EFFECTS OF IONOPHORE ANTIBIOTICS ON RUMEN FERMENTATION

by

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INTRODUCTION

As the world's population increases, the ruminant's importance also increases. The ruminant's unique ability to ferment its diet allows utilization of non-protein nitrogen and fiber which man can not utilize. However, fermentation can be inefficient resulting in losses such as nitrogen and methane. Manipulating fermentation is not just a good idea but a necessity.

Lasalocid and monensin, ionophore antibiotics, alter rumen fermentation and thereby increase the efficiency in ruminants. They affect the proportion of ruminal acids, nitrogen metabolism, methane production and overall health of the animal. The question has arisen whether these antibiotics exert their influence on rumen fermentation over a long period of time or have rumen bacteria become resistant to them.

The purpose of this study was to:

1. test the effect of a new ionophore antibiotic on rumen fermentation.
2. test the effect of ionophore antibiotics on ammonia production and utilization from in vitro fermentation of two nitrogen sources.
3. test the effect of lasalocid and monensin on the rumen microbial population to see if the microorganisms adapt to the antibiotics when they are fed for a period of time.

EFFECT OF MONENSIN, LASALOCID AND A NEW IONOPHORE RO22-6924/004 ON IN VITRO RUMEN FERMENTATION

Summary

The effects of monensin sodium, lasalocid sodium and a new ionophore antibiotic, RO22-6924/004 on rumen fermentation were tested in vitro. The substrate for the in vitro fermentation consisted of ground corn with soybean meal or corn starch with urea. Antibiotic concentrations tested were 0, 15, 30, 60 and 120 $\mu g \cdot ml^{-1}$. Fermentation characteristics determined were gas production, microbial cell protein yield, volatile fatty acid and ammonia concentrations. All three antibiotics decreased microbial activity as evidenced by decreased total gas production and decreased microbial cell protein yield. Ionophore RO22-6924/004 appeared to be more effective than either lasalocid or monensin. All three antibiotics increased the molar proportion of propionate and decreased the molar proportions of acetate and butyrate. Total volatile fatty acid concentration decreased with ground corn and soybean meal, but remained unchanged with starch and urea substrate. Ammonia concentration decreased with ground corn and soybean meal but increased with starch and urea substrate. The results suggest that RO22-6924/004 is more potent than either monensin or lasalocid in altering the in vitro rumen fermentation characteristics.

Introduction

Two feed additives, lasalocid (Bovatec®, Hoffman-LaRoche, Inc., Nutley, NJ) and monensin (Rumensin®, Ely Lilly, Greenfield, IN) have had great economic impact on the beef cattle industry. Several studies have shown lasalocid and monensin behave similarly in regard to rumen fermentation and feed efficiency (Bartley et al, 1979; Brethour, 1979; Herod et al, 1979; Berger and Ricke, 1980). Their enhancement of cattle performance has encouraged the testing of related compounds such as narasin (Potter et al., 1979), avoparcin (Johnson et al., 1979), thiopeptin (Gill et al., 1979),

and salinomycin (Zinn and Axe,1983), that may also alter ruminal fermentation and improve feed efficiency and(or) weight gain.

Our objective was to compare the effects of monensin sodium and lasalocid sodium with a new ionophore RO22-6924/004 (Hoffman-LaRoche, Inc., Nutley, N.J.) on rumen fermentation in vitro.

Materials and Methods

The effects of lasalocid sodium, monensin sodium and RO22-6924/004 (6924) on in vitro rumen fermentation were compared. The fermentation substrate contained either 3,880 mg corn starch and 120 mg of urea or 3,296 mg ground corn and 704 mg soybean meal. The antibiotics dissolved in methanol were added at 0, 15, 30, 60, and 120 $\mu g \cdot ml^{-1}$.

Rumen fluid inoculum for the in vitro rumen fermentation was obtained from a rumen-fistulated Holstein steer 8 h after feeding. The steer was fed twice daily 1.8 kg alfalfa hay (IFN 1-00-096) and 1.35 kg concentrate diet composed of 40.6% grain sorghum (IFN 4-05-643), 40.6% corn (IFN 4-02-931), 17.5% soybean meal (IFN 5-04-604), .5% dicalcium phosphate (IFN 6-01-080), .5% trace mineral salt, and .2% Vitamin A and D supplement containing 1,000,000 IU of A and 500,000 IU of D per 454 grams. The rumen fluid was immediately strained through four layers of cheesecloth. Microbial activity was estimated by the el-Shazly and Hungate (1965) method. Four grams substrate, 100 ml mineral buffer and 50 ml rumen fluid inoculum were incubated at 39 C for 6 hours. Activity was measured by gas produced directly by fermentation and indirectly by CO_2 released from the buffer due to volatile fatty acid (VFA) production. Gas production was measured by a water displacement apparatus (Bartley et al, 1979). At the end of incubation, pH of the mixture was measured and samples removed for VFA and ammonia analyses.

VFA was determined by acidifying rumen fluid with 6N HCl and centrifuging at 25,400 x g for 20 minutes. The supernatant was extracted with ether (Holdeman et al., 1977) and 1 µl was injected into a gas chromatograph (Sigma 3B, Perkin Elmer, Norwalk, CT) with a 1.83 m x .64 cm column packed with SP 1000 (oven temp 145 C, 75 $ml \cdot min^{-1}$ gas flow, thermal conductivity detector). Ammonia concentration was determined by the procedure of Conway as modified by Webb (1971).

Microbial protein synthesis was measured by adding 10 ml rumen fluid inoculum and 20 ml mineral buffer to .97 g corn starch and .03 g urea previously weighed into 50 ml plastic centrifuge tubes. The tubes were flushed with CO_2, capped with bunsen valves and incubated for 6 h at 39 C. The quantity of microbial protein synthesized during fermentation was determined by the method of Barr et al. (1974). The fermentation mixture was centrifuged at 25,400 x g for 15 minutes. The centrifugate was washed twice with methanol to remove urea and polysaccharide slime. The Kjeldahl nitrogen content of the washed centrifugate was determined (AOAC 1970).

A general linear model procedure was used to analyze data followed by comparison of treatment means that were of interest using the least square design techniques (Snedecor and Cochran, 1967).

Results

Final pH of the antibiotic-treated fermentation of both substrates was lower than the control with no antibiotic (tables 1 and 2). Gas production was depressed ($P<.001$) by the antibiotics at all concentrations. Except for monensin at 60 $\mu g \cdot ml^{-1}$, the depression was proportional to the antibiotic concentration.

