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D-Lactic Acidosis in Calves as a Consequence of Experimentally Induced Ruminal Acidosis

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With 5 tables

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Summary

In order to test the hypothesis that ruminal drinking in calves can lead to D-lactic metabolic acidosis, ruminal acidosis was induced in nine calves by intraruminal application of untreated whole milk via a stomach tube. The amount of the daily force-fed liquid was 3 × 1 l. The experimental design called for an end of intraruminal applications if two or more of the following signs were observed: severe depression, estimated degree of dehydration > 10%, absence of sucking reflex, lack of appetite for two consecutive feedings, severe metabolic acidosis with calculated Actual Base Excess (ABE) < −15 mmol/l. The procedure was scheduled to be discontinued on the 17th day of experiment. The onset of ruminal acidification occurred rapidly, and mean pH value fell from 6.70 (± 0.48) to 4.90 (± 0.38) after the first application. The following days the pH values varied between 4 and 5. Rumen acidity was characterized biochemically by a significant increase in both isomers of lactic acid. The effects of the intraruminal administration on the calves were detrimental; eight of nine calves showed an acute disease process. According to the pre-established clinical standard, seven of nine calves were removed from the intraruminal feeding schedule. All but one of the calves developed severe systemic acidosis. The increase in anion gap demonstrated the net acid load. In all the calves D-lactate levels were found to show a significant and rapid increase. On the contrary, L-lactate never deviated from physiological levels. These observations confirm that, in young calves as in adult cattle, ruminal acidosis may lead to a clinically manifested D-lactic metabolic acidosis.

Introduction

In milk-fed calves ruminal acidosis is caused by fermentative processes, which occur in the rumen when, as a consequence of failure of the oesophageal groove reflex, milk, instead of being delivered directly to the abomasum, spills into the reticulo-ruminal cavity (ruminal drinking). The most detrimental product that is produced by bacterial fermentation of the carbohydrate fraction of the milk is lactic acid. Contrary to eukaryotic cells, which produce L-lactic acid predominantly (D-lactic acid may be synthesized in mammalian tissue from the methylglyoxal pathway in minimal amounts only), prokaryotic cells, such as bacteria, are able to produce L- and D-lactic acid in substantial amounts. High quantities of both these isomers can therefore accumulate in the rumen. This can lead to an increased lactic acid load in the bloodstream, with the consequence of the onset of a metabolic acidosis. It has been known for decades that in adult cattle the D-isomer of lactic acid is the cornerstone of the metabolic derangements that occur in acute ruminal acidosis (Dunlop and Hammond, 1965; Dunlop, 1972). It can be hypothesized that in calves, which experience ruminal acidosis, D-lactic acid can have the same detrimental metabolic effects as seen in adult cattle. However, no study so far seems to have addressed this issue.

The objective of this study was to test the hypothesis that ruminal acidosis in calves, as in adult cattle, leads to D-lactic metabolic acidosis. In the experiment, oesophageal groove reflex dysfunction was simulated by intraruminal application of untreated whole milk via a stomach tube.

Materials and Methods

The study took place under the supervision of the local 'Centralised Veterinary Service for the Welfare of Experimental Animals' at the University of Bologna and with the approval of the 'Ethical Review Committee of Animal Experimentation' of the University of Bologna.

Animals and nutrition

Eleven healthy Holstein-Friesian male calves, kept in a free housing system with straw bedding, were included in this study. Their age at the beginning of the study ranged between 5 and 23 days. Before the start of the study the calves were fed 2 l of untreated whole milk at 08.00, 13.00 and 18.00 h. Milk was offered from nipple pails.

During the whole experimental period the calves were not allowed access to water, or to solid feed.

Experimental design

Ruminal acidosis was defined as pH < 6.00. Nine calves were randomly chosen and assigned to the experimental group (group A: calves nos 2, 4, 5, 6, 7, 11, 12, 13 and 15). The other two calves (group B: calves nos 9 and 16) served as controls.

Rumen acidification was obtained by intraruminal application of milk via a stomach tube, each time with half of the scheduled milk feeding. This means that during the phase of ruminal acidification calves of group A were force-fed three times a day with 1 l milk. The remaining part of the ration was offered through a nipple pail immediately after each intraruminal application. Calves assigned to group B were fed milk only by nipple pail.

Daily clinical examination of all calves was performed; among the common clinical parameters, particular attention was given to sensorium, sucking reflex, skin turgor, eyeball position, peripheral (fetlock) temperature, heart rate, respiratory rate, appetite and faeces' characteristics. The physical examination was carried out always by the same observer (A.G.), which was not blinded to treatment groups. After having assigned each parameter a predetermined score, the observer gave a total clinical score ranging in value from 0 to 3 (0 = absence of relevant clinical findings; 1, 2, 3 = presence of slight, moderate or severe clinical findings, respectively).

