EXPERIMENTALLY INDUCED ACUTE D(-) LACTIC ACIDOSIS IN GOAT (CAPRA HIRCUS)

by

Javier Castillejos E.

B. Sci., Universidad Nacional Autonoma de Mexico

D.V.M., Universidad Nacional Autonoma de Mexico, 1971

A Thesis

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCES

Department of Anatomy and Physiology

Kansas State University

Manhattan, Kansas

1978

Approved by:

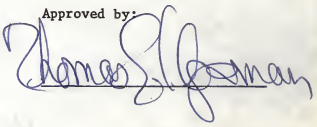


TABLE OF CONTENTS

SUMMARY

Four goats (Capra hircus) weighing from 35 to 50 kg each, were infused either via naso gastric tube or rumen fistula with pure corn starch to produce D (-) lactic acidosis. Specific enzymatic analysis for D (-) lactic acid, L (+) lactic acid and glucose in plasma, rumen fluid and urine were carried out. Blood packed cell volume data was also estimated as well as pH values for blood, rumen fluid and urine. It was found that the accumulation of free glucose in rumen up to 50 mg % for the next 24 hours post infusion along with rumen pH values of 5.5 or less were a necessary condition for the production of D (-) lactic acid up to 20-600 mg %. Levels of D (-) lactic in plasma up to 5 mg % were closely related to clinical disturbances observed. The elimination of the D (-) isomer from the animal body appeared to be carried out by urinary system.

INTRODUCTION

D (-) lactic acidosis, also known as acute indigestion, acidosis, overeating disease, cereal poisoning, founder, acute impaction, acute acid indigestion, grain engorgement, overloading, lactic acidosis and/or over-ingestion is defined as an acid balance disturbance of ruminant species caused by the excessive consumption of readily available carbohydrates. The source of carbohydrates is varied and foods, such as grains or fruits are common offenders in animals not accustomed to such diets (9,11). Diarrhea, anorexia and postration are the symptoms commonly observed (21). Anuria has also been reported to occur (15). This disease often terminates with death of the animal (20).

During the last two decades the feedlot industry has been using an almost pure grain ration for feeding cattle during the "finishing" period

hence increasing the risk of D (-) lactic acidosis occurrence. The elevated starch content of grains makes them readily fermentable carbohydrates whose fermentation in the rumen, when consumed in excess, gives rise to abnormal increase of organic acids, beginning with volatile fatty acid (VFA) followed by L (+) lactic acid. As free glucose accumulates in rumen and pH declines even more, D (-) lactic concentration increases. This condition is a known cause of death in feedlot cattle (11).

The sequence of events taking place in the rumen after grain overload results initially in an abnormal increase of VFA concentration which diminishes rumen pH (33). Further decrease in pH is attributed to an increasing production of both lactic acid isomers (31). The resulting low pH values in the rumen of these animals result in the almost total disappearance of the preponderantly normal cellulolytic population and gives rise to the overgrowth of lactic acid producing bacteria such as Streptococcus bovis and Lactobacillus sp. (20,25). Lactobacillus sp. appears to be more resistant to low pH than S. bovis and is a potent D (-) lactic acid producer (19). S. bovis is able to ferment sugar to a final pH of 4.5 under in vitro conditions (24).

Chemistry of Lactic Acid

D (-) Lactic Acid

L (+) Lactic Acid

The symbols (-) and (+) indicate the direction of rotation of a plane of polarized light. The capital letters D and L refer to the molecular structural relationship with the isomer of glyceric acid (23). Lactic acid is a stronger acid than VFA (pK's 3.86 and 4.7, respectively). It is held that the lipid solubility of lactic acid is less when compared to VFA because of the hydroxyl group found on its alpha carbon (38). Therefore, this would account for the lower absorption of lactic acid when compared to VFA. It has been reported that the concentration of L (+) isomer in the rumen fluid of healthy cattle fed a pure roughage diet can be as high as 20 mg % at one hour post-feeding period whereas the same isomer concentration was twice as high one hour after an all grain diet was fed (37). At normal rumen pH, 6.7-8.2, the L (+) isomer of lactic acid is converted to propionate and acetate (5) therefore resulting in no accumulation of ruminal lactate. The D (-) lactic acid which is formed after readily fermentable carbohydrate is consumed in excess may disappear via various routes. It may pass through on the lower digestive tract and be partially converted to L (+) lactate, carbon dioxide and protein by tissues of the abomasum, small intestine and colon (26). It may be converted to L (+) lactic acid by some rumen microorganisms and then absorbed (19) or converted to any other metabolite. It may also be absorbed as the D (-) isomer (15). This latter route appears to be favored under acid conditions (26,34,38).

Induced D (-) lactic acidosis has been produced by overfeeding ruminant species, either forcibly or ad libitum, with cereal grains or fruits (1,10, 34) resulting in environmental changes in the reticulo rumen at different degrees (20,25). It has been suggested that the accumulation of free glucose in the rumen is a necessary prerequisite for the development of D (-) lactic acid producing microorganisms. Slyter (1976) reported that once the low

ruminal pH's (between five and six) are attained, free amylase of the ruminal ingesta may increase and microbial glucose utilization rates decrease; in the same paper it is reported that the increased rate of glucose production and decreased rates of its utilization leads to glucose accumulation in the rumen. The purpose of this study was to clarify the genesis and role of D (-) lactic acid during acute lactic acidosis syndrome.

Material and Methods

Four goats (Capra hircus) weighing 35 to 50 kg each were used in these experiments. Their diet consisted of alfalfa hay fed ad libitum. All animals were starved 24 hours before being infused. Pure corn starch was administered within 10 minutes after preinfusion samples were taken, via either nasagastric tube or rumen fistula. The time between repeated infusions made on the same animal was 30 to 45 days. This was considered to be sufficient time for the normal cellulolytic microbial rumen population to totally recuperate (31). Starch to be infused was diluted in 1000 ml of water. All animals were surgically prepared with jugular canulas in the right jugular vein. To asses systemic acid base status, venous blood samples were analyzed. The advantage of drawing venous blood instead of arterial blood is to allow one to easily monitor pH values.

The goal of initial trials was to find out the dose of starch required to induce this disturbance. Increasing doses of pure corn starch[a] from 3 to 18 g per kg body weight were given in separate experiments. In most experiments a permanent rubber tube[b] was attached to the fistula for drawing out rumen liquor. Blood samples collected for the determination of D (-) lactic acid, L (+) lactic acid and glucose were kept under refrigeration for no more than five minutes after being obtained. They were then

centrifuged at 5000 rpm. Protein free filtrates of plasma were prepared with 4.5% perchloric acid for lactic acid analysis or tungstic acid for glucose analysis. Identical procedures were used for rumen fluid and urine. Rumen fluid samples were collected in graduated tubes containing either hydrochloric or tungstic acids. The glucose determinations were done by the Raabo and Terkildsen modified enzymatic procedure (27). The method used for the determination of L (+) lactic acid was based on the enzymatic procedure described by Hohorst (1963). This laboratory adapted the method used for the enzymatic determination of D (-) lactic acid in wine and fruit juices (12) to rumen fluid, plasma and urine specimens. D (-) lactic acid standards[d] as well as specific D (-) lactic acid dehydrogenase[e] were required for such analysis. Blood pH values were determined within two minutes after collection using a Corning gas blood analyzer Model 165. The pH values of urine and rumen fluid were measured in a Beckman Spandomatic pH meter at about one minute after collection. The determination of urine pH was done as soon as practical after collection. This was usually within 10 minutes.

Results

The infusion of pure corn starch in doses below 9 g per kg body weight, as in trials 1, 2 and 3, appeared to be insufficient to cause evident physiological alterations. Concentrations of plasma D (-) lactic acid in those animals increased from preinfusion levels of less than 1.5 mg % to around 4 mg % at the end of the trials. When in further trials starch concentrations were increased up to 11 g per kg body weight the animal's responses varied from lack of consistency in feces to severe depression up to the point in which the inability to stand was observed. In trial number four, in which 11 g pure corn starch per kg body weight was ad-

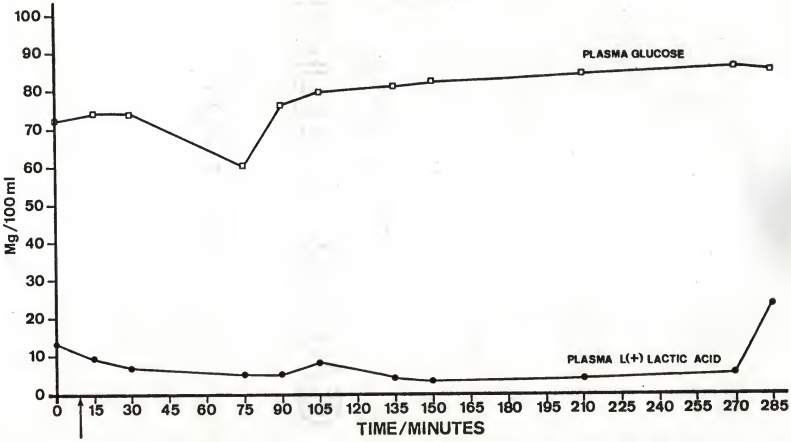
Trial # 1. Physiological changes in 50 kg goat with unsuccessful experimentally induced D(-) lactic acidosis.