Although there was no difference in total VFA concentration compared to the control with starch and urea substrate, 6924 at 15 $\mu g \cdot ml^{-1}$ had lower total VFA ($P<.05$) compared to monensin and lasalocid at the same concentration (table 3).

TABLE 1: EFFECT OF IONOPHORE ANTIBIOTICS ON IN VITRO RUMEN FERMENTATION[a]

Antibiotic concentration (μg/ml)	Final pH	Gas production (ml)	Microbial cell protein[b] (mg)	Ammonia-N (mg/dl)
Control				
0	5.70[c]	151.7[c]	75.6[c]	25.9[c]
Monensin				
15	5.48[f]	123.3[e]	73.8[c]	28.2[d]
30	5.47[f]	116.7[f]	74.4[c]	28.1[d]
60	5.47[f]	98.5[f]	71.3[d]	28.7[d]
120	5.46[f]	99.8[f]	71.9[d]	28.9[e]
Lasalocid				
15	5.45[f]	99.8[f]	71.9[d]	29.5[f]
30	5.49[f]	89.5[f]	71.9[d]	28.9[e]
60	5.49[f]	80.8[f]	68.8[e]	29.4[f]
120	5.52[f]	68.7[f]	64.4[e]	30.3[f]
RO22-6924/004				
15	5.46[f]	93.3[f]	70.6[d]	28.9[e]
30	5.42[f]	86.8[f]	68.1[f]	29.9[f]
60	5.41[f]	71.5[f]	65.6[f]	30.7[f]
120	5.44[f]	61.7[f]	60.6[f]	30.9[f]

[a]Substrate was corn starch and urea.

[b]Microbial cell protein expressed as mg protein synthesized per gram substrate.

[c,d]Means in the same column with different superscripts differ ($P<.05$).

[c,e]Means in the same column with different superscripts differ ($P<.01$)

[c,f]Means in the same column with different superscripts differ ($P<.001$).

TABLE 2: EFFECT OF IONOPHORE ANTIBIOTICS ON IN VITRO RUMEN FERMENTATION[a]

Antibiotic concentration (µg/ml)	Final pH	Gas production (ml)	Ammonia-N (mg/dl)
Control			
0	5.53[b]	129.3[b]	7.3[b]
Monensin			
15	5.47[b]	78.2[d]	5.3[c]
30	5.45[c]	71.7[d]	5.3[c]
60	5.44[c]	64.2[d]	5.3[c]
120	5.43[c]	56.0[d]	5.3[c]
Lasalocid			
15	5.45[c]	80.5[d]	6.3[b]
30	5.47[b]	73.0[d]	5.7[b]
60	5.46[b]	63.5[d]	5.5[b]
120	5.49[b]	50.2[d]	5.2[c]
RO22-6924/004			
15	5.49[b]	78.8[d]	5.2[c]
30	5.46[b]	67.7[d]	5.0[c]
60	5.44[c]	63.0[d]	4.8[c]
120	5.43[c]	45.8[d]	6.3[b]

[a]Substrate was ground corn and SBM.

[b,c]Means in the same column with different superscripts differ ($P<.05$).

[b,d]Means in the same column with different superscripts differ ($P<.001$).

TABLE 3. EFFECT OF IONOPHORE ANTIBIOTICS ON IN VITRO VOLATILE FATTY ACIDS CONCENTRATIONS[a]

Antibiotic concentration (μg/ml)	Total (μmoles/ml)	moles/100 moles			
		Acetate	Propionate	Butyrate	Others[b]
Control					
0	80.70	66.5[c]	20.4[c]	11.0[c]	2.0
Monensin					
15	85.08[f]	64.9[c,f]	25.2[e,f]	8.1[f]	1.9
30	84.59	64.6[d,f]	25.5[e,f]	8.0[f]	1.9
60	76.26	64.7[d,f]	25.4[e,f]	7.9[f]	2.0
120	82.11	65.6[c,f]	24.9[e,f]	7.6[f]	2.1
Lasalocid					
15	83.42[f]	63.3[e,f]	26.6[e,g]	8.3[f]	1.9
30	77.40	62.2[e,g]	27.8[e,g]	8.3[f]	1.8
60	71.68	61.8[e,g]	27.2[e,g]	8.6[f]	2.3
120	75.30	62.5[e,g]	26.8[e,g]	8.4[f]	2.3
RO22-6924/004					
15	73.53[g]	62.2[e,g]	27.7[e,g]	8.0[f]	2.0
30	76.80	62.3[e,g]	28.1[e,g]	7.7[f]	2.0
60	74.44	63.0[e,g]	27.5[e,g]	7.5[f]	2.0
120	74.45	63.4[e,g]	26.4[e,g]	7.9[f]	2.4

[a]Substrate was starch and urea.

[b]Others include isobutyrate, isovalerate and valerate.

[c,d]Means in the same column with different superscripts differ (P<.05).

[c,e]Means in the same column with different superscripts differ (P<.001).

[f,g]Means in the same antibiotic concentration with different superscripts differ P<.05).

Total VFA concentration decreased (P<.05) with corn and SBM substrate at all antibiotic concentrations except at 15 $\mu g \cdot ml^{-1}$ of lasalocid and monensin (table 4). Each antibiotic decreased acetate (P<.05) and butyrate (P<.001) and increased propionate (P<.001) with both substrates. At 15 and 120 $\mu g \cdot ml^{-1}$, monensin did not decrease (P>.05) acetate.

Final ammonia concentration was consistently higher with starch and urea in antibiotic-treated fermentation than the control (P<.05). Lasalocid and 6924 had greater increases than monensin although they were not significant. However, with corn and SBM, ammonia concentration decreased (P<.05) with monensin and 6924 producing the greatest response.

Each antibiotic decreased microbial cell protein synthesis. Reductions by monensin occurred at 60 and 120 $\mu g \cdot ml^{-1}$ (P<.05), whereas lasalocid (P<.05) and 6924 (P<.01) decreased synthesis at all concentrations.