The experiment was divided into three phases:

Phase 1 = pre-induction phase (days −1 and 0): evaluation of the clinical state and determination of the baseline values;

Phase 2 = phase of induction of ruminal acidosis: intraruminal applications of milk in the calves of group A (day 1 onwards);

Phase 3 = recovery phase: end of the intraruminal applications in the group A and evaluation of the recovery from the induced disease.

The experimental design called for an end of intraruminal milk applications (phase 2) if two or more of the following signs or observations were recorded:

severe depression with prolonged recumbency,
estimated degree of dehydration >10%,
complete absence of sucking reflex,
lack of appetite for two consecutive feedings,
severe systemic metabolic acidosis with calculated Actual Base Excess (ABE) <−15 mmol/l.

In any case, the procedure was scheduled *a priori* to be discontinued on the 17th day of the experiment.

During the entire experiment the sequence of the clinical examinations and sampling followed the order illustrated in Table 1.

Rumen fluid examination

After a macroscopic examination (colour, odour, consistency, possible presence of casein coagula), the pH-value was measured with a portable pH meter (Hanna Instruments HI9024 microcomputer pH meter, Hanna Instruments Italia S.r.l., Padova, Italy). Afterwards the rumen fluid was frozen until analysed for D- and L-lactate (spectrophotometric method).

Examined blood parameters

Blood gas analysis (ABL 700, Radiometer, Copenhagen, Denmark) was performed on heparinized blood immediately after sampling. The following parameters were determined: pH, pCO2, $[HCO_3^-]$, ABE. Blood concentrations of sodium (Na^+), potassium (K^+) and chloride (Cl^-) were also determined (ABL 700, Radiometer) in heparinized blood. The anion gap value was calculated as follows: $([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$.

The concentration of D- and L-lactate was determined in plasma samples (spectrophotometric method) prepared from blood collected only in the morning, and frozen until analysis.

Table 1. Sequence of clinical examinations and sampling during the entire experimental period

07.30 hours:	physical examination
07.45 hours:	blood collection through a permanent catheter (18 gauge) fixed by surgical suture in the external jugular vein
07.50 hours:	ruminal fluid collection through a modified foal's stomach tube the distal end of which was made heavy by a leaden head. The tube was introduced through the mouth and suction was applied to obtain a sample
07.55 hours	**only in the calves of group A, during phase 2: intraruminal application of 1 l milk** via the same stomach tube used for the ruminal fluid collection
08.00 hours:	morning feeding
12.45 hours:	blood collection sampling
12.50 hours:	ruminal fluid collection
12.55 hours:	**only in the calves of group A, during phase 2: intraruminal application of 1 l milk** via the same stomach tube used for the ruminal fluid collection
13.00 hours:	midday feeding
17.45 hours:	blood collection sampling
17.50 hours:	ruminal fluid collection
17.55 hours:	**only in the calves of group A, during phase 2: intraruminal application of 1 l milk** via the same stomach tube used for the ruminal fluid collection
18.00 hours:	evening feeding

D-Lactate determination

D-Lactate concentration was measured in ruminal fluid and in plasma by quantitating substrate conversion (spectrophotometrically 340 nm; Hitachi Automatic Analyzer, Hitachi, Tokyo, Japan) to reduced nicotinamide adenine dinucleotide using D-lactate dehydrogenase (D-LDH, Lorenz et al., 2003).

Statistical analysis

The results of the different determinations are presented as mean values and SD. Simple linear regression analysis was used to test the relationship between D-lactataemia and ABE, as well as between D-lactataemia and anion gap.

Results

Phase 1 = pre-induction phase (days −1 and 0)

During the pre-induction phase (days −1 and 0) all calves of both groups were clinically healthy and showed physiological ruminal and biochemical values.

Rumen fluid was found to have a light milky-to-beige colour, a stale smell and a watery consistency. The pH values ranged between 6.28 and 7.05 (mean value for both groups A and B = 6.69 ± 0.18). Concentration of both isomers of lactate were below 1 mmol/l.

Blood ABE and anion gap values ranged between −0.1 and 5.7 mmol/l (mean value for both groups A and B = 3.17 ± 1.61) and 7.5 and 18.2 mmol/l (mean value for both groups A and B = 11.61 ± 2.14) respectively. The concentration of the two isomers of lactate in the blood were found to be below 1.08 mmol/l (D-lactate) and 1.34 mmol/l (L-lactate) respectively.