Table # 1

Sample	Time hours	Plasma D(-)Lactic Acid	Plasma L(+)Lactic Acid	Plasma Glucose
0	0	0	13.0	72.4
1	15'	0	9.6	74.6
2	30'	0	7.45	74.3
3	1.15	0	5.46	73.0
4	1.30	0	5.41	77.0
5	1.45	0	8.47	79.3
6	2.15	0	3.80	80.75
7	2.30	0	3.53	82.2
8	3.30	0	4.23	84.2
9	4.30	0	5.10	85.6
10	4.45	0	23.64	85.6

Trial # 1. Pure corn starch, 3 g per kg body weight infused at ↑ .

FIGURE 1


PLASMA GLUCOSE
PLASMA L(+) LACTIC ACID
Mg/100 ml
TIME/MINUTES
0
10
20
30
40
50
60
70
80
90
100
0
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
240
255
270
285

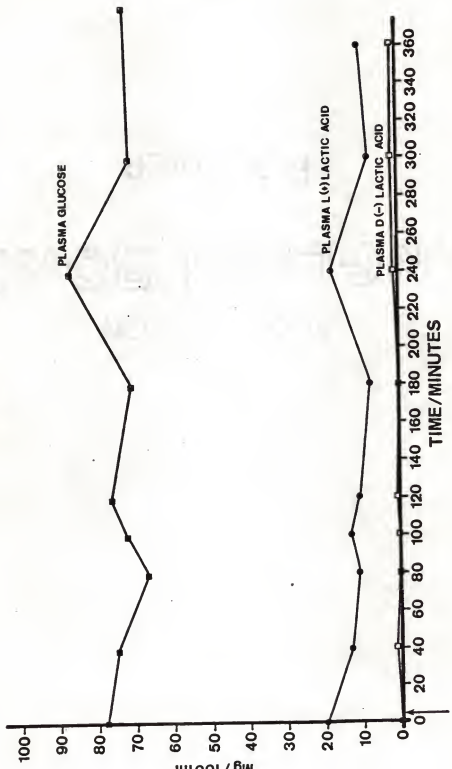
Trial # 2. Physiological changes in 50 kg goat with unsuccessful experimentally induced D(-) lactic acidosis.

Table # 2

Sample	Time hours	Plasma D(-)Lactic Acid	Plasma L(+)Lactic Acid	Plasma Glucose
0	0	.295	20.18	79.22
1	40'	.590	12.45	75.96
2	1.20	0	10.92	67.20
3	1.40	0	13.25	72.30
4	2	.295	10.89	75.76
5	3	0	7.89	71.07
6	4	.738	17.66	87.16
7	5	.738	8.23	71.48
8	6	1.33	10.11	71.89

Trial # 2. Pure corn starch, 6.5 g per kg body weight infused at ↑ .

FIGURE 2


PLASMA GLUCOSE
PLASMA L(+) LACTIC ACID
PLASMA D(-) LACTIC ACID
Mg/100ml
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
TIME/MINUTES

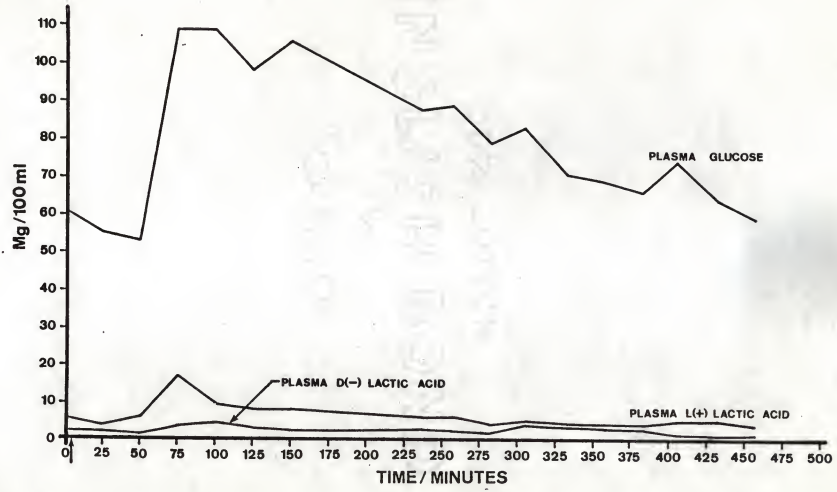
Trial # 3. Physiological changes in 50 kg goat with unsuccesful experimentally induced D(-) lactic acidosis.

Table # 3

Sample	Time hours	Plasma D(-)Lactic Acid	Plasma L(+)Lactic Acid	Plasma Glucose
0	0	2.64	5.15	61.3
1	25'	2.47	4.0	55.6
2	50'	1.76	5.85	53.2
3	1.15	3.52	16.74	109.6
4	1.40	4.23	9.44	108.9
5	2.05	3.0	7.78	98.0
6	2.3	2.47	8.0	105.5
7	3.55	3.0	6.0	87.9
8	4.20	2.11	6.0	88.3
9	4.45	1.58	5.0	79.4
10	5.10	4.17	5.69	83.0
11	5.35	3.68	4.13	70.7
12	6.0	2.94	3.70	68.9
13	6.25	2.94	3.49	65.8
14	6.50	1.71	4.77	74.5
15	7.15	0.98	5.0	64.0
16	7.40	0.98	3.75	68.3

Trial # 3. Pure corn starch, 9 g per kg body weight infused at  .

FIGURE 3


Mg/100 ml
110
100
90
80
70
60
50
40
30
20
10
0
PLASMA GLUCOSE
PLASMA D(−) LACTIC ACID
PLASMA L(+) LACTIC ACID
0
25
50
75
100
125
150
175
200
225
250
275
300
325
350
375
400
425
450
475
500
TIME / MINUTES

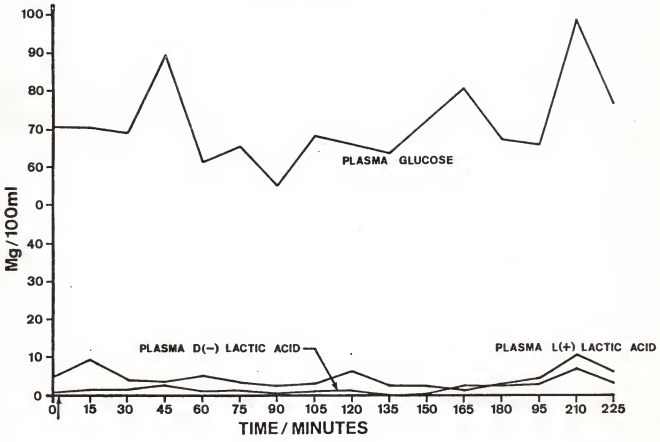
Trial # 4. Physiological changes in 51 kg goat with unsuccessful experimentally induced D(-) lactic acidosis.

Table # 4

Sample	Time Hours	Plasma D(-)Lactic Acid	Plasma L(+)Lactic Acid	Plasma Glucose
0	0	0.8	4.88	70.58
1	15'	1.45	9.07	70.39
2	30'	1.45	3.86	69.25
3	45'	2.61	3.43	89.37
4	1	0.1	3.81	60.91
5	1.15	1.16	3.22	65.27
6	1.30	0.8	2.63	54.26
7	1.45	1.16	3.16	67.93
8	2	1.45	6.22	65.65
9	2.15	0.0	2.68	63.56
10	2.30	0.1	2.73	71.91
11	2.45	2.50	1.34	80.45
12	3	2.32	3.0	67.17
13	3.15	2.90	4.67	65.84
14	3.30	6.68	10.6	98.48
15	3.45	3.19	6.92	76.66

Trial # 4. Pure corn starch, 10 g per kg body weight infused at  .