Discussion

Final pH of the fermentation with either ground corn and SBM or corn starch and urea was lower with antibiotic-treated compared to the control. Because total VFA production remained unchanged or decreased, the pH decrease may be due to increased lactate production. Lactate concentrations in these experiments were not measured. However, both lasalocid and monensin are known inhibitors of lactate production from soluble sugars and ground grains (Dennis et al., 1981a) because of their ability to inhibit Streptococcus bovis and Lactobacillus species (Dennis et al., 1981b). It is possible to have increased lactate production from starch in the presence of ionophore antibiotics (T.G. Nagaraja, unpublished data). Lasalocid and monensin have been shown to reduce protozoal population in the rumen and inhibit protozoal activity (T.G. Nagaraja, unpublished data). Protozoa engulf starch and

TABLE 4. EFFECT OF IONOPHORE ANTIBIOTICS ON IN VITRO VOLATILE FATTY ACIDS CONCENTRATIONS[a]

Antibiotic concentration (µg/ml)	Total (µmoles/ml)	moles/100 moles			
		Acetate	Propionate	Butyrate	Others[b]
Control					
0	81.30[c]	68.5[c]	17.3[c]	11.7[c]	2.6
Monensin					
15	73.91[c]	64.4[e]	24.8[f]	8.7[f]	2.2
30	70.95[d]	65.1[d]	25.1[f]	8.2[f]	1.8
60	68.29[e]	64.2[f]	26.5[f]	8.4[f]	1.8
120	69.56[d]	63.9[f]	26.0[f,g]	8.3[f]	1.8
Lasalocid					
15	72.19[c]	64.6[e]	24.1[f]	9.2[f]	2.3
30	70.18[d]	64.5[e]	24.7[f]	8.7[f]	2.1
60	69.99[d]	63.3[f]	26.1[f]	8.7[f]	1.9
120	65.72[e]	63.1[f]	26.5[f,g]	8.5[f]	2.0
RO22-6924/004					
15	67.92[e]	63.3[f]	25.8[f]	9.3[f]	1.7
30	69.01[d]	62.4[f]	26.8[f]	8.9[f]	2.0
60	67.19[e]	61.8[f]	28.0[f]	8.3[f]	1.9
120	65.79[e]	61.3[f]	28.5[f,h]	8.5[f]	1.8

[a]Substrate was ground corn and SBM.

[b]Others include isobutyrate, isovalerate and valerate.

[c,d]Means in the same column with different superscripts differ (P<.05).

[c,e]Means in the same column with different superscripts differ (P<.01).

[c,f]Means in the same column with different superscripts differ P<.001).

[g,h]Means in the same antibiotic concentration with different superscripts differ (P<.05).

thereby protect starch against bacterial attack (Hungate, 1966). Therefore, in the presence of lasalocid or monensin more starch is available for bacterial fermentation leading to higher lactate production than the control. In addition to lactate, the lower ammonia concentation in ground corn and SBM substrate may also account for lower pH of antibiotic-treated fermentation. Because the final ammonia concentration in the fermentation mixture of corn starch and urea was higher in antibiotic-treated than the control, the lower pH must be due to increased lactate production.

All three antibiotics depressed microbial activity as evidenced by decreased gas production and microbial cell protein synthesis. These results are in agreement with the report of Van Nevel and Demeyer (1977) for monensin and Bartley et al. (1979) for lasalocid. Decreased gas production in the in vitro fermentation was reflective of decreased CO_2 and methane production as well as reduced VFA production. Both lasalocid and monensin inhibit methane formation in the rumen (Thornton et al., 1976; Van Nevel and Demeyer, 1977; Bartley et al., 1979; Chen and Wolin, 1979). Total VFA production in the in vitro fermentation remained unchanged only with corn starch and urea but decreased with ground corn and SBM. This is in contrast to Bartley et al., (1979) who reported no change in VFA production with ground corn, ground brome hay and urea substrate in in vitro fermentation. Each of the three antibiotics increased propionate proportion and decreased acetate and butyrate proportions which are consistent with other studies (Bartley et al., 1979; Fuller and Johnson, 1981; Gutierrez et al., 1982; Shell et al., 1983). The extent of propionate increase was highest with ionophore 6924 at all concentrations excepting 120 $mg \cdot ml^{-1}$ with starch and urea substrate.

Final ammonia concentrations with starch and urea substrate was higher in antibiotic-treated than the control fermentation. Because Starnes et al. (1984) have

shown that rumen ureolytic activity is inhibited by monensin and lasalocid, the increase in ammonia concentration is probably due to decreased assimilation by the microorganisms. However, Dinius (1978) has reported that monensin does not affect ammonia assimilation by bacteria. Final ammonia concentration with ground corn and SBM substrate was lower in antibiotic-treated than the control fermentation. The decrease is probably due to inhibition of proteolysis (Poos et al., 1979) as well as deamination (Van Nevel and Demeyer, 1977).

Based on the in vitro fermentation results, ionophore RO22-6724/004 appears to be more potent in altering rumen fermentation characteristics than either lasalocid or monensin. Therefore, additional testing of the new ionophore is warranted.

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EFFECT OF IONOPHORE ANTIBIOTICS ON AMMONIA CONCENTRATION FROM IN VITRO FERMENTATION OF PROTEIN AND NONPROTEIN NITROGEN

Summary

Two in vitro rumen fermentation experiments were conducted to test the effects of ionophore antibiotics on ammonia production and utilization. The fermentation substrate was ground corn with soybean meal or starch with urea. Ammonia concentration was measured at .5, 1.0, 2.0, 4.0 and 6.0 hours. In Expt. 1, lasalocid sodium, monensin sodium and a new ionophore antibiotic RO22-6924/004 were tested at 0, 15 and 60 $\mu g \cdot ml^{-1}$. With ground corn and soybean meal the antibiotics reduced the ammonia concentration at 1.0, 2.0 and 4.0 h but at 6.0 h only 6924 at 60 $\mu g \cdot ml^{-1}$ was significant. Ammonia concentration was higher in antibiotic-treated fermentation than the control with urea as the nitrogen source. The increase appeared to be because of decreased utilization. In Expt. 2, lasalocid sodium and monensin sodium were tested in vitro at 0 or 15 $\mu g \cdot ml^{-1}$ with rumen fluid inoculum from cattle fed lasalocid, monensin or no antibiotic (control). Addition of lasalocid or monensin to inoculum from control cattle decreased ammonia concentration from SBM and ground corn and increased from urea and starch. Addition of monensin to inoculum from lasalocid-fed cattle decreased ammonia concentration from SBM and ground corn, but had no effect on ammonia concentration with starch and urea. Addition of antibiotics to inoculum from monensin cattle had no effect on ammonia concentration from either substrate. Results indicate ruminal microorganisms from cattle adapted to lasalocid or monensin are resistant to the effect of ionophores on ammonia metabolism.