Phase 2 = phase of intraruminal applications of milk (day 1 onwards)

Clinical repercussions

Immediately before the first intraruminal application, at the time of the physical examination on day 1, calf no. 2 showed diarrhoeic faeces. This sign disappeared within 1 day (Table 2).

The effects of the intraruminal administration on the clinical conditions of the calves of group A were detrimental; eight of nine calves showed acute disease with clinical scores as high as 3. According to the study design, seven calves were removed from the intraruminal feeding: calf no. 4 on the fourth experimental day (10 administrations); calf no. 5 on the sixth day (16 administrations); calf no. 12 on the seventh day (19 administrations); calves nos 6 and 15 on the 10th day (28 administrations); calf no. 13 on the 11th day (31 administrations); calf no. 11 on the 12th day (34 administrations).

Calf no. 2 was in a severe clinical condition (clinical score = 3) on day 4. It did not by itself meet the criteria for discontinuing the experiment. Moreover, it recovered spontaneously during the following days. Therefore, the experimental treatment was continued. Calf no. 7 remained completely healthy during the entire phase and, similarly to calf no. 2, was rumen-fed until the 17th experimental day.

The most frequent clinical findings were depression (in eight of nine calves), reduction (three of nine) or absence (three of nine) of sucking reflex, dehydration (five of nine; in one case with hypovolaemic shock), recumbency (three of nine), inappetence (three of nine).

Ruminal acidosis

Prior to the first intraruminal application, at the time of the physical examination of day 1, the rumen content of calf no. 7 was found to be slightly acidic (pH = 5.78) (Table 3). On the

Table 2. Clinical scores during the pre-induction phase (days −1 and 0) and the phase of induction of ruminal acidosis (day 1 onwards)

Day	−1	0	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Group A																			
Calf 2	0	0	1[d]*x*	1 *x*	2 *x*	3 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *y*
Calf 4	0	0	0 *x*	1 *x*	2 *x*	3 *y*													
Calf 5	0	0	0 *x*	1 *x*	1 *x*	2 *x*	2 *x*	3 *y*											
Calf 6	0	0	0 *x*	1 *x*	1 *x*	2 *x*	2 *x*	2 *x*	2 *x*	2 *x*	2 *x*	3 *y*							
Calf 7	0	0	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *x*	0 *y*
Calf 11	0	0	0 *x*	1 *x*	2 *x*	2 *x*	2[d]*x*	2[d]*x*	2[d]*x*	2 *x*	2 *x*	2 *x*	2 *x*	3 *y*					
Calf 12	0	0	0 *x*	1 *x*	2 *x*	2 *x*	2[d]*x*	2[d]*x*	3[d]*y*										
Calf 13	0	0	0 *x*	0 *x*	0 *x*	1 *x*	1 *x*	1 *x*	1 *x*	1 *x*	1 *x*	2 *x*	3 *y*						
Calf 15	0	0	0 *x*	0 *x*	0 *x*	0 *x*	1 *x*	1 *x*	1 *x*	1 *x*	1 *x*	3 *y*							
Number of intubated calves	–	–	9	9	9	9	8	8	7	6	6	6	4	3	2	2	2	2	2

The Table refers exclusively to the calves of group A.
[a]Clinical check up was performed before the first intraruminal administration.
Clinical score: 0 = absence of relevant clinical findings; 1 = presence of slight clinical signs; 2 = presence of moderate clinical signs; 3 = presence of severe clinical signs.
d = presence of diarrhoea; *x* = intraruminal application of 1 l of milk via stomach tube t.i.d. (8.00, 13.00 and 18.00 hours); *y* = intraruminal application of 1 l of milk via stomach tube s.i.d. (8.00 hours).

Table 3. Ruminal pH and ruminal concentration of L- and D-lactate (mmol/l) during the pre-induction phase (days −1 and 0) and the phase of induction of ruminal acidosis (day 1 onwards)