FIGURE 4


100
90
80
70
60
0
40
30
20
10
0
Mg/100ml
PLASMA GLUCOSE
PLASMA D(−) LACTIC ACID
PLASMA L(+) LACTIC ACID
0
15
30
45
60
75
90
105
120
135
150
165
180
95
210
225
TIME / MINUTES

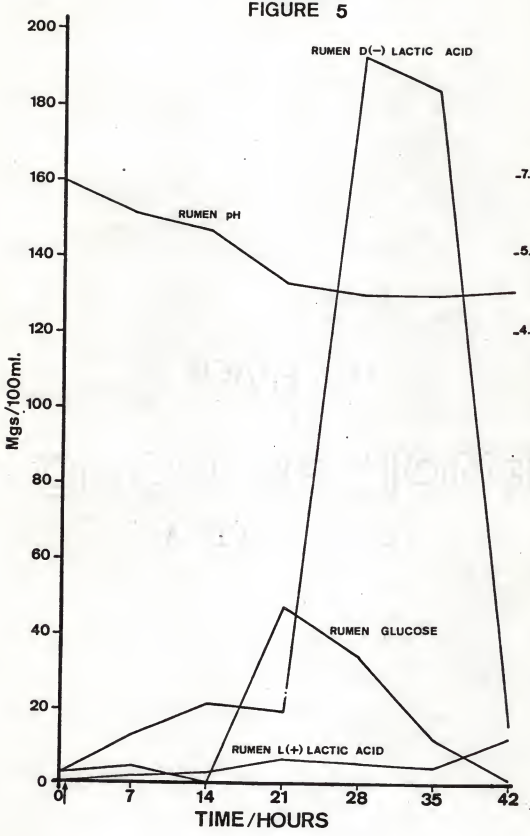
Trial # 5. Physiological changes in 50 kg goat moderately affected by experimentally induced D(-) lactic acidosis.

Table # 5

Sample	Time hr	Rumen D(-) lactic acid	Rumen L(+) lactic acid	Rumen glucose	Rumen pH	Plasma D(-) lactic acid	Plasma L(+) lactic acid	Plasma glucose	PCV
0	0	2.10	0.32	3.12	7.2	0.57	4.76	68.16	34
1	7	12.45	2.31	4.29	6.2	1.53	2.89	44.14	34
2	14	20.88	2.73	0.19	5.7	1.53	3.86	80.46	34
3	21	19.15	6.95	46.67	5.0	2.10	5.69	77.24	35
4	28	193.0	5.53	33.98	4.7	6.13	8.24	39.06	35
5	35	184.5	4.79	11.71	4.7	5.17	8.50	38.67	35
6	42	15.3	11.72	1.56	5.8	2.10	8.11	70.11	35

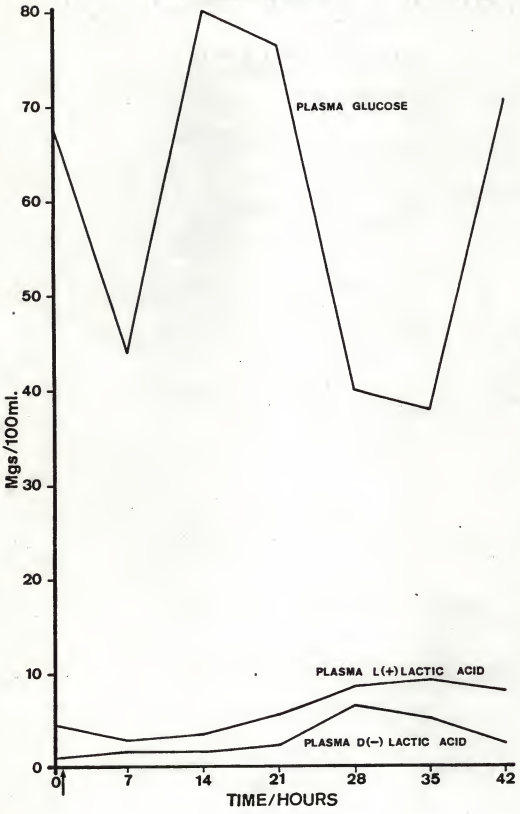
Trial # 5. Pure corn starch, 11 g per kg body weight infused at ↑ .

FIGURE 5


RUMEN D(−) LACTIC ACID
RUMEN pH
RUMEN GLUCOSE
RUMEN L(+) LACTIC ACID
Mgs./100ml.
TIME/HOURS
200
180
160
140
120
100
80
60
40
20
0
0
7
14
21
28
35
42
7.0
5.5
4.0

Trial # 5. Pure corn starch, 11 g per kg body weight infused at  .

FIGURE 5


80
70
60
50
40
30
20
10
0
Mgs/100ml.
PLASMA GLUCOSE
PLASMA L(+) LACTIC ACID
PLASMA D(−) LACTIC ACID
0
7
14
21
28
35
42
TIME/HOURS

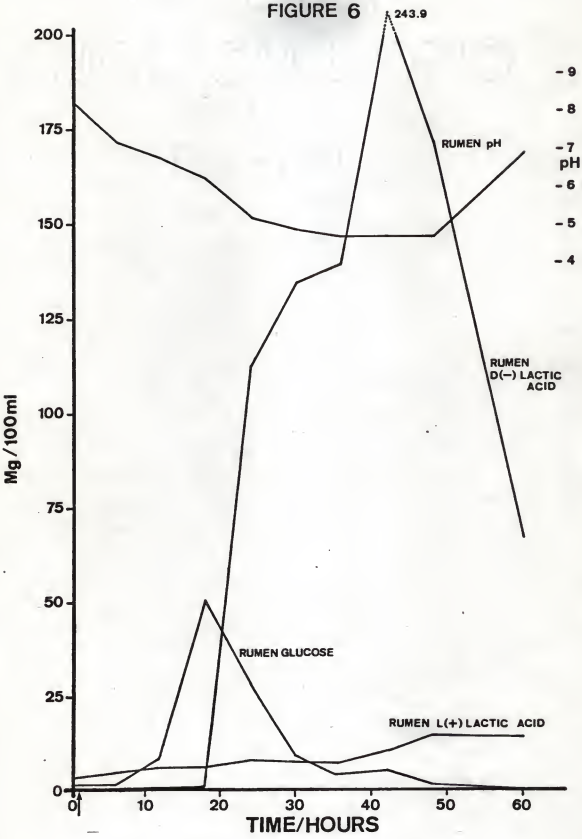
Trial # 6. Physiological changes in 50 kg goat acutely affected by experimentally induced D(-) lactic acidosis.

Table # 6

Sample	Time hr	Rumen D(-) lactic acid	Rumen L(+) lactic acid	Rumen glucose	Rumen pH	Plasma D(-) lactic acid	Plasma L(+) lactic acid	Plasma glucose	Blood pH	PCV
0	0	0.0	2.99	1.34	8.3	0.0	4.63	26.92	7.34	37
1	6	0.0	4.95	1.34	7.2	2.91	3.73	52.88	7.35	39
2	12	0.0	6.11	8.46	6.8	1.11	6.21	31.92	7.30	40
3	18	1.34	6.47	50.57	6.2	2.01	3.96	55.96	7.38	36
4	24	112.65	8.01	28.84	5.2	10.97	6.89	51.92	7.37	38
5	30	134.60	8.62	9.03	4.9	12.54	8.37	42.30	7.36	40
6	36	139.7	7.14	4.61	4.7	10.07	7.05	49.42	7.33	40
7	42	243.90	10.91	5.76	4.7	21.72	8.98	32.69	7.32	40
8	48	171.33	14.87	1.73	4.7	14.55	3.96	57.11	7.27	46
9	60	67.4	14.61	0.0	6.85	8.51	3.92	48.14	7.37	39

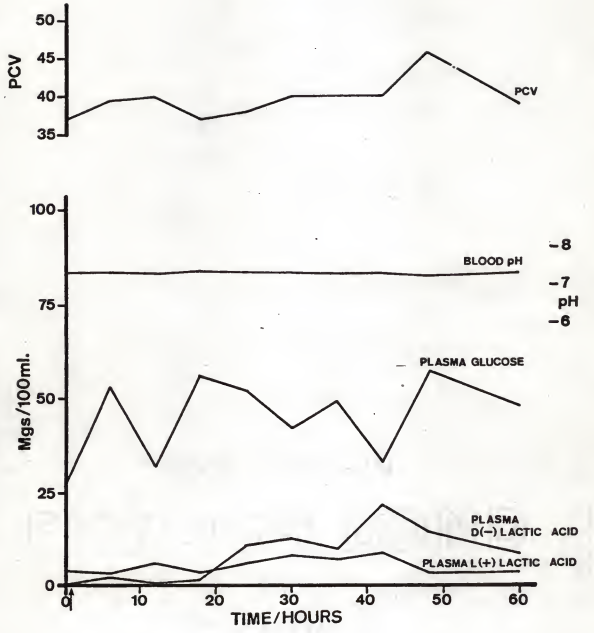
Trial # 6. Pure corn starch, 12 g per kg body weight infused at ↑ .