Introduction

Ammonia is the major product of ruminal fermentation of nitrogen compounds (McDonald, 1952). It is also the major nitrogen source for microbial protein synthesis (Bryant and Robinson, 1962). Therefore, the ruminal ammonia concentration is important for assessing the efficiency of nitrogen utilization in ruminants. Ruminal ammonia concentration is in a dynamic equilibrium between production and utilization. Ammonia nitrogen sources include: 1) degradation of dietary protein and non-protein nitrogen, 2) hydrolysis of urea recycled to the rumen and 3) degradation of microbial cell protein. Utilization includes: 1) assimilation by microbes, 2) absorption through the rumen wall and 3) passage to the omasum (Chalupa, 1972; Owens and Bergen, 1983). The concentration of ruminal ammonia can be influenced by any one of those factors. Ionophore antibiotics have an effect on ruminal nitrogen metabolism. They decrease ruminal proteolysis (Hanson and Klopfenstein, 1979; Poos et al., 1979), inhibit ruminal amino acid deamination (Van Nevel and Demeyer, 1977; Schelling et al., 1978) and decrease ruminal urease activity (Starnes et al., 1984). The objectives of this study were to test the effect of ionophore antibiotics on ammonia production and utilization from in vitro fermentation of protein and non-protein nitrogen sources and to determine if rumen microorganisms from antibiotic-fed cattle are resistant to the effect of additional antibiotics on ammonia production and utilization.

Material and Methods

Expt. 1. The effects of ionophore antibiotics - lasalocid, monensin and RO22-6924/004 on ammonia production and utilization from protein and nonprotein nitrogen were tested in vitro. Rumen fluid inoculum for the in vitro fermentation was obtained from a Holstein steer fed twice daily 1.8 kg alfalfa hay (IFN 1-00-091) and 1.4 kg concentrate diet composed of 40.6% grain sorghum (IFN

4-05-043), 40.6% corn (IFN 4-02-931), 17.5% soybean meal (IFN 5-04-604) .5% dicalcium phosphate (IFN 6-01-080), .5% trace mineral salt and .2% vitamin A and D supplement containing 1,000,000 IU vitamin A and 500,000 IU vitamin D per 454 grams. The rumen fluid was strained through four layers of cheese cloth. Four grams of substrate were incubated with 100 ml phosphate buffer (pH 6.8) and 50 ml strained rumen fluid at 39 C for 6 hours. The fermentation substrates consisted of 3,296 mg ground corn and 704 mg soybean meal (SBM) or 3,880 mg corn starch and 120 mg urea. Antibiotics dissolved in methanol were added at 0, 15 or 60 $\mu g \cdot ml^{-1}$. Ammonia concentration was measured at .5, 1.0, 2.0, 4.0 and 6.0 h incubaton by the procedure of Conway as modified by Webb (1971). The experiment was repeated three times with rumen fluid inoculum obtained from the same cattle on three different occasions.

Expt. 2. To determine if microorganisms from lasalocid- or monensin-fed cattle have developed resistance to the effects of antibiotics on ammonia production and utilization, in vitro fermentation similar to Expt. 1 was done with rumen fluid obtained from lasalocid- or monensin-fed cattle. Six cattle grouped into three groups of two each were used as sources of rumen fluid. One group was fed lasalocid (.66 $mg \cdot kg^{-1}$ body weight d^{-1}), the second group was fed monensin (.66 $mg \cdot kg^{-1}$ body weight d^{-1}) and the third group fed no antibiotic served as control. Each animal was fed a diet of 20% alfalfa hay and 80% concentrate diet. The composition of the concentrate diet was as in Expt. 1. Rumen fluid was sampled after the cattle had received the antibiotics for about 100 days. Antibiotics for the in vitro fermentation were tested at 0 or 15 $\mu g \cdot ml^{-1}$. The substrate sampling time and procedure for ammonia determination were as described in Expt. 1. A general linear model procedure was used to analyze data followed by comparison of treatment means that were of interest using the least square design techniques (Snedecor and Cochran, 1967).

Results

Exp. 1. Ammonia concentration in the in vitro fermentation system increased with time during the first 4 h incubation period. In almost all instances (except ionophore 6424 at 60 $\mu g \cdot ml^{-1}$ with starch and urea substrate), the concentration peaked at 4 h incubation (figures 1 and 2). Ammonia concentration was consistently higher with corn starch and urea then with ground corn and SBM substrate. The difference in ammonia concentration between the two nitrogen sources was approximately three- to four-fold at 4 h incubation.

In the in vitro fermentation system containing SBM as the nitrogen source all three antibiotics at 15 or 60 $\mu g \cdot ml^{-1}$ decreased ammonia concentration at 1.0, 2.0 and 4.0 h ($P<.01$). The difference at .5 and 6 h between 0 and 15 $\mu g \cdot ml^{-1}$ antibiotic concentration was not significant. However, at 60 $\mu g \cdot ml^{-1}$ level, the difference at .5 h was significant only with monensin and ionophore 6924; while at 6.0 h the difference was significant only with ionophore 6924 (figure 1). Among the three antibiotics tested, lasalocid appeared to be least effective in reducing ammonia concentration although the difference was significant only at 4 h incubation. Ammonia concentrations in the fermentation treated with monensin or 6924 were similar at all incubation times.

In the in vitro fermentation with urea as the nitrogen source, ammonia concentration throughout the incubaton was higher in antibiotic-treated fermentation than the control containing no antibiotic (figure 2). The difference in ammonia concentration was not significant at the .5 and 1.0 h incubation periods. Lasalocid-treated fermentation at 15 or 60 $\mu g \cdot ml^{-1}$ concentration had higher ammonia concentration than the control only at the 6 h incubation. At 15 $\mu g \cdot ml^{-1}$ concentration, ammonia concentration in 6924-treated fermentation was higher ($P<.05$) than the control at 2.0, 4.0 and 6.0 h and in monensin-treated fermentation the concentration was higher ($P<.05$) than the control only at 6 h incubation. At 60

Figure 1. The effect of 0 (●——●), 15 and 60 µg/ml of lasalocid (□——□), monensin (▽——▽) and RO22-6924/004 (*——*) on ammonia concentration from in vitro fermentation with ground corn and soybean meal.

a, b - means at the same sampling time with different superscripts differ (P<.05).

a, c - means at the same sampling time with different superscripts differ (P<.01).

a, d - means at the same sampling time with different superscripts differ (P<.001).

e, f - means at the same sampling time with different superscripts differ (P<.05).

