RUMEN MICROBIAL CHANGES IN IONOPHORE ANTIOBIOTIC – TREATED STEERS WITH EXPERIMENTALLY INDUCED ACIDOSIS

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Introduction

Ionophore antibiotics intervene primarily in rumen microbial metabolism to make fermentation more favorable for the ruminant animal (Bergen and Bates, 1984). Among the favorable alterations in ruminal fermentation is decreased lactic acid production, attributed to inhibition of gram-positive, lactic acid-producing bacteria (Nagaraja and Taylor, 1987). Tetronasin, produced by *Streptomyces longisporoflavus*, is a new ionophore antibiotic that is being investigated as a possible feed additive for ruminants. Tetronasin has an antibacterial spectrum similar to that of monensin and recently was shown to be effective in preventing the accumulation of lactic acid in a continuous coculture of lactic acid-producing and -fermenting ruminal bacteria (Newbold and Wallace, 1988). The objective of this experiment was to determine the relative effectiveness of tetronasin in the prevention of experimentally induced lactic acidosis.

Materials and Methods

Five ruminally-cannulated steers (weighing 250 to 300 kg) fed a diet of alfalfa hay and grain (80:20) were used. The experimental design was a Latin square, with each steer receiving each of the following five treatments: control (no antibiotic), .15 mg of tetronasin/kg body weight (B.W.), .25 mg of tetronasin/kg B.W., .25 mg of salinomycin/kg B.W. and 1.0 mg of lasalocid/kg B.W. Acidosis was induced by intraruminal administration (6.25 g/kg body wt.; twice daily) of a slurry of finely ground corn and corn starch (50:50) alone or with antibiotics. The carbohydrate-antibiotic mixture was administered twice a day for up to 4 days or until the cattle exhibited ruminal (rumen pH $<$ 4.5) or systemic acidosis, as evidenced by decreases in blood pH, bicarbonate concentration and base excess values. At the end of each period, the rumen of each steer was emptied and washed with warm water and inoculated with ruminal contents from a healthy donor steer.

Ruminal fluid samples were obtained from each steer before (0 h) and at 12, 24, 36, 48, 60, 72, 84, and 96 h after the initial carbohydrate-antibiotic mixture dosing. Rumen fluid was analyzed for pH, lactic acid, volatile fatty acid, total viable anaerobic bacteria, lactic acid-producing bacteria (anaerobic lactobacilli), lactic acid-fermenting bacteria, total and generic composition of ciliated protozoa. Culture media used to enumerate ruminal bacteria included modified MRS medium for anaerobic Lactobacilli, complete carbohydrate agar (CA) for total viable anerobic counts and selective agar medium with Na-D, L-lactate (.3%) for counts of lactate-fermenting bacteria (Anderson et al., 1987). Microbial enumeration was not done for the tetronasin-treated steer fed the low dose (.15 mg). Statistical interpretation was by analysis of variance for a Latin square design, with a split plot in time, performed using a General Linear Models procedure of Statistical Analysis Systems.

Results and Discussion

Intraruminal administration of the ground corn and corn starch mixture for 3 days was sufficient to cause control steers to become ruminally acidotic (pH $<$ 5.0). Although, ionophore antibiotic-treated steers occasionally exhibited ruminal pH below 5.0, there was no evidence of systemic acidosis as evidenced by near normal blood pH and no marked base deficit.

Ruminal pH of control steers was not different from that of steers treated with antibiotics for up to 60 h. Lasalocid-, salinomycin-, and tetronasin (.15 mg) -treated steers had higher ruminal pH than the control at 72 h (table 1). Control steers became ruminally acidotic at 72 h as evidenced by the low pH and high lactic acid concentration. Although ruminal lactic acid concentrations tended to be higher initially in antibiotic-treated steers the difference was not significant. Ruminal

lactate concentrations in samples collected after 60 h in antibiotic-treated steers were extremely low (table 1). Total VFA concentration increased in all steers after intraruminal administration of the grain and starch mixture and tended to be higher in antibiotic-treated steers than control (table 1). The molar proportion of propionate was higher between 24 to 60 h in antibiotic-treated steers than in control (table 2). The molar proportion of butyrate tended to be lower in antibiotic-treated steers than in control initially but after 60 h, the butyrate proportion increased substantially in all antibiotic-treated steers (table 2).