FIGURE 6


243.9
200
175
150
125
100
75
50
25
0
Mg/100ml
- 9
- 8
- 7
pH
- 6
- 5
- 4
RUMEN pH
RUMEN D(−) LACTIC ACID
RUMEN GLUCOSE
RUMEN L(+) LACTIC ACID
0
10
20
30
40
50
60
TIME/HOURS

Trial # 6. Pure corn starch, 12 g per kg body weight infused at ↑ .

FIGURE 6

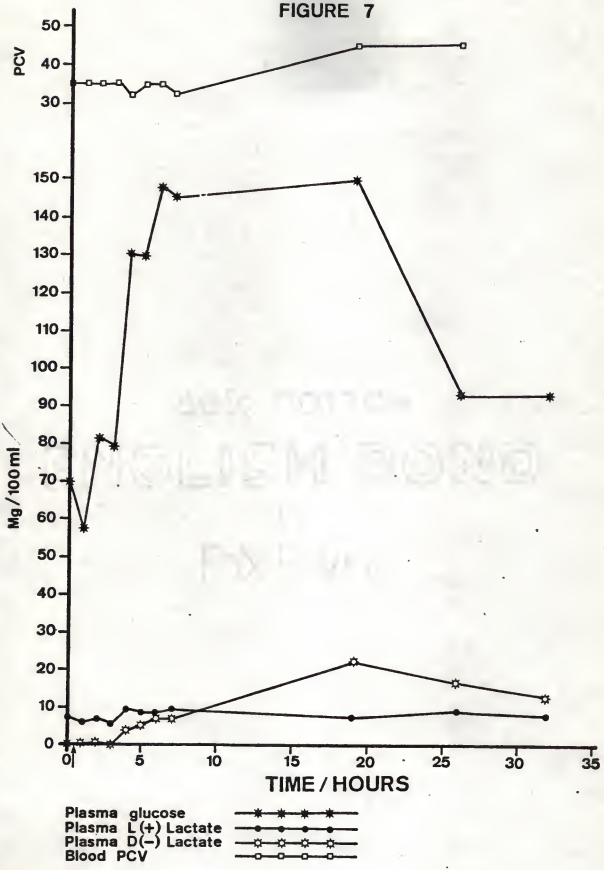

PCV
50
45
40
35
PCV
100
75
50
25
0
Mgs/100ml.
BLOOD pH
-8
-7
pH
-6
PLASMA GLUCOSE
PLASMA D(-) LACTIC ACID
PLASMA L(+) LACTIC ACID
0
10
20
30
40
50
60
TIME/HOURS

Trial # 7. Physiological changes in 45 kg goat acutely affected by experimentally induced D(-)lactic acidosis.

Table # 7

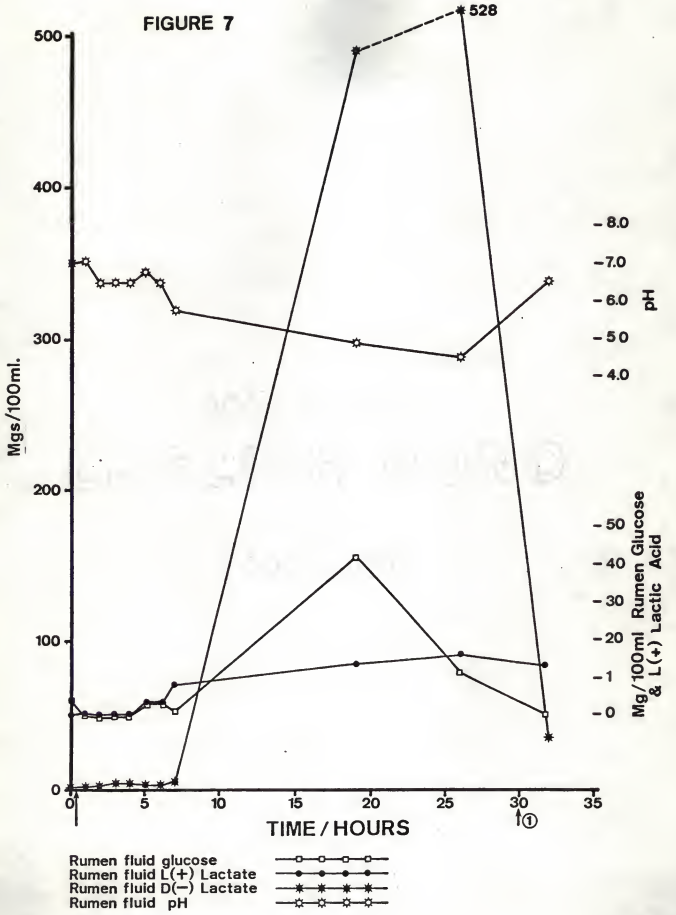
Sample	Time hr	Rumen D(-) lactic acid	Rumen L(+) lactic acid	Rumen glucose	Rumen pH	Plasma D(-) lactic acid	Plasma L(+) lactic acid	Plasma glucose	PCV
0	0	1.31	0.03	4.5	7.0	0.0	7.72	70.06	35.5
1	1	1.31	0.86	0.0	7.1	0.0	6.52	57.0	35.4
2	2	2.62	0.0	0.0	6.5	0.0	7.0	82.23	35.4
3	3	3.93	0.22	0.0	6.5	0.0	5.90	79.0	35.5
4	4	3.93	0.12	0.0	6.5	3.93	9.40	130.75	32.6
5	5	2.62	3.92	3.0	6.8	5.24	8.59	130.76	35.4
6	6	2.62	3.73	3.80	6.5	5.72	8.53	148.07	35.4
7	7	4.0	8.69	1.92	5.7	5.24	9.01	125.38	32.9
8	19	491.1	13.74	42.0	4.9	22.27	7.40	150.0	45.7
9	26	528.06	16.0	11.5	4.50	17.03	9.85	93.26	45.8
10	32	34.06	13.5	0.49	6.55	13.10	8.50	93.26	-

FIGURE 7


PCV
50
40
30
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Mg/100 ml
0
5
10
15
20
25
30
35
TIME / HOURS
Plasma glucose
Plasma L (+) Lactate
Plasma D(−) Lactate
Blood PCV

Trial # 7. Pure corn starch, 15 g per kg body weight infused at ↑ .

FIGURE 7


528
500
400
300
200
100
0
Mgs/100ml.
8.0
7.0
6.0
5.0
4.0
pH
50
40
30
20
1
0
Mg/100ml Rumen Glucose & L(+) Lactic Acid
0
5
10
15
20
25
30
35
TIME / HOURS
1
Rumen fluid glucose
Rumen fluid L(+) Lactate
Rumen fluid D(−) Lactate
Rumen fluid pH

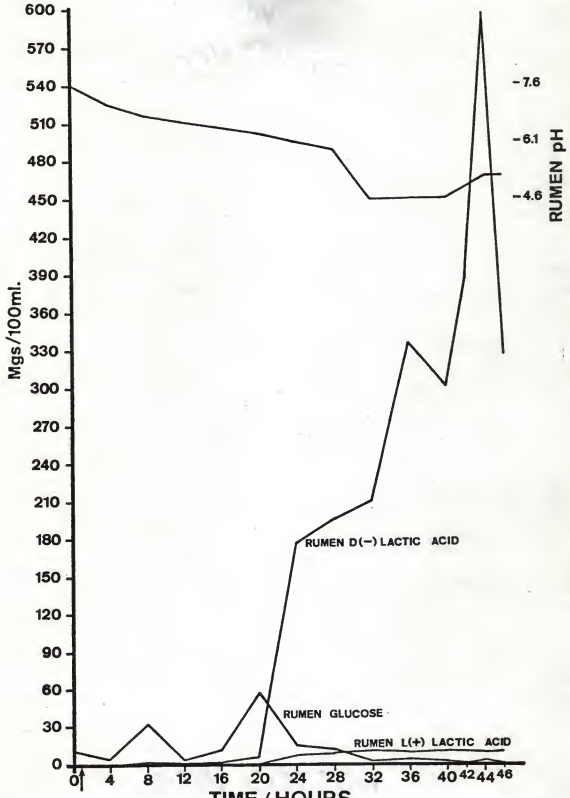
Trial # 8. Physiological changes in 45 kg goat acutely affected by experimentally induced D(-) lactic acidosis.