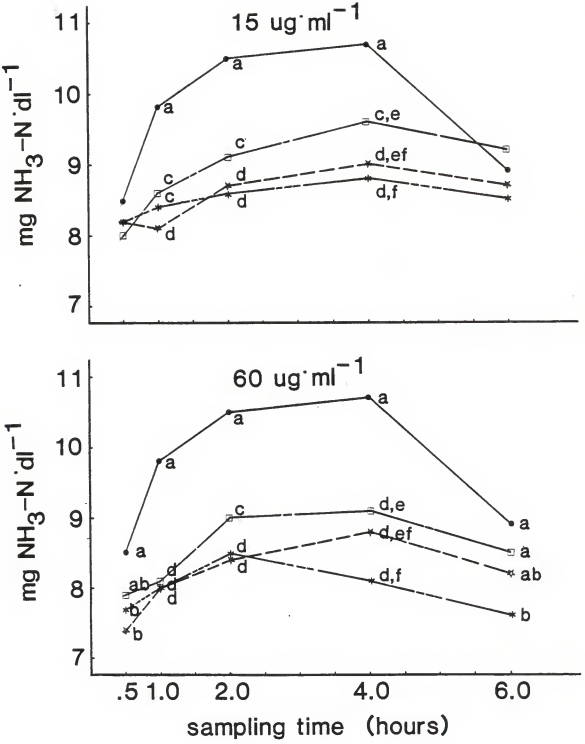

15 ug·ml⁻¹
60 ug·ml⁻¹
mg NH3-N·dl⁻¹
11
10
9
8
7
a
c,e
d,ef
d,f
c
d
ab
b
.5 1.0 2.0 4.0 6.0
sampling time (hours)

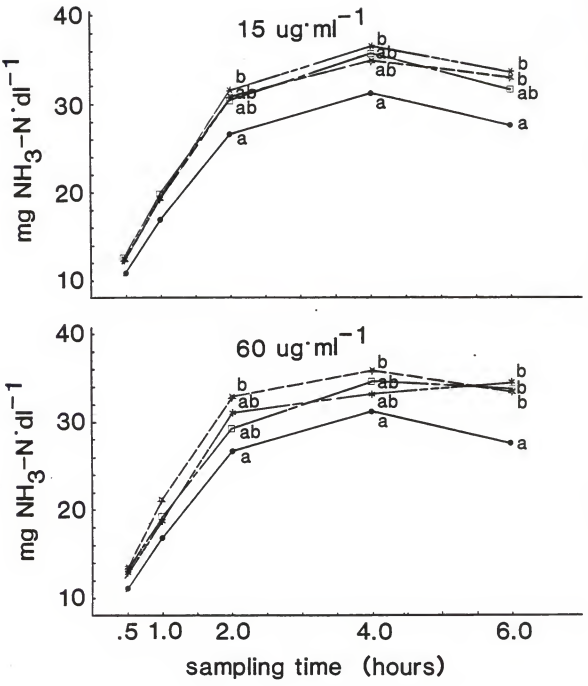
Figure 2. The effect of 0 (●————●), 15 and 60 µg/ml of lasalocid (⊡— — —⊡), monensin (✱— — —✱) and RO22-6924/004 (✱— — — —✱) on ammonia concentration from in vitro fermentation with corn starch and urea.

a, b - means at the same sampling time with different superscripts differ (P<.05).

a, c - means at the same sampling time with different superscripts differ (P<.01).

a, d - means at the same sampling time with different superscripts differ (P<.001).

e, f - means at the same sampling time with different superscripts differ (P<.05).


15 ug·ml⁻¹
60 ug·ml⁻¹
mg NH3-N·dl⁻¹
40
30
20
10
b
ab
a
.5 1.0 2.0 4.0 6.0
sampling time (hours)

$\mu g \cdot ml^{-1}$ concentration, ammonia concentrations in 6924-treated and monensin-treated fermentation were higher at 2.0, 4.0 and 6.0 hours.

Exp. 2. The effects of lasalocid and monensin on ammonia concentration in the in vitro fermentation of either substrate incubated with rumen fluid from control (no antibiotic) cattle were similar to that of Exp. 1. Ammonia concentration was higher with SBM but lower with urea in lasalocid or monensin treated fermentation than the control fermentation (figures 3 and 4).

In the in vitro fermentation with rumen fluid inoculum obtained from lasalocid-fed cattle, addition of lasalocid (15 $\mu g \cdot ml^{-1}$) had no effect on ammonia concentration from either SBM or urea. However, addition of monensin (15 $\mu g \cdot ml^{-1}$) had no effect on ammonia concentration from urea but reduced the ammonia concentration from SBM at 1.0 ($P<.05$), 2.0, 4.0 ($P<.01$) and 6 hours ($P<.05$). Addition of lasalocid or monensin to the in vitro system with rumen fluid inoculum obtained from monensin-fed cattle had no effect on ammonia concentration from either SBM or urea (figures 3 and 5).

Ammonia concentration in the in vitro fermentation of ground corn and SBM or corn starch and urea with no additional antibiotic added (0 $\mu g \cdot ml^{-1}$) was lower with rumen fluid inoculum obtained from control cattle than either lasalocid- or monensin-fed cattle (figure 4). The extent of ammonia decrease with additional lasalocid or monensin (15 $\mu g \cdot ml^{-1}$) was less with rumen fluid inoculum obtained from lasalocid or monensin-fed cattle than the cattle fed no antibiotic (figure 5). Ammonia concentration was higher in lasalocid- or monensin-treated fermentation (15 $\mu g \cdot ml^{-1}$) with rumen fluid from lasalocid- or monensin-fed cattle than the control cattle (figure 6). The difference was significant only during the first 2 h incubation.

Figure 3. The effect of 0 (●———●) and 15 µg/ml of lasalocid (⊟— – —⊟) and monensin (✱— — — —✱) on ammonia concentration from in vitro fermentation with ground corn and soybean meal.

a, b - means at the same sampling time within a group with different superscripts differ (P<.05).

a, c - means at the same sampling time within a group with with different superscripts differ (P<.01).

a, d - means at the same sampling time within a group with different superscripts differ (P<.001).

e, f - means at the same sampling time within a group with different superscripts differ (P<.05).