Total anaerobic bacterial counts increased in 60 h. However, low ruminal pH in antibiotic treated steers was associated with high VFA concentra-

TABLE 1. RUMINAL pH, LACTIC ACID AND VOLATILE FATTY ACID CONCENTRATIONS IN CONTROL OR IONOPHORE ANTIBIOTIC-TREATED STEERS WITH EXPERIMENTALLY INDUCED LACTIC ACIDOSIS

Sampling time, h	Control	Tetronasin[a]		Lasalocid[a]	Salinomycin[a]	SE
		.15 mg	.25 mg	.25 mg	.25 mg	
pH						
0	7.11	7.14	7.10	7.15	7.08	.04
12	6.66	6.55	6.41	6.60	6.44	.08
24	6.47	6.27	5.87	6.16	5.95	.18
36	6.17	6.04	5.08	5.92	5.89	.30
48	5.61	5.88	5.17	5.61	6.10	.25
60	5.33	6.13	5.21	5.56	5.78	.38
72	4.98[d]	5.47[c]	5.13[d]	5.67[e]	5.61[c]	.09
84	–	5.74	4.83	5.70	6.31	–
96		5.60	5.20	5.50	6.40	
L(+) and D (-) Lactic acid, mM						
0	.1	0	.1	.1	.1	.1
12	.2	.2	.6	.2	.3	.3
24	.2	.1	5.1	1.0	20.8	3.7
36	.3	.2	31.6	.6	22.1	11.4
48	12.3	4.7	1.8	.3	1.0	4.1
60	11.5	18.4	1.5	4.7	12.1	8.2
72	29.1	1.3	5.1	.3	1.4	7.7
84	–	.1	7.2	.1	.2	–
96	–	0	0	.2	0	–
Volatile fatty acid, mM						
0	38.4	40.4	42.3	38.5	49.2	3.4
12	61.9	73.3	73.9	65.7	75.3	4.4
24	76.6[d]	88.3[de]	94.3[e]	90.8[e]	75.8[d]	4.1
36	74.5	86.8	54.4	84.2	64.1	10.3
48	60.7[b]	88.7[bc]	106.7[c]	87.7[bc]	73.7[b]	10.0
60	60.5[b]	81.9[bc]	108.3[c]	104.4[c]	77.0[bc]	12.2
72	73.8[d]	105.9[ef]	113.8[e]	89.5[df]	78.3[d]	6.3
84	–	75.9	131.0	92.6	73.0	–
96	–	71.6	98.0	98.4	65.9	–

[a]Kg^{-1} body wt/d^{-1}
SE = Standard error of the mean
[bc]Means in the same row with different superscripts differ ($P < .1$).
[def]Means in the same row with different superscripts differ ($P < .05$).

TABLE 2. RUMINAL PROPIONATE AND BUTYRATE PROPORTIONS IN CONTROL OR IONOPHORE ANTIBIOTIC-TREATED STEERS WITH EXPERIMENTALLY INDUCED LACTIC ACIDOSIS

Sampling time, h	Control	Tetronasin[a]		Lasalocid[a]	Salinomycin[a]	SE
		.15 mg	.25 mg	1.0 mg	.25 mg	
				mol/100 mol		
Propionate						
0	11.9	12.7	12.9	12.2	13.3	.3
12	24.7	27.1	28.8	29.5	27.8	1.5
24	24.5^{d}	33.7^{ef}	39.1^{e}	40.2^{e}	29.7^{de}	2.5
36	18.6^{d}	35.5^{d}	32.7^{de}	41.7^{e}	33.8^{e}	4.4
48	13.4^{d}	29.0^{ef}	26.6^{e}	38.5^{f}	34.2^{ef}	3.2
60	13.3^{d}	21.5^{def}	15.5^{de}	33.5^{f}	27.4^{ef}	4.4
72	17.6	16.7	18.4	27.3	21.7	6.3
84	–	20.1	14.1	25.7	25.8	–
96		21.5	5.6	26.5	27.8	–
Butyrate						
0	9.3	8.8	9.2	9.7	8.0	.8
12	9.5^{d}	10.4^{d}	8.8^{de}	9.6^{d}	7.7^{e}	.5
24	12.6^{d}	7.7^{e}	6.1^{ef}	5.9^{ef}	4.9^{f}	.9
36	15.7^{d}	7.4^{e}	3.0^{f}	6.1^{ef}	5.4^{ef}	1.1
48	11.7	7.8	14.6	10.8	5.9	3.1
60	16.6^{bc}	9.6^{c}	32.0^{b}	11.0^{c}	13.6^{c}	5.4
72	8.4	19.2	29.2	14.9	20.2	6.4
84	–	20.4	27.5	18.0	11.3	–
96	–	17.1	32.0	18.0	11.8	–