Table # 8

Sample	Time hr	Rumen D(-)lactic acid	Rumen L(+)lactic acid	Rumen glucose	Rumen pH	Plasma D(-)lactic acid	Plasma L(+)lactic acid	Plasma glucose	Blood pH	Urine D (-)lactic acid	Urine L (+)lactic acid	Urine glucose	Urine pH	PCV
0	0	0.0	0.28	13.14	7.6	2.5	2.70	61.63	7.42	*	*	*	*	36.5
1	4	0.0	0.41	3.56	6.8	0.5	3.60	66.24	7.414	*	*	*	*	36.5
2	8	1.46	0.48	33.12	6.3	1.2	5.18	69.39	7.484	0.76	0.74	0.0	7.6	37.5
3	12	0.36	0.32	3.35	6.08	6.0	4.99	100.41	7.497	*	*	*	*	35.0
4	16	1.46	0.32	12.51	6.8	2.8	5.53	71.90	7.535	*	*	*	*	34.0
5	20	5.84	0.54	57.44	5.5	6.0	3.22	71.48	7.520	*	*	*	*	33.0
6	24	174.6	11.68	14.04	5.3	2.8	4.08	91.40	7.501	*	*	*	*	36.5
7	28	196.5	12.49	12.57	5.1	13.48	4.83	73.16	7.427	11.45	0.64	0.0	6.8	38.0
8	32	210.0	13.84	2.93	4.6	11.45	4.54	86.37	7.39	*	*	*	*	42.8
9	36	342.7	13.91	2.30	4.6	11.45	2.57	95.38	7.365	*	*	*	*	47.5
10	40	298.1	14.0	0.83	4.6	10.68	2.57	115.51	7.369	*	*	*	*	45.0
11	42	384.3	14.10	0.41	4.75	11.90	2.54	113.52	7.374	*	*	*	*	52.0
12	44	590.0	13.58	1.25	5.1	4.07	2.70	123.48	7.375	*	*	*	*	52.0
13	46	325.2	14.07	0.83	5.1	4.32	3.38	79.24	7.372	*	*	*	*	52.0

Trial # 8. Pure corn starch, 16.5 g per kg body weight infused at ↑ .

FIGURE 8


600
570
540
510
480
450
420
390
360
330
300
270
240
210
180
150
120
90
60
30
0
Mgs/100ml.
7.6
6.1
4.6
RUMEN pH
RUMEN D(−) LACTIC ACID
RUMEN GLUCOSE
RUMEN L(+) LACTIC ACID
0
4
8
12
16
20
24
28
32
36
40
42
44
46
TIME / HOURS

Trial # 8. Pure corn starch, 16.5 g per kg body weight infused at ↑ .

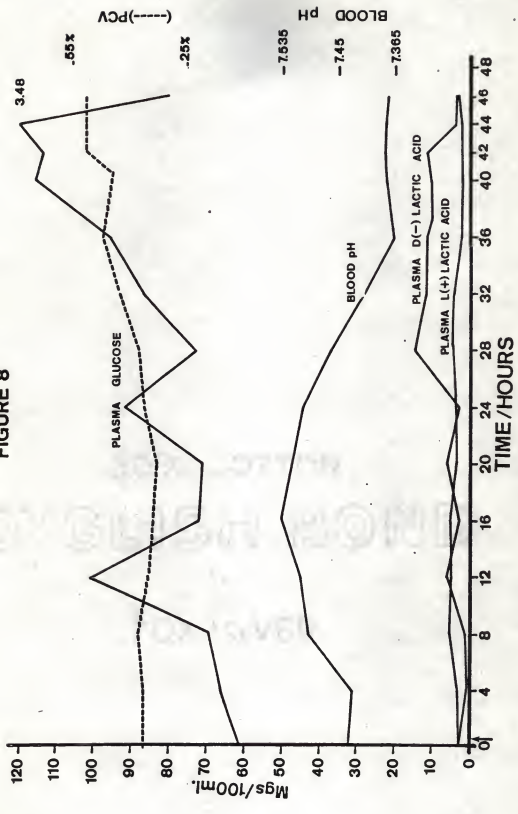


FIGURE 8
Mgs/100ml.
120
110
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
40
42
44
46
48
TIME/HOURS
PLASMA GLUCOSE
BLOOD pH
PLASMA D(−) LACTIC ACID
PLASMA L(+) LACTIC ACID
3.48
.55%
.25%
7.535
7.45
7.365
BLOOD pH
(-----)PCV

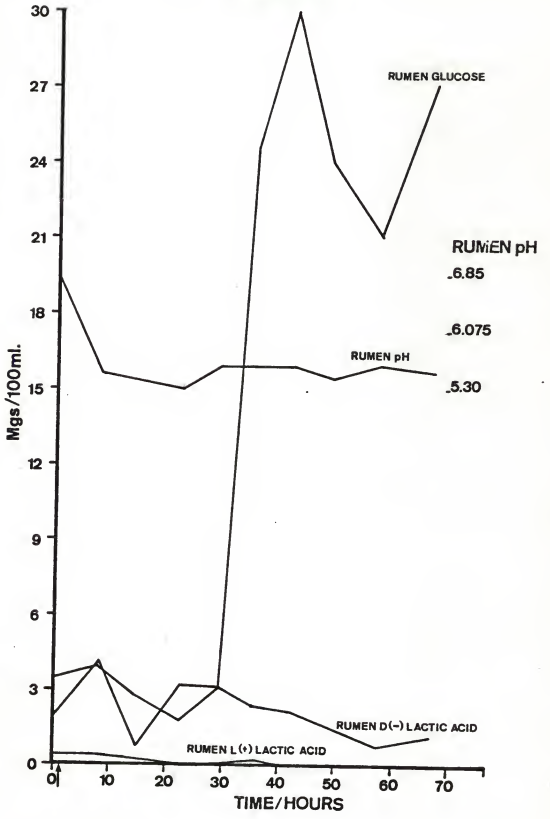
Trial # 9. Physiological changes in 46 kg goat with unsuccessfully induced D(-) lactic acidosis.

Table # 9

Sample	Time hr	Rumen D(-)lactic acid	Rumen L(+)lactic acid	Rumen glucose	Rumen pH	Plasma D(-)lactic acid	Plasma L(+)lactic acid	Plasma glucose	Blood pH	Urine D(-)lactic acid	Urine L(+)lactic acid	Urine glucose	Urine pH	PCV
0	0	1.82	0.22	3.42	6.85	0.0	32.2	68.40	7.5	0.0	0.86	0.36	8.0	35.5
1	7	4.02	0.22	3.96	5.50	0.0	2.67	64.12	7.423	0.0	0.86	0.0	7.10	35.5
2	14	0.73	0.19	2.88	5.35	0.0	4.15	56.88	7.420	0.0	0.09	0.54	7.07	28.5
3	21	3.29	0.0	1.98	5.30	0.0	2.76	54.64	7.392	0.0	0.0	1.62	7.07	33.0
4	28	3.29	0.03	3.24	5.6	0.0	6.18	57.80	7.381	0.0	0.0	2.16	7.07	32.5
5	35	2.55	0.12	24.32	5.6	0.0	2.51	57.62	7.433	0.0	0.19	0.0	7.30	35.0
6	42	2.42	0.0	29.72	5.6	0.0	3.76	63.19	7.429	0.0	0.03	0.0	7.40	32.0
7	49	0.0	0.0	23.90	5.4	0.0	8.88	34.38	7.420	0.0	0.32	0.0	7.40	32.2
8	56	0.73	0.0	21.10	5.6	0.0	4.92	26.39	7.400	0.0	0.32	0.54	7.40	32.1
9	66	1.10	0.0	27.0	5.5	0.0	5.98	25.10	7.457	0.0	0.12	0.98	7.7	30.0

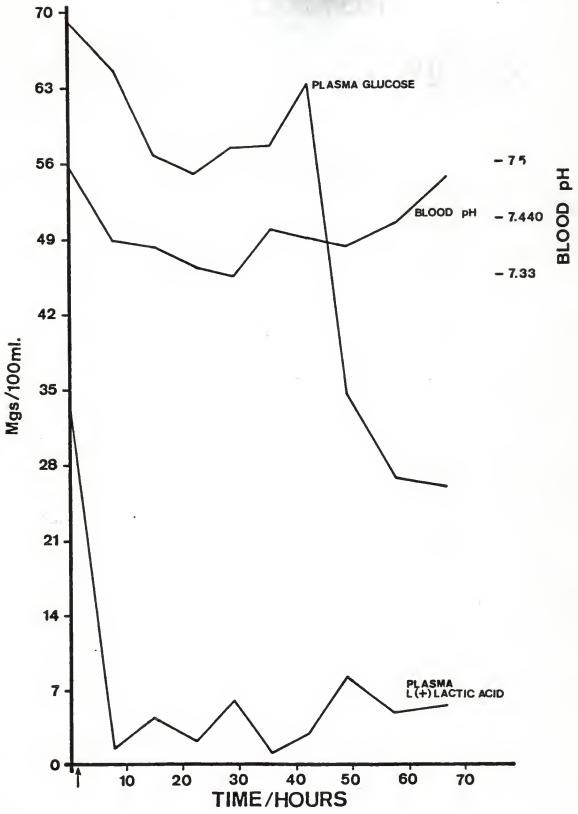
Trial # 9. Pure corn starch, 18 g per kg body weight infused at .