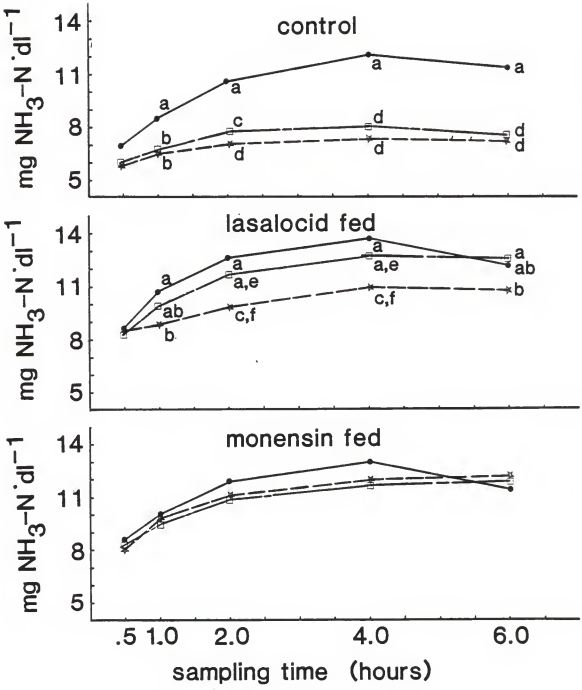

control
lasalocid fed
monensin fed
mg NH3-N·dl-1
14
11
8
5
.5 1.0 2.0 4.0 6.0
sampling time (hours)

Figure 4. The effect of 0 (●——————●) and 15 $\mu g \cdot ml^{-1}$ of lasalocid (□—— – ——□) and monensin (✱—— — ——✱) on ammonia concentratin from in vitro fermentation with corn starch and urea.

a, b means at the same sampling time with different superscripts differ ($P<.05$).

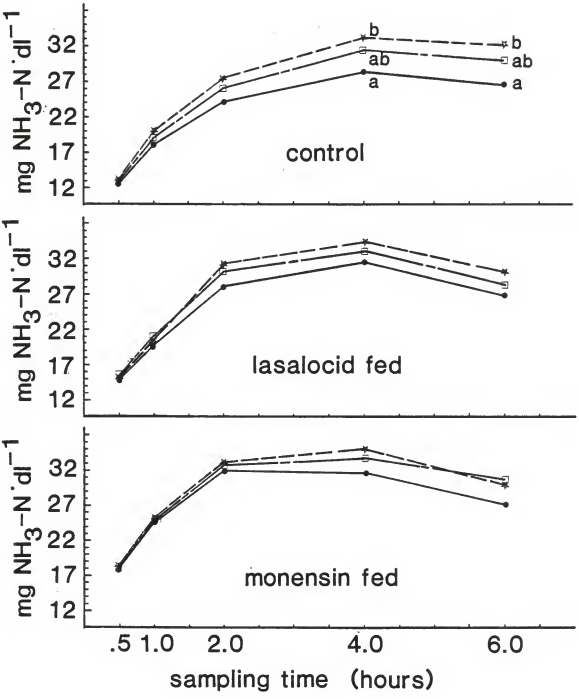

mg NH3-N·dl-1
32
27
22
17
12
b
ab
a
control
lasalocid fed
monensin fed
.5 1.0 2.0 4.0 6.0
sampling time (hours)

Figure 5. The effect of lasalocid and monensin on ammonia concentration from in vitro fermentation with ground corn and soybean meal and inoculum from control cattle (●——●), lasalocid cattle (□— - —□) and monensin cattle (✕— — —✕).

a, b - means at the same sampling time within a treatment with different superscripts differ (P<.05).

a, c - means at the same sampling time within a group with with different superscripts differ (P<.01).

a, d - means at the same sampling time within a group with different superscripts differ (P<.001).

e, f - means at the same sampling time within a group with different superscripts differ (P<.05).

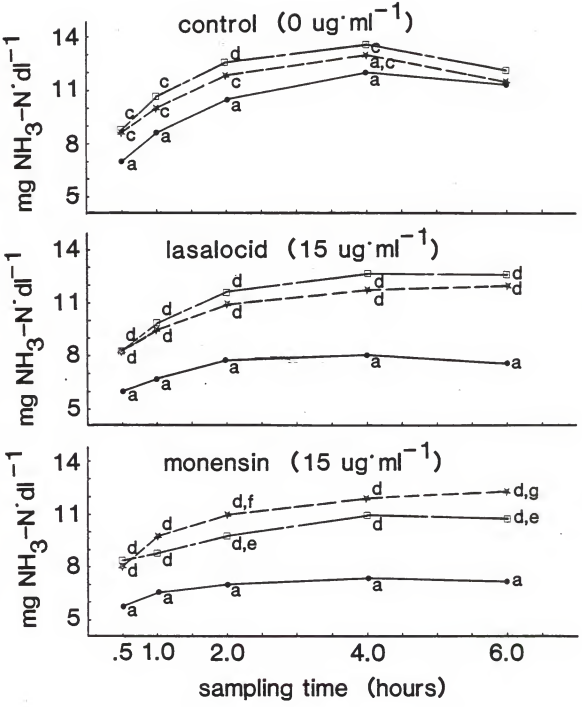

control (0 ug·ml⁻¹)
lasalocid (15 ug·ml⁻¹)
monensin (15 ug·ml⁻¹)
mg NH3-N·dl⁻¹
14
11
8
5
.5 1.0 2.0 4.0 6.0
sampling time (hours)

Figure 6. The effect of lasalocid and monensin on ammonia concentration from in vitro fermentation with corn starch and urea and inoculum from control cattle (●———●), lasalocid cattle (⊟– –⊟) and monensin cattle (✕— —✕).