Day		−1	0	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
pH	Mean	6.93	6.92	6.70	4.31	4.33	4.37	4.42	4.22	4.15	4.11	4.09	3.96	4.28	4.60	4.21	3.98	4.19	3.83	4.03
	±SD	0.19	0.35	0.48	0.64	0.32	0.34	0.57	0.41	0.53	0.26	0.21	0.28	0.54	0.47	0.45	0.11	0.33	0.04	0.30
D-Lactate	Mean	0.2	0.1	2.0	32.7	36.8	31.9	32.1	33.0	31.4	43.9	45.0	38.9	36.0	31.6	37.3	46.1	40.4	48.4	43.2
	±SD	0.2	0.0	5.7	16.1	10.9	11.1	9.0	4.7	3.1	19.1	17.3	11.7	10.6	4.5	7.8	11.6	4.2	12.6	4.2
D-Lactate	Mean	0.1	0.1	2.1	32.7	37.4	35.5	32.0	32.4	37.4	41.4	43.0	37.9	34.3	31.6	32.7	44.3	47.2	45.6	41.3
	±SD	0.1	0.1	5.9	13.1	13.3	12.9	8.4	3.9	7.7	11.3	15.4	8.7	6.9	5.0	0.0	1.6	1.4	1.4	7.3
		_[b]	_[b]	_[b]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
Number of intubated calves		–	–	9	9	9	9	8	8	7	6	6	6	4	3	2	2	2	2	2

The Table refers exclusively to the calves of group A at the morning examination (7.55 AM).
[a]Samples were obtained before the first intraruminal administration.
[b]Mean value of all calves of group A.
[c]Mean value calculated only from the intubated calves.

basis of the macroscopic characteristics (milky white colour and acidic smell) and of the increased concentration of lactate (L-lactate 17.1 mmol/l; D-lactate 17.9 mmol/l) it was assumed that the calf had 'drunk' a small quantity of milk spontaneously into the rumen during the last feeding of day 0. It was decided to continue the experimental trial in this calf despite these observations.

As anticipated, the onset of ruminal acidification as a consequence of force-feeding, occurred rapidly. At 12.50 h on day 1 (i.e. about 5 h after the first intraruminal application of milk) all group A calves had acidic rumen contents, with pH values ranging between 4.20 and 5.71 (mean for the group A = 4.90 ± 0.38). The acidity increased following the second administration, reaching pH-values between 3.81 and 4.68 at 17.50 h on day 1 (mean for the group A = 4.35 ± 0.35). Rumen pH remained constant below pH 6.0 during the entire period of ruminal intubation.

During the phase of acidification the rumen juice was macroscopically characterized by a milky white colour, an acidic smell, a yoghurt-like consistency and by the presence of white casein curds.

Rumen acidity was characterized biochemically by a lactic acid fermentation. The concentration of both isomers of lactate (D+, and L−) increased in all calves from baseline values below 1 mmol/l to mean values between 30 and 50 mmol/l for each isomer, although a wide range was observed.

Metabolic acidosis

Force-feeding milk into the rumen was accompanied by a decrease in blood pH; a significant reduction of ABE was observed in all but one (calf no. 7) of the group A calves (Table 4). Metabolic acidosis (defined as ABE < −3.0) appeared in calves nos. 2 and 12 on the second experimental day (ABE = −6.8 and −3.1 mmol/l respectively), in calves nos. 4 and 11 on the third day (ABE = −8.3 and −4.3 mmol/l respectively), in calves nos. 5, 6 and 15 on the fourth day (ABE = −6.8, −5.4 and −3.7 mmol/l respectively) and in calf nos. 13 on the fifth experimental day (ABE = −4.8 mmol/l). Once established, metabolic acidosis persisted in all calves until the end of phase. Calf no. 7 never showed signs of acid–base disturbances.

Table 4. Blood values (Actual Base Excess, mmol/l; anion gap, mmol/l; D- and L-lactate concentration, mmol/l) during the pre-induction phase (days −1 and 0) and the phase of induction of ruminal acidosis (day 1 onwards)

Day		−1	0	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ABE	Mean	3.2	3.7	1.7	−0.1	−2.8	−4.6	−6.0	−7.9	−8.6	−7.3	−8.5	−10	−8.5	−7.7	−2.7	−4.0	−5.1	−5.5	−6.0
	SD	2.32	1.95	2.70	3.16	3.61	4.40	4.08	4.88	5.92	6.47	7.26	7.89	8.44	9.01	5.30	6.08	6.93	6.29	5.66
AG	Mean	12.2	12.8	13.5	14.7	16.6	17.9	20.3	19.8	20.5	22.3	21.8	22.5	21.0	21.4	20.3	20.5	20.2	20.9	20.5
	SD	3.71	1.95	1.59	2.73	2.40	3.26	1.65	3.23	2.74	3.33	2.98	3.31	3.16	4.05	5.87	4.03	2.97	4.88	0.78
D-Lactate	Mean	0.1	0.2	0.3	1.8	4.5	5.8	5.9	6.7	7.5	7.6	8.1	8.5	8.1	7.7	6.2	6.9	7.9	8.2	8.6
	SD	0.1	0.1	0.6	1.3	1.5	2.2	1.8	3.0	2.4	2.6	2.1	2.1	2.1	2.4	3.7	3.7	3.4	2.4	2.3
L-Lactate	Mean	0.7	0.7	0.7	0.6	0.6	0.6	0.5	0.4	0.4	0.5	0.5	0.4	0.5	0.4	0.4	0.3	0.3	0.3	0.4
	SD	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0
		_[b]	_[b]	_[b]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]	_[c]
Number of intubated calves		–	–	9	9	9	9	8	8	7	6	6	6	4	3	2	2	2	2	2