FIGURE 9


30
27
24
21
18
15
12
9
6
3
0
Mgs/100ml.
0
10
20
30
40
50
60
70
TIME/HOURS
RUMEN GLUCOSE
RUMEN pH
6.85
6.075
5.30
RUMEN pH
RUMEN D(−) LACTIC ACID
RUMEN L(+) LACTIC ACID

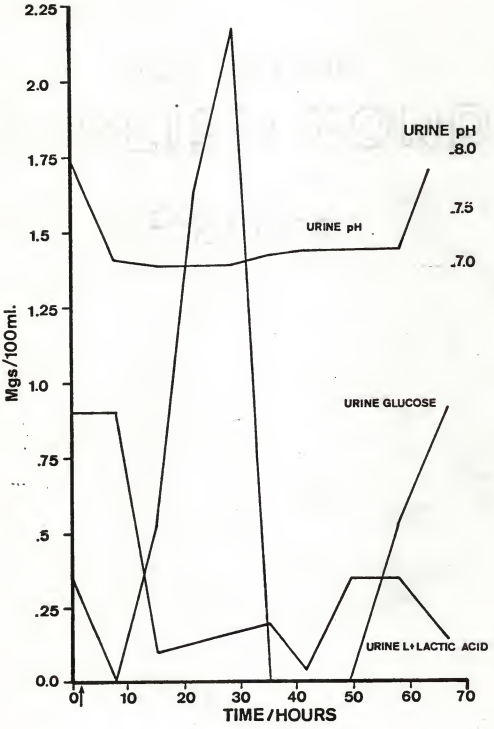
Trial # 9. Pure corn starch, 18 g per kg body weight infused at  .

FIGURE 9


70
63
56
49
42
35
28
21
14
7
0
Mgs/100ml.
PLASMA GLUCOSE
BLOOD pH
7.5
7.440
7.33
BLOOD pH
PLASMA
L(+)LACTIC ACID
1
10
20
30
40
50
60
70
TIME/HOURS

Trial # 9. Pure corn starch, 18 g per kg body weight infused at ↑ .

FIGURE 9


2.25
2.0
1.75
1.5
1.25
1.0
.75
.5
.25
0.0
Mgs/100ml.
URINE pH
8.0
7.5
7.0
URINE pH
URINE GLUCOSE
URINE L+LACTIC ACID
0
10
20
30
40
50
60
70
TIME/HOURS

ministered, an increase in the concentration of plasma D (-) lactic acid from 0.8 to 6.68 mg % was observed along with moderate anorexia and lack of feces consistency for the next 48 hours post infusion. The relationship between the appearance of plasma D (-) lactic acid, at concentrations up to 5 mg observed at about 24-30 hours post infusion, and the clinical disturbances such as anorexia and postration were evident when the dose of starch infused was 11 g/kg or greater. It was demonstrated, in fistulated animals, that rumen pH began to decline as soon as the infusion of the carbohydrate was ended. This decline by one or two pH units occurred during the first 19-24 hours post infusion. At this time rumen glucose and D (-) lactic acid levels were below 30-35 and 10-30 mg % respectively. As rumen pH decreased below an average value of 5.5 a sudden accumulation of free glucose in the rumen up to 50 mg % occurred. This was followed by a dramatic increase in rumen D (-) lactic acid from 1-20 mg % to 200-600 mg % (Tables 5, 6, 7). The lower the pH in the rumen fluid, the higher its D (-) lactic acid concentration.

In trial number 7, in which 14 g pure corn starch per kg body weight was administered it was observed that at approximately 26 hours post infusion the pH of rumen fluid was 4.5. This was the lowest pH value recorded during this work.

At this time rumen and plasma concentrations of D (-) lactic acid were 528.06 mg % and 22.27 mg % respectively. This animal was the most severely affected. Clinical signs were dorsal recumbancy, clonic movements, marked taquipnea and salivation. Because this animal was so severely affected the rumen contents were washed out. Within 20-25 minutes after the rumen was emptied normal appetite and behavior resumed, then rumen and plasma D (-) lactic acid levels dropped to 34.06 mg % and 13.10 mg % respectively and

rumen pH reached normal values (from 4.5 to 6.55).

It was found that when the rumen concentrations of D (-) lactic acid were 150 mg % or greater the rumen contents became abnormally watery. This was undoubtedly due to the influx of water from the animal tissue. Tissue dehydration was indicated by increasing PCV in trials 7 and 9. Copious diarrhea that lasted about 24 hours was seen in one animal (trial 8). Such diarrhea appeared about 56-60 hours after starch was infused. Animals from trials 4, 5, 6 and 7 seemed by their external appearance to be markedly dehydrated. The peak values for plasma D (-) lactic acid were reached at the same time (Fig. 8).

The peak values for rumen L (+) lactic acid after 24 hours of starch infusion in trials 5, 6, 7 and 8 were between 30 and 45 times smaller than their correspondant rumen D (-) lactic acid values. The values for L (+) lactic acid in plasma appeared to be fairly erratic. On trials 6 and 8, in which animals were severely affected, a systemic alkalosis was observed during the first 16 hours post infusion. This was followed by a decrease in pH which was directly related to the increase in plasma D (-) lactic acid and general disruption of animal physiology. In trial number 8 the urinary concentration of D (-) lactic acid at 28 hours post infusion period was 11.45 mg % compared to 13.48 mg % of the same isomer in plasma. This suggests that the urinary system is the main elimination route of D (-) lactic acid from the animal body. Urine pH decreased as systemic acidosis became more extreme.

The largest amount of starch infused was in trial 9. Paradoxically, no physiological changes appeared. It seemed that the accumulation of free glucose in the rumen, 29.72 mg %, recorded 42 hours post starch infusion, was insufficient to cause the production of D (-) lactic acid by rumen bacteria. Therefore D (-) lactic acid in plasma did not increase.

It is indicated that values of plasma L (+) lactic acid as high as 32.20 mg % and 18-20 mg %, for trials 9 and 2 respectively, were recorded before infusion was done.

DISCUSSION AND CONCLUSIONS

In trials 1, 2, and 3, the absence of clinical disturbances, as well as the loss levels of plasma D (-) lactic acid maximum, 0.0, 1.33 and 4.17 mg % respectively, are thought to be the result of the small amounts of starch infused. On trial 4, in which the same infusion procedure was used, and significantly larger amounts of starch were administered (see table 4) and increase in plasma D (-) lactic acid up to 5 mg % was recorded. This level of D (-) lactic acid was correlated with visible, although not severe, clinical alterations which consisted of the lack of normal responses to the surrounding environment as well as anorexia.

It is interesting to observe that before any significant levels of D (-) or L (+) lactic acid appeared in rumen fluid a marked decrease in ruminal pH had occurred. This situation was invariably observed in all fistulated animals during the first 20-24 hours post infusion. Even lower pH levels in rumen fluid were observed after 30 to 50 hours post infusion. This apparently depended on the amount of starch infused. See tables 5, 6, 7, 8. It has been demonstrated that the low rumen pH values initially observed after overfeeding with readily fermentable carbohydrates such as grains (34) or fruits (8) are caused by the excessive production of VFA (34). These organic acids which under normal feeding conditions are produced by the usual cellulolytic microbial rumen population serve as the main energy source for such animal species when absorbed to the bloodstream and taken by the liver. This VFA, when produced in excess, has also been incriminated as the causative agent for the development of rumen atony (32,34). Rumen atony occurred in trials 4, 5, 6, 7, 8 and 9 within the first 20 hours post infusion and lasted for about four hours. In trials

1, 2, and 3 inhibition of rumen motility was observed around 10 minutes after infusion was ended and lasted for no more than one hours.