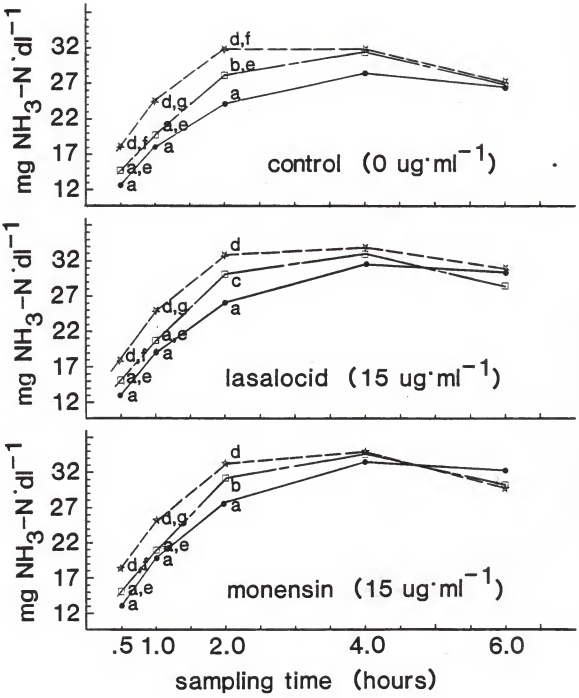
a, b - means at the same sampling time within a treatment with different superscripts differ (P<.05).

a, c - means at the same sampling time within a treatment with different superscripts differ (P<.01).

a, d - means at the same sampling time within a treatment with different superscripts differ (P<.001).

e, f - means at the same sampling time within a treatment with different superscripts differ (P<.05).

e, g - means at the same sampling time within a treatment with different superscripts differ (P<.01).


mg NH3-N·dl-1
32
27
22
17
12
d,f
b,e
d,g
a,e
a
control (0 ug·ml-1)
lasalocid (15 ug·ml-1)
monensin (15 ug·ml-1)
d
c
b
.5 1.0 2.0 4.0 6.0
sampling time (hours)

Discussion

The effects of ionophore antibiotics, lasalocid, monensin and RO22-6924/004, on ammonia production and utilizaton were dependent on the nitrogen source. With natural protein such as SBM, the ammonia concentration was lower and with nonprotein nitrogen such as urea, the ammonia concentration was higher in antibiotic-treated fermentation than the control without antibiotic. Decreased ammonia concentration with SBM in antibiotic-treated fermentation was probably caused by reduced protein degradation (Van Nevel and Demeyer, 1977; Hanson and Klopfenstein, 1979; Poos et al., 1979; Whetstone et al., 1981) as well as reduced deamination (Tolbert et al., 1977; Van Nevel and Demeyer, 1977; Shelling et al., 1978). Among the three antibiotics tested, the new ionophore RO22-6924/004 appeared to be more inhibitory than monensin with lasalocid being the least effective antibiotic in reducing proteolysis and deamination. Ricke et al. (1984) have reported higher ammonia concentration in lasalocid-fed sheep than monensin-fed sheep on a diet containing SBM as the nitrogen source.

Increased ammonia concentration with urea as the nitrogen source in antibiotic- treated fermentation was probably because of increased urea hydrolysis and(or) decreased ammonia assimilation by rumen microorganisms. Starnes et al. (1984) have reported that lasalocid and monensin inhibit urease activity in the rumen. Therefore, it appears that increased ammonia concentration was because of decreased assimilation by the microorganisms. However, Dinius (1978) has reported that monensin does not affect ammonia assimilation by rumen bacteria. All three antibiotics were similar in regard to their inhibition of ammonia assimilation.

In Exp. 2 the effects of lasalocid and monensin on ammonia production and utilization were similar to that of Exp. 1. However, the magnitude of difference between the control and antibiotic-treated fermentation was less in Exp. 2 than in Exp. 1. The difference between the two experiments is probably reflective of

dietary influence. In Exp. 2, the control group was on a high-grain diet while in Exp. 1 the rumen fluid for the in vitro fermentation was obtained from cattle fed a low-grain diet. Rumen microbial population from lasalocid- or monensin-fed cattle appears to be resistant to the effects of lasalocid and monensin in regard to ammonia production and utilization. This was evidenced by the fact that addition of lasalocid or monensin had no effect on ammonia concentration in the in vitro fermentation with rumen fluid from lasalocid- or monensin-fed cattle. However, addition of monensin did further depress ammonia production from SBM fermented with rumen fluid obtained from lasalocid-fed cattle. This suggests that the two ionophores to some extent behave differently in their effects on ruminal ammonia metabolism. Similar rumen microbial adaptation to nitrogen metabolism in monensin-fed cattle has been observed by Poos et al. (1979) who reported that ruminal ammonia concentration in monensin-fed cattle approached the control at approximately 45 days.

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RUMEN MICROBIAL ADAPTATION TO LASALOCID OR MONENSIN

Summary

Six rumen fistulated cattle, divided into three groups (control, lasalocid-fed and monensin-fed), were used to determine if rumen bacteria adapt to lasolocid or monensin. Sampling periods consisted of 1) pre-treatment: 50% alfalfa hay and 50% concentrate diet and no antibiotic 2) treatment: 20:80 diet with lasalocid and monensin administered daily at .65 $mg^{*}kg^{-1}$ body weight 3) post-treatment: diet 20:80 and no antibiotic. No differences were measured in ruminal pH, total or individual VFA, ammonia or electrolyte concentration between the groups except monensin-fed had higher propionate than control cattle. Differences noted between treatment periods were probably due to diet. Lasalocid and monensin were tested (0 or 15 $\mu g^{*}ml^{-1}$) in vitro with rumen fluid inoculum from each group. In antibiotic-treated fermentation, final pH and ammonia concentration increased slightly, and gas production, microbial cell protein synthesis, acetate and butyrate concentrations decreased slightly. Only propionate proportion consistently increased during the pre-treatment and treatment periods. Results suggest that a microbial population shift alone does not account for propionate enhancement and continuous feeding of lasalocid or monensin is required to elicit the altered fermentation response.

Introduction

Ionophore antibiotics, lasalocid and monensin, alter rumen fermentation characteristics. Both antibiotics decrease ruminal acetic and butyric acids, increase ruminal propionic acid and decrease methane production (Richardson et al., 1976; Van Nevel and Demeyer, 1977; Poos et al., 1979; Bartley et al., 1979). It is proposed that the effects of lasalocid or monensin are due to selection of a resistant rumen microbial population. Rumen bacteria that are the primary acetate, butyrate, hydrogen and formate producers are inhibited by the antibiotics. Rumen

bacteria that produce succinate and propionate are resistant (Chen and Wolin, 1979; Dennis et al., 1981). However, there is no evidence of such altered rumen microbial population in cattle fed lasalocid or monensin. Dawson and Boling (1983) have reported a greater proportion of monensin-resistant bacteria in the rumen of monensin-fed cattle. The objective of this study was to determine if microbial adaptation to lasalocid and monensin occurs when these antibiotics are fed for a period of 120 days.