[a]Kg^{-1} body weight d^{-1}.
SE = Standard error of the mean.
[bc]Means in the same row with unlike superscripts differ ($P < .1$).
[def]Means in the same row with unlike superscripts differ ($P < .05$).

tion and not with high lactic acid concentration. The increased VFA concentration was probably reflective of increased lactic acid fermentation. This was evidenced by higher counts of lactic acid-fermenting bacteria in antibiotic-treated than in control steers. Also, antibiotic-treated steers had higher propionate proportion than the control initially. After 36 to 48 h, the propionate proportion declined with a concurrent increase in butyrate proportion particularly in tetronasin-treated steers. Butyrate may be a major product of lactate fermentation in steers treated with ionophore antibiotics.

Although ruminal lactobacilli are extremely sensitive to ionophore antibiotics (Nagaraja and Taylor, 1987), antibiotic-treated steers had high all steers following intraruminal administration of the carbohydrate mixture. Antibiotic treatment had no effect on total bacterial counts (table 3). Lactobacilli counts increased in all steers following the carbohydrate dosing. Salinomycin and tetronasin-treated steers had higher Lactobacilli counts ($P < .05$) than control at 36 h (table 3). Lactate-fermenting bacterial counts tended to decline in the control steers following the intraruminal administration of carbohydrate. Antibiotic-treated steers had higher ($P < .05$) counts of lactate-fermenting bacteria than the controls after 48 h (table 3).

(Key Words: Ionophores, Rumen, Acidosis)

TABLE 3. RUMINAL TOTAL ANAEROBIC, LATIC ACID-PRODUCING AND – FERMENTING BACTERIAL COUNTS IN CONTROL OR IONOPHORE ANTIBIOTIC-TREATED STEERS WITH EXPERIMENTALLY INDUCED LACTIC ACIDOSIS

Sampling time, h	Control	Tetronasin[a] .25 mg	Lasalocid[a] 1.0 mg	Salinomycin[a] .25 mg	SE[b]
Total counts, x10^{10}/g of DM					
0	4.7	5.8	5.1	6.6	1.2
12	12.5	13.5	32.1	18.4	1.0
24	11.4	16.7	38.7	33.1	1.7
36	30.3	21.0	17.1	11.4	1.5
48	30.3	26.9	28.5	16.1	1.8
60	13.1	17.1	18.2	16.6	1.4
72	29.6	57.9	62.4	30.0	1.4
84		25.7	13.5	56.2	
96	–	–	30.2	44.2	
Lactobacillus, x10^{8}/g of DM					
0	9.0	3.9	4.3	1.4	1.9
12	3.0	3.1	1.1	4.0	2.5
24	10.0	67.0	12.2	44.0	2.4
36	5.1[b]	130.6[c]	25.0[d]	71.8[cd]	1.8
48	88.7	209.9	66.1	90.0	2.3
60	101.4	349.1	86.7	350.8	2.3
72	439.9	524.8	761.8	327.1	2.3
84	–	–	134.9	558.0	–
96		–	149.6	489.8	
Lactate-fermenting, x10^{9}/g of DM					
0	8.6	17.8	10.7	13.5	1.3
12	5.4	8.9	6.6	4.6	1.6
24	7.1	16.6	10.0	3.9	1.9
36	3.4	5.0	21.9	10.6	2.1
48	3.6[b]	8.6[c]	22.2[cd]	14.1[cd]	1.4
60	1.6[b]	21.0[c]	25.2[c]	21.7[c]	1.5
72	.3	45.0	15.4	17.3	2.6
84	–	–	23.3	28.8	–
96	–	–	26.9	–	

[a]Kg^{-1} body wt/d^{-1}.
SE = Standard error of mean.
[bcd]Means in the same row with different superscripts differ ($P < .05$).

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