One physiological explanation for this last situation is that not excessive production of VFA in the rumen took place, due to the low levels of substrate (starch) infused. No clinical signs of lactic acidosis were seen in these first three trials. The excessive production of VFA is probably the first stage of the pathogenesis of this syndrome.

In the last five trials (5 through 9) it was observed that after the atonic period, rumino reticular motility was resumed by sporadic but potent contractions for at least the next 15-20 hours. It has been suggested that the ruminoreticular atony observed in acidotic animals can be a protective mechanism against lactic acid absorption (19). The inhibitory effect of VFA on rumen motility appears to be carried about through the central nervous system (33). However, unlike the inhibitory effect of VFA, lactic acid has been shown to be unable to cause any inhibition of rumen motility when found in rumen fluid (33). When it is left in contact with duodenal mucosa, rumen atony is observed. Such an acidification has been demonstrated to be a potent stimulus for the release of secretin. Endogenous secretin may be an important etiological factor in the development of ruminal atony observed during D (-) lactic acidosis (4).

The finding that during the first 24 hours post infusion only minute amounts of both isomers of lactic acid along with ruminal pH values and rumino-reticular atony are in good agreement with some other investigators (3,34). Lee and Matrone (22) found higher concentrations of L (+) lactate and propionate in rumen liquor when Na^+ and K^+ were supplemented in purified diets given ad libitum whereas D (-) lactic acid and acetate reached greater concentrations, as compared to L (+) lactate and propionate, when neither Na^+ nor K^+ were supplemented.

Excessive management during preinfusion periods in trials 1, 2 and 9 gave rise to abnormally high levels of plasma L (+) lactic acid at that time.

After starch was given, levels of L (+) lactic acid in rumen fluid and plasma increased only slightly, if at all, and were fairly erratic (see graphs 1 through 9). The lack of significant increases in rumen fluid of L (+) lactic acid could have been due to its rapid conversion by rumen microbes, rapid absorption into body fluids, or insignificant production of this L (+) isomer by rumen microbial population. If significant concentrations of L (+) lactic were absorbed from the rumen after starch was infused it could be suggested that an increased rate of tissue metabolism was responsible for the low levels of L (+) isomer recorded in plasma. The increase in plasma glucose levels observed between 4 and 30 hours post infusion (Tables 1 through 8) may have been caused by increased gluconeogenesis and hepatic glucose release in response to the increased availability of both propionic and L (+) lactic acid isomer during this period.

Rumen and systemic alterations became evident when starch infusions were increased to 11 g per kg body weight. When this dose of starch was given, there was a drop in pH of rumen fluid below 5.5 concommitant with the accumulation of free glucose in rumen fluid around 50 mg % followed by D (-) lactic acid production in the rumen up to 200-550 mg %. It was a characteristic feature of this work that the pH of the rumen fluid decreased after one hour of starch infusion. Both lactic acid isomers did not increase during 16 hours post infusion period. Therefore it is assumed that cellulolytic microbial rumen population did use starch. Then the low rumen pH values at that time appeared to be the result of excessive production of VFA (31,34). As it was earlier mentioned, cellulolytic microbes tend to disappear as acidity increases in rumen fluid. Later as rumen pH lowers

even more, from 6 to 5.5 or less, a sudden increase in the production of free glucose in rumen fluid from about 0.0-5.0 mg % ml to around 50 mg % ml was observed. This was followed by a dramatic increase in the production of rumen D (-) lactic acid from 0.0-3.0 mg % to 200-600 mg %. These findings, glucose and D (-) lactic acid production and accumulation, occurred within 24 hours after infusion was done (graphs 5 through 8). The low rumen pH observed in these trials has been reported to be a proper environment for the overgrowth of acid resistant bacteria lactic acid producers such as Streptococcus bovis and Lactobacillus sp. (20). It is well known that Lactobacillus are the D (-) lactic acid producers (19), using free glucose as the main substrate (31).

The relationship between the amount of starch infused and the production of D (-) lactic acid showed not to be necessarily linear. This was seen in trial 9. In this animal the highest amount of starch was infused, 18 g per kg body weight, showing neither accumulation of free glucose in the rumen nor significant production of the D (-) isomer (Fig. 9-a). The peak value for free glucose concentration in the rumen fluid of this animal was 29.72 mg % at 42 hours after starch infusion. The possibility of cellulolytic rumen microbial accommodation to the surrounding environment is not excluded. This animal showed no clinical alterations. The lack of expected changes in the rumen and systemic physiology supports the fact that the accumulation of free rumen glucose within the first 24 hours post infusion, around 50 mg %, along with low pH in the rumen fluid below 5.5, are prerequisites for the development of acute D (-) lactic acidosis.

Low rumen pH values and high D (-) lactic acid concentrations in rumen fluid give rise to increased absorption of this isomer to the blood (26,38). Plasma D (-) lactic acid concentrations were invariably related to clinical

disturbances such as anorexia and postration at different degrees (trials 4 through 8). It seemed that physiological buffers were unable to neutralize such an hydrogen ion addition. In trial 6 a concentration of plasma D (-) lactic acid of 21.72 mg % along with a blood pH of 7.328 was found. Trial 9 showed no appearance of D (-) lactic acid in plasma, nor did blood pH changes.

It is well known that for lactate to be metabolized it has to be converted to pyruvate. This requires a specific L (+) lactic acid dehydrogenase enzyme to carry out the process. On the other hand, the "unphysiological" D (-) lactic acid isomer appears to be scarcely produced in animals (28) and the specific D (-) lactic dehydrogenase required for its metabolism seems also to be found only in minute amounts (36).

Dunlop and Hammond (9) showed that, after the intravenous injection of both isomers of lactic acid, the D (-) isomer disappeared from the bloodstream at considerably lower rates than the L (+) isomer. The conversion of D (-) lactate to labeled protein has been reported to occur in tissues from the abomasum, small intestine and colon of cattle overfed with grains (26). They also found that a significant interconversion of D (-) to L (+) lactate took place in colon tissue, as well as the production of carbon dioxide from D (-) lactate in abomasal tissue was reported. The presence of a D-alpha-hydroxydehydrogenase in liver and kidney mitochondria in ox and some other mammalian species was reported by Tubbs (1965). The physiological significance of this enzyme has not been completely elucidated.

Metabolic acidosis is usually associated with a rise in plasma potassium concentration and increased membrane permeability (13). It has been shown that as the hydrogen ion concentration of extracellular fluid is increased, an exchange takes place between the extracellular H^+ and the intracellular K^+ (13). Although in the present work no attempt was made to measure blood

potassium levels, it can be thought that the observed clinical disturbances, as related to systemic acidosis, can be due to hyperkalemia.

By the time rumen D (-) lactic acid reached its peak value, the rumen contents were abnormally watery (trials 5 through 9). It is postulated that as lactic acid accumulates in the rumen, resulting hypertonicity draws fluid from body tissues causing severe dehydration (35). In this experiment, only two animals, namely 5 and 8, showed evidence of this systemic dehydration.

Two possible factors have been incriminated in causing increased PCV values during acute acidosis in ruminant species: a) efflux of water from the bloodstream into the rumen (9), and b) release of red blood cells due to splenocontractions caused under stress by adrenaline release (2). This research, given the watery consistency of rumen digesta, supported the first situation. Dehydration can be responsible for the low systemic blood pressure leading to death of ruminants after grain overload (19). It is well recognized that low systemic blood pressure lowers the glomerular filtration rates, leading to anuria (19). Marked anuria was seen in severely acidotic animals. It was found that approximately the same concentration of D (-) lactic acid in urine as those found in plasma at a given time (Table 8). A similar finding was also reported by Giesecke (15).

The role that D (-) lactic acid plays in the pathogenesis of acute lactic acidosis seems to be largely based on the addition of hydrogen ions to the blood, by this way challenging the buffer capacity of the animal, as well as resulting in hyperkalemia.

LITERATURE CITED

1. Ahrens, A. F.: Histamine, lactic acid, and hypertonicity as factors in the development of rumenitis in cattle. Am. J. Vet Res. 28 (1967): 1335-1342.

2. Braide, V. B. C. and R. H. Dunlop: Pharmacological differences between sodium D-lactate and sodium L-lactate in sheep. Am. J. Vet Res. 30 (1969): 1281-1288.