The Table refers exclusively to the calves of group A at the morning examination (7.55 AM).
[a]Samples were obtained before the first intraruminal administration.
[b]Mean value of all calves of group A.
[c]Mean value calculated only from the intubated calves.

Table 5. Concentration of D-lactate (mmol/l) in the blood of the single calves during the pre-induction phase (days −1 and 0) and the phase of induction of ruminal acidosis (day 1 onwards)

Day	−1	0	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Group A																			
Calf 2	0.17	0.38	1.98	3.87	4.74	6.36	6.19	5.77	7.63	5.80	6.33	6.95	9.42	9.35	8.80	9.32	10.3	9.90	10.3
Calf 4	0.40	0.30	0.22	3.03	6.21	9.79													
Calf 5	0.10	0.15	0.08	1.16	4.45	6.88	4.99	6.75											
Calf 6	0.07	0.09	0.07	2.82	6.60	7.35	9.24	11.2	9.93	11.5	11.1	11.1							
Calf 7	0.04	0.12	0.15	2.56	3.58	2.01	3.14	0.72	2.68	4.03	5.19	5.35	5.18	5.01	3.58	4.51	5.43	6.47	6.97
Calf 11	0.01	0.04	0.01	0.75	4.47	4.66	4.93	6.13	7.07	7.48	8.18	8.12	8.02	8.86					
Calf 12	0.07	0.08	0.17	1.81	4.54	5.63	6.94	8.58	9.25										
Calf 13	0.08	0.16	0.07	0.09	1.52	3.96	6.00	6.77	7.44	8.44	8.17	9.10	9.74						
Calf 15	0.06	0.08	0.02	0.27	4.61	5.52	6.14	7.81	8.47	8.62	9.58	10.3							
Number of intubated calves	–	–	9	9	9	9	8	8	7	6	6	6	4	3	2	2	2	2	2

The Table refers exclusively to the calves of group A at the morning examination (7.55 AM).
[a]Samples were obtained before the first intraruminal administration.

The increase in the anion gap reflected the net acid load; although with differences in the time of appearance, all but two calves (nos 5 and 7) showed anion gap values higher than 20 mmol/l. The anion gap was increased by 10.93 ± 4.09 mmol/l (this value represents the mean of the single differences between the highest anion gap value during phase 2 and the mean value during phase 1).

D-*Lactataemia*

In all the calves of group A there was a significant increase in plasma D-lactate concentration (Tables 4 and 5). Four calves showed values higher than 2 mmol/l at the second day of phase 2. On day 3 only one calf, and on day 4 no calf had a D-lactate concentration below 2 mmol/l (mean value: day 3 = 4.5 ± 1.5 mmol/l; day 4 = 5.8 ± 2.2 mmol/l). All the calves reached a concentration of at least 6 mmol/l at one point. Six calves showed maximum values higher than 9 mmol/l and the highest value recorded was 11.5 mmol/l (calf no. 6 on day 8). Calf no. 2 presented an abnormal D-lactate concentration (1.98 mmol/l) already before the first intraruminal application (08.00 hours of day 1).

Blood D-lactataemia showed a statistically significant linear correlation with the ABE value ($P < 0.001$; $r = 0.8321$) and with the anion gap ($P < 0.001$; $r = 0.8633$).

Unlike the D-lactate, the value of blood L-lactate remained in the normal range for the whole period.

Control calves

Throughout phase 2 calves nos. 9 and 16 (group B) presented clinical findings, ruminal condition and metabolic state that never varied from those of phase 1.

Phase 3 = recovery phase

After the cessation of the intraruminal milk feeding the calves of group A recovered spontaneously within 2 days; only calf no. 4 needed to have its rumen flushed with lukewarm water to remove any abnormal contents.

