assistance in sample collection. In addition, we thank Dr Wallace W. Jones for providing samples used in the preliminary stages of this study. This work was presented in abstract form at the 46th annual meeting of the Canadian Federation of Biological Sciences in Ottawa, Canada, June 2003.

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