3. Briggs, P. K., J. P. Hogan and R. J. Reid: The effect of volatile fatty acids, lactic acid, and ammonia on rumen pH in sheep. Aust. J. Agric. Res. 8 (1957): 674-690.

4. Bruce, L. A. and Huber, T. L.: Inhibitory effect of acid in the intestine on rumen motility in sheep. J. Anim. Sci. 37 (1973): 164-168.

5. Bruno, C. F. and W. E. C. Moore: Duodenal acidification: Stimulus for the release of intestinal hormones in sheep. J. An. Sci. 41 (1975): 1199-1205.

6. Church, D. C.: Digestive physiology and nutrition of ruminants. Vol. 1 (1976): 323-331.

7. Dirksen, G.: Acidosis. Physiology of digestion and metabolism in the ruminant. Oriel Press (1970):612-625.

8. Dunlop, R. H.: Pathogenesis of ruminant lactic acidosis. Adv. Vet. Sci. Comp. Med. 16 (1972): 259-302.

9. Dunlop, R. H. and P. B. Hammond: D-lactic acidosis of ruminants. A. of New York Ac. of Sci. 119 (1965): 1109-1141.

10. Dunlop, R. H., Hammond, P. B. and Stevens, C. E.: D-lactate in bovine blood following carbohydrate engorgement. Fed. of Am. Soc. for Exp. Bio. 23 (1964): 262.

11. Elam, C. J.: Acidosis in feedlot cattle practical observations. J. Anim. Sci. 43 (1976): 898-900.

12. Gawehn, K. and Bergmeyer, H. V.: D (-) lactate. Methods of Enzymatic Analysis. Weinhein: Verlag Chemie; New York Acad. Press. Second Ed., Vol 2 (1974): 1492-1495.

13. Gericke, D. M. and P. C. Belonge: Aspects of forced exercise and the therapy thereof in sheep. J. S. Afr. Vet Assoc. 46 (1975): 353-357.

14. Gibbons, W. J.: Rumen intoxication in cattle. Mod. Vet. Practice 43 (1962): 74.

15. Giesecke, D., C. Barthelmus and M. Stangassinger: Undersuchungen zur Genese und Biochemie der Pansenacidose. Zentralblatt fur Veterinarmedizin A, 23 (1976): 353-363.

16. Hinkson, R. S., W. H. Hoover and B. R. Poulton: Metabolism of lactate isomers by rat and sheep liver and rumen epithelial tissue. J. An. Sci. 26 (1967): 799-803.

17. Hohorst, H. J. In methods of enzymatic analysis. H. V. Bergmeyer, ed. Academic Press, Inc. New York, p. 266 (1963).

18. Huber, T. L.: Effect of acute indigestion on compartmental water volumes and osmolity in sheep. Am. J. Vet Res. 32 (1971):887-890.

19. Huber, T. L: Physiological effects of acidosis in feedlot cattle. J. of Anim. Sci. 43 (1976): 902-909.

20. Hungate, R. E., R. W. Dougherty, M. P. Bryant, and R. M. Cello: Microbiological and physiological changes associated with acute indigestion in sheep. Cornell Vet 42 (1952): 423-449.

21. Jensen, R. and D. R. Mackey: Diseases of feedlot cattle, 2nd edition. Lea and Febiger (1971): 292-295.

22. Lee, D., Jr. and G. Matrone: Influences of monovalent cations on

growth and lactic acid metabolism of sheep fed purified diets. J. Nutr. 101 (1971): 967-974.

23. Lockwood, L. B., D. E. Yoder and M. Zientz: Lactic acid. Annals of the N. Y. Academy of Sciences, 119 (1965): 854-864.

24. Mac Phearson, M. J.: Isolation and identification of amylolytic Streptococci from the rumen of sheep. J. Pathol. Bacterial. 66 (1953): 95-102.

25. Milton J. Allison, I. M. Robinson, R. W. Dougherty and J. A. Bucklin: Grain overload in cattle and sheep: Changes in microbial populations in the cecum and rumen. Am. J. Vet. Res. 36 (1967): 181-184.

26. Preston, A. M. and C. H. Noller: Metabolism of D (-) lactate by tissues of the ruminant digestive tract. J. An. Sci. 37 (1973): 1403-1407.

27. Raabo, E. and Terkildsen, T. C.: On the determination of blood glucose. Scand. J. Clin. and Lab Invest. 12 (1960): 402-407.

28. Ross, A.: Intracellular pH and distribution of weak acids across cell membranes. A study of D- and L-lactate and of DMO in rat diaphragm. Physiol. 249 (1975): 1-25.

29. Ryan, R. K.: Concentrations of glucose and low molecular weight acids in the rumen of sheep changed gradually from a hay to a hay-plus-grain diet. Am. J. Vet. Res. 25 (1964): 653-659.

30. Slyter, L. L., J. Bond, Z. S. Rumsey and J. M. Weaver: Ruminal bacteria, lactic acid and glucose in heifers fasting and refeeding. J. Anim. Sci. 39 (1974): 252.

31. Slyter, L. L.: Influence of acidosis in rumen function. J. Anim. Sci. 43 (1976): 910-929.

32. Stern, J. S., C. A. Baile and J. Mayer: Are propionate and butyrate physiological regulators of plasma insulin in ruminants? Amer. J.

Physiol. 219 (1970):84-91.

33. Svendsen, Per: The effect of volatile fatty acids and lactic acid on rumen motility in sheep. Nord. Vet. Med. 25 (1973): 226-231.

34. Svendsen, P.: An introduction to animal physiology. West Point, Conn. (1974): 205.

35. Telle, P. P. and R. L. Preston: Ovine lactic acidosis: Intraruminal and systemic. J. Anim. Sci. 33 (1971): 698-704.

36. Tubbs, P. K.: The metabolism of D-L hydroxy acids in animal tissues. Annals of the N. Y. Academy of Sci. 119 (1965): 920-926.

37. Waldo, D. R. and L. H. Schultz: Lactic acid production in the rumen. J. Dairy Sci. 38 (1955): 1453-1460.

38. Williams, V. J. and D. D. S. Mackenzie: The absorption of lactic acid from the reticulorumen of the sheep. Aust. J. Bio. Sci. 18 (1965): 917-934.

39. Whanger, P. D. and G. Matrone: Metabolism of lactic, succinic and acrylic acids by rumen microorganisms from sheep fed sulfur-adequate and sulfur deficient diets. Biochem. Biophys. ACTA 98 (1965): 454.

ORIGINS OF PRODUCTS

a) Corn starch

Sigma

St. Louis, Missouri 63178 U.S.

b) Pezzer Catheter, 76 French

Davol Rubber Company

Providence, R. I. U.S.

c) $(D\ (-)\ Lactate)_2\text{-}Ca\cdot 5H_2O$

Boehringen Mannheim Corp.

San Francisco, Cal. 94104 U.S.

d) D (-) Lactic Dehydrogenase, from L. leichmannii

Sigma

St. Louis, Missouri 63178 U.S.

ACKNOWLEDGEMENTS

The author wholeheartedly thanks his family for support and encouragement throughout his graduate studies. A special thanks to Dr. R. A. Frey, Head, Department of Anatomy and Physiology for his human qualities. Without the help of Mrs. Audyne Self this degree couldn't have been possible.

Isabel Lopez Almansa Lopez
Yo te amo Chatis

EXPERIMENTALLY INDUCED ACUTE D(-) LACTIC ACIDOSIS IN GOAT (CAPRA HIRCUS)

by

Javier Castillejos E.

B. Sci., Universidad Nacional Autonoma de Mexico

D.V.M., Universidad Nacional Autonoma de Mexico, 1971

AN ABSTRACT

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCES

Department of Anatomy and Physiology

Kansas State University

Manhattan, Kansas

1978

ABSTRACT

Four goats (Capra hircus) weighing from 35 to 50 kg each, were infused either via naso gastric tube or rumen fistula with pure corn starch to produce D(-) lactic acidosis. Specific enzymatic analysis for D(-) lactic acid, L(+) lactic acid and glucose in plasma, rumen fluid and urine were carried out. Blood packed cell volume data was also estimated as well as pH values for blood, rumen fluid and urine. It was found that the accumulation of free glucose in rumen up to 50 mg % for the next 24 hours post infusion along with rumen pH values of 5.5 or less were a necessary condition for the production of D (-) lactic acid up to 20-600 mg %. Levels of D (-) lactic in plasma up to 5 mg % were closely related to clinical disturbances observed. The elimination of the D (-) isomer from the animal body appeared to be carried out by urinary system.