Discussion

This study represents an attempt to elucidate the role of D-lactic acid in the metabolic evolution of ruminal acidosis in milk-fed calves. Previous studies in experimentally induced ruminal acidosis investigated clinical aspects (van Weeren-Keverling Buisman et al., 1990a; Baur, 1993; Gentile et al., 1996), biochemical changes in the rumen (van Weeren-Keverling Buisman et al., 1990a; Baur, 1993; Gentile, 1995; Gentile et al., 1996), morphological alterations of the rumen wall (van Weeren-Keverling Buisman et al., 1990a) as well as of the intestinal mucosa (van Weeren-Keverling Buisman et al., 1990b), immunological responses (Dall'Ara et al., 1996) and metabolic repercussions (Gentile et al., 1996).

Regarding the last one, Gentile et al. (1996) failed to detect significant acid–base disorders in experimentally induced ruminal acidosis. By contrast, acute, and sometimes severe, disturbances of systemic acid–base balance were found to be a common finding in calves admitted to the clinic (Second Medical Animal Clinic, Munich) with evidence of ruminal drinking (Gentile and Baur, 1995; Gentile et al., 1998). This apparent discrepancy was attributed to the fact that in the experimental trial calves were forced-fed with flavofosfolipol containing milk replacer, whereas almost all spontaneous clinical cases had been fed whole milk.

Likewise all the calves suffering from spontaneous chronic indigestion because of ruminal drinking described by Stocker et al. (1999) presented with metabolic acidosis. These authors, however, did not describe the kind of feed the patients had received.

Grude et al. (1999) found comparably elevated blood-D-lactate levels in high anion gap acidaemic 'ruminal drinkers' patients, but not in 'ruminal drinkers' without acidaemia. In the same investigation similarly high D-lactate concentrations were found in acidaemic patients without evidence of ruminal drinking. The authors suggested that although consistent with a reticulo-rumen origin, the hyper-D-lactataemia could be attributed primarily to an intestinal source, such as in cases of bacterial fermentative processes in osmotic diarrhoea. The contribution of D-lactate to metabolic acidosis in diarrheic calves has been discussed by other authors (Omole et al., 2001; Lorenz, 2002; Naylor et al., 2002).

Bacterial overproduction of D-lactic acid in the gut has been described extensively in human patients suffering from the 'short bowel syndrome' (Oh et al., 1979) as a consequence of small bowel resection or jejunoileal bypass surgery. In these cases, colonic fermentation and lactic acid formation may increase if the small intestine is resected or bypassed to an extent which causes malabsorption of carbohydrates.

In the 1990s attention was drawn to the pathogenetical similarities between bovine acute ruminal lactic acidosis and human short bowel syndrome, with special reference to acid–base homeostasis and lactic acid metabolism (Anonymous, 1990; Gentile and Rademacher, 1998). In both conditions only the D-isomer tends to accumulate in the blood, despite formation of both isomers in the gastrointestinal tract. The commonly claimed mechanism of this unilateral accumulation is that the mammalian organism lacks D-LDH, while L-lactate is metabolized efficiently by L-LDH. D-2-Hydroxy acid dehydrogenase (Tubbs, 1965; Cammack, 1969), an unspecific enzyme in the liver and kidney, is supposed to compensate for the lack of a D-LDH, but it has only a limited efficiency.

As D-lactate does cross the blood–brain barrier it can also accumulate in the brain, where it can cause central nervous system toxicity; encephalopathic manifestations in humans, mostly characterized by confusion, ataxia, weakness, lethargy and slurred speech, have in fact been clearly correlated with D-lactic acidosis (Carr et al., 1982; Dahlquist et al., 1984; Hudson et al., 1990; Koletzko et al., 1994). Data are required to determine the threshold level of D-lactate necessary to produce these symptoms. Some authors have assumed that clinical manifestations are associated with D-lactate concentrations higher than 3–4 mmol/l (Thurn et al., 1985). Whether D-lactate accounts for a part or all of the encephalopathic changes remains to be determined.

In this context Dahlquist et al. (1984) suggested two different hypotheses for the activity of D-lactate: an acute effect on one or more enzymes that affect energy metabolism, or a direct neurotransmitter activity.

The clinical similarity between the neurological syndrome and acquired abnormalities of pyruvate metabolism has been emphasized and the possible interference by D-lactate on pyruvate metabolism and thus central nervous system function

has been postulated (Cross and Callaway, 1984). Nevertheless, it is equally possible that an altered mental status is caused by another unidentified toxic by-product of large intestinal bacteria.

Major findings of the study permit the following considerations:

1 The presence of milk in the reticulo-ruminal cavity inevitably induces a process of local acidification, mainly characterized by lactic fermentation.
2 Both lactic acid isomers are involved in this ruminal acidification.
3 Rumen pH value remains constant in an acidic range as long as milk is permitted to enter the forestomachs (ruminal drinking); in our experiment this occurred through force feeding.
4 Persistence of ruminal acidosis can be associated with the appearance of systemic disease; in our experiment this happened in eight of nine calves.
5 It can be assumed that ruminal acidosis can lead to death; in our study, in order to prevent unnecessary suffering, we established clinical criteria that would prevent such an outcome. Seven of nine experimental calves met these criteria and therefore intraruminal feeding was terminated.
6 Metabolic acidosis is a possible outcome of ruminal acidosis; in our study it occurred in all but one calf of the experimental group.
7 The acid–base disturbance is due to an increased load of organic acids from the gastrointestinal tract, as is confirmed in our study by an increase of the anion gap; in our experiment this increase was on average higher than 10 mmol/l.
8 Among the organic acids both isomers of lactic acid formed in the rumen can be considered primarily responsible for the formation of metabolic acidosis. However, because of the different metabolic pathways, only D-lactate tends to accumulate in the blood; in our experiment hyper-D-lactataemia was evident in all the calves of the group A.
9 Hyper-D-lactataemia can be detected also in non-acidotic patients. In fact, although a statistical correlation was observed between blood ABE value and D-lactate concentration, the lack of an evident metabolic acidosis in calf no. 7 confirms that hyper-D-lactataemia may occur without acidaemia. This can occur if the buffering systems are able to mitigate the fall of pH.
10 Hyper-D-lactataemia can also have intestinal origin. The slight increase of blood D-lactate in calf 2 before induction of ruminal acidosis might be attributed to lactic acid fermentation in the large bowel as a consequence of carbohydrate malabsorption in the small intestine.

Conclusion

Our work confirms the hypothesis that persistent ruminal acidosis in calves is likely associated with a D-lactic metabolic acidosis. The presented data fit into the following model. During ruminal acidosis, L- and D-lactic acid are produced abundantly by bacterial fermentative activity. Lactose and other carbohydrates contained in milk fed to calves represent the main compounds for this process. Both isomers of lactic acid are absorbed from the rumen or from the intestinal lumen into the bloodstream where they exert an acidotic effect. The acid–base disturbance is counteracted by compensatory buffers that may or may not restore a physiological extracellular pH.

Serum L- and D-lactate concentrations result from kinetic metabolical processes, which are strongly isomer specific. L-Lactate can be metabolized very quickly by the body, and therefore does not accumulate despite the continuous influx into the bloodstream. On the contrary, due to the lack of a specific metabolic pathway, absorbed D-lactate cannot be eliminated at the same rate, with the consequence of the risk of hyper-D-lactataemia. Whether D-lactate accounts for part or all of the clinical findings remains to be determined.

Further studies in D-lactate metabolism are necessary to elucidate its role in the development of neurological signs which may be observed in calves with ruminal acidosis or diarrhoea. D-Lactic acidosis in ruminants also appears to be an excellent model for recurrent acidosis in human disorders associated with short bowel syndrome.

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References

Anonymous, 1990: The colon, the rumen, and D-lactic acidosis (Editorial). Lancet **336,** 559–600.

Baur, T., 1993: Untersuchungen über den Einfluß der intraruminalen Verabreichung von Milch beim jungen Kalb. Vet. Med. Diss., München, Germany.

Cammack, R., 1969: Assay, purification and properties of mammalian D-2-hydroxy acid dehydrogenase. Biochem. J. **115,** 55–64.

Carr, D. B., V. E. Shih, J. M. Richter, and J. B. Martin, 1982: D-Lactic acidosis simulating a hypothalamic syndrome after bowel bypass. Ann. Neurol. **11,** 195–197.

Cross, S. A., and C. W. Callaway, 1984: D-Lactic acidosis and selected cerebellar ataxias. Mayo Clin. Proc. **59,** 202–205.

Dahlquist, N. R., J. Perrault, C. W. Callaway, and J. D. Jones, 1984: D-Lactic acidosis and encephalopathy after jejunoileostomy: response to overfeeding and to fasting in humans. Mayo Clin. Proc. **59,** 141–145.

Dall'Ara, P., P. A. Martino, A. Gentile, and S. Testoni, 1996: Valutazione dei parametri dell'immunità innata, non-specifica, in vitelli con acidosi ruminale. Atti. Soc. It. Sci. Vet. **50,** 259–260.

Dunlop, R. H., 1972: Pathogenesis of ruminant lactic acidosis. Adv. Vet. Sci. Comp. Med. **16,** 259–302.

Dunlop, R. H., and P. B. Hammond, 1965: D-Lactic acidosis of ruminants. Ann. N. Y. Acad. Sci. **119,** 1109–1132.

Gentile, A., 1995: Untersuchungen über die Azidität der Pansenflüssigkeit von Kälbern nach intraruminaler Verabreichung von Rehydratationslösungen. Dtsch. tierärztl. Wschr. **102,** 241–244.

Gentile, A., and T. Baur, 1995: Conseguenze della somministrazione intraruminale di soluzioni reidratanti o di latte. Atti. Soc. It. Buiatria **27,** 571–582.

Gentile, A., and G. Rademacher, 1998: Vergleichende Betrachtung der Veränderungen des Säure-Basenhaushaltes bei der Pansenazidose des Rindes und beim sogenannten Kurzdarmsyndrom des Menschen. Proceedings of the 10th Magyar Buiatrikus Kongresszus Közèp-Európai Buiatrikus Találkozó, Middle European Buiatrics Congress, 21–23/05/1998, Siófok, Ungheria, 47–51.

Gentile, A., S. Testoni, C. Guglielmini, G. Gerardi, A. Boari, and P. Famigli-Bergamini, 1996: Acidosi ruminale cronica nel vitello

lattante indotta da somministrazione intraruminale di latte ricostituito. Atti. Soc. It. Buiatria **28,** 293–309.

Gentile, A., G. Rademacher, G. Seeman, and W. Klee, 1998: Systemische Auswirkungen der Pansenazidose im Gefolge von Pansentrinken beim Milchkalb. Tierärztl Prax. **26**(G)**,** 205–209.

Grude, T., I. Lorenz, G. Rademacher, A. Gentile, and W. Klee, 1999: Levels of D- and L-lactate in rumen liquid, blood, and urine in calves with and without evidence of ruminal drinking. Proceedings 32nd Annual Convention AABP, 23–26/09/1999, Nashville, Tennessee (USA), 213–214.

Hudson, M., R. Pocknee, and N. A. G. Mowat, 1990: D-Lactic acidosis in short bowel syndrome – an examination of possible mechanisms. Q. J. Med. **74,** 157–163.

Koletzko, S., K. L. Waag, and B. Koletzko, 1994: Rezidivierende D-lactat-acidosen mit Enzephalopathie bei einem Jungen mit Kurzdarmsyndrom. Dtsch. Med. Wschr. **119,** 458–462.

Lorenz, I., 2002: Influence of serum D-lactate concentration on clinical signs in calves with metabolic acidosis. Congress Abstracts of the 22nd World Buiatrics Congress, 18–23/08/2002, Hannover (Germany) Abstract no. 542–326 (CD-rom).

Lorenz, I., I. Hartmann, and A. Gentile, 2003: Determination of D-lactate in calf serum samples – an automated enzymatic assay. Comp. Clin. Pathol. **12,** 169–171.

Naylor, J. M., J. B. Ewaschuk, G. A. Zello, 2002: A new hypothesis for the development of acidosis in diarrheic calves – the role of novel acids Congress Abstracts of the 22nd World Buiatrics Congress, 18–23/08/2002, Hannover (Germany), Abstract no. 543–432 (CD-rom).

Oh, M. S., K. R. Phelps, M. Traube, J. L. Barbosa-Saldivar, C. Boxhill, and H. J. Carroll, 1979: D-Lactic acidosis in a man with the short-bowel syndrome. N. Engl. J. Med. **301,** 249–252.

Omole, O. O., G. Nappert, J. M. Naylor, and G. A. Zello, 2001: Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. J. Nutr. **131,** 2128–2131.

Stocker, H., H. Lutz, and P. Rüsch, 1999: Clinical, haematological and biochemical findings in milk-fed calves with chronic indigestion. Vet. Rec. **145,** 307–311.

Thurn, J. R., G. L. Pierpont, C. W. Ludvigsen, and J. H. Eckfeldt, 1985: D-Lactate encephalopathy. Am. J. Med. **79,** 717–721.

Tubbs, P. K., 1965: The metabolism of D-α-hydroxy acids in animal tissue. Ann. N. Y. Acad. Sci. **119,** 920–926.

van Weeren-Keverling Buisman, A., Th. Wensing, T. S. G. A. M. Van den Ingh, and H. J. Breuking, 1990a: Intraruminal administration of milk in the calf as a model for ruminal drinking: clinical aspects and biochemical and morphological changes in the rumen. J. Anim. Physiol. a. Anim. Nutr. **63,** 255–266.

van Weeren-Keverling Buisman , A., J. M. V. M. Mouwen, T. Wensing, and H. J. Breuking, 1990b: Intraruminal administration of milk in the calf as a model for ruminal drinking: morphological and enzymatical changes in the jejunal mucosa. Vet. Res. Comm. **14,** 129–140.