Materials and Methods

Animals and Diet. Six rumen-fistulated mature cattle were randomly assigned to three treatments. One treatment group received lasalocid (.66 $mg \cdot kg^{-1}$ body weight). The second received monensin (.66 $mg \cdot kg^{-1}$ body weight) in the diet and the third group receiving no antibiotic served as the control. Each animal was fed twice daily a diet of alfalfa hay (IFN 1-00-096) and a concentrate diet composed of 40.6% grain sorghum (IFN 4-05-643), 40.6% corn (IFN 4-02-931), 17.5% soybean meal (IFN 5-04-604), .5% dicalcium phosphate (IFN 6-01-080), .5% trace mineral salt and .2% vitamin A and D supplement containing 1,000,000 IU vitamin A and 5,000,000 IU vitamin D per 454 gram.

Dietary change and sampling schedule

Cattle were fed the hay and grain diet at 50:50 ratio for a period of 4 weeks. Rumen fluid samples (pre-treatment samples) were collected twice at two week intervals. Lasalocid[a] and monensin[b] premixes were hand mixed with the grain portion of the diet and fed at .33 $mg \cdot kg^{-1}$ body weight with each feeding. The diet

[a]Bovatec premix 68 g/454 g

[b]Rumensin premix 60 g/454 g

was then changed from 50:50 to 20:80 hay to grain ratio. Rumen fluid samples were collected at 12 and 16 weeks after the antibiotic feeding (treatment samples). After the second treatment sample, antibiotic feeding was discontinued. Two additional rumen fluid samples at two week intervals were collected (post-treatment samples). On each sampling day rumen fluid samples were collected just before (pre-feeding) and 3 h after (post-feeding) the morning feeding. Rumen contents were hand mixed as thoroughly as possible before collecting the sample and strained through 4 layers of cheese cloth immediately after collection. The pre-feeding rumen fluid sample was used to determine microbial activity and microbial cell protein yield. The post-feeding rumen fluid samples were used to measure pH, ammonia, volatile fatty acids (VFA) and soluble electrolytes.

Analytical Procedures

Ammonia was determined by the procedure of Conway as modified by Webb (1971). VFA was analyzed by acidifying rumen fluid with 6N HCl and centrifuging at 25,400 xg for 20 minutes. One microliter of the supernatant was injected into a gas chromatograph (Shimadzu GC[C] - Mini 3) with a 1.83 m x .64 cm column packed with SP 1200 (oven temperature 140 C, 21 ml per min helium carrier gas flow) and a flame ionization detector. Microbial activity was estimated by the el-Shazly and Hungate (1965) method. Rumen fluid from each animal was incubated in vitro with ground alfalfa hay and grain and with 0 or 15 $\mu g \cdot ml^{-1}$ lasalocid or monensin. The composition of grain and the ratio of hay to grain in the in vitro fermentation was the same as the diet fed to the cattle at the sampling time. Fermentation rate was measured by total gas production during 6 h incubation. Gas production was measured by a water displacement apparatus. At the end of 6 h incubation, samples were taken for pH, VFA and ammonia determinations.

[C]Shimadzu Scientific Instruments, Inc., Columbia, MD.

Microbial cell protein yield was measured according to Barr et al. (1974). The procedure consisted of incubating strained rumen fluid (10 ml) and phosphate buffer (pH 6.8, 20 ml) with corn starch (970 mg) and urea (30 mg) at 39 C, under anaerobic conditions. After 6 h incubation, the mixture was centrifuged at 25,400 x g for 20 min. The sediment containing microbial cells and unfermented substrate was washed with methanol to remove urea. The Kjeldahl nitrogen (AOAC, 1970) of the washed sediment was measured to determine the microbial cell protein yield.

Rumen fluid electrolytes were determined with cell-free rumen fluid. Calcium and magnesium were determined by atomic absorption spectrophotometry[d] and sodium and potassium were determined by atomic emission spectrophotometry[d]. Phosphorus was determined colorimetrically (Fiske and Subba Row, 1925). Chloride was determined titrimetrically based on the method of Schales and Schales (1941)[e].

A general linear model procedure was used to analyze data followed by comparison of treatment means that were of interest using the least square design techniques (Snedecor and Cochran, 1967).

Results

Ruminal pH of control cattle decreased ($P<.05$) during treatment and post-treatment periods (table 1). The decrease was because of dietary change from high roughage to high grain. Rumen pH of lasalocid-fed cattle did not change but in monensin-fed cattle, pH in the treatment period was lower ($P<.05$) than the pre-treatment period. Total VFA in control cattle was unchanged but was lower ($P<.05$) in lasalocid-fed cattle during the treatment and post-treatment period and

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[d] Jarrell Ash Atomic Absorption Emmision Spectrophotometer Model 82-270, Fisher Scientific, Waltham, MA).

[e] Sigma Technical Bulletin No. 830, Sigma Chemical Co., St. Louis, MO).

TABLE 1: RUMINAL MEASUREMENTS IN CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Item	Control cattle			Lasalocid-fed cattle			Monensin-fed cattle		
	Pre-treatment	Treatment	Post-treatment	Pre-treatment	Treatment	Post-treatment	Pre-treatment	Treatment	Post-treatment
Total VFA (μ moles/ml)	90.04	93.21	86.00	106.36^{b}	86.53^{c}	80.41^{c}	96.80^{b}	89.45^{bc}	81.12^{c}
Acetate (moles/100 moles)	63.8^{b}	58.0^{c}	59.4^{c}	59.4	58.3	60.0	62.4^{b}	54.1^{d}	59.9^{c}
Propionate (moles/100 moles)	20.4	19.6*	19.4	22.9^{b}	20.3^{c}	19.0^{d}	21.9^{c}	23.8^{d}*	19.4^{b}
Butyrate (moles/100 moles)	10.9^{b}	15.9^{e}	15.1^{e}	11.6^{b}	14.0^{e}	14.2^{e}	10.7^{b}	14.2^{e}	12.6^{d}
Others[a] (moles/100 moles)	5.1	6.8	6.4	6.2	7.6	7.2	5.3	8.1	8.5
pH	6.87^{b}	6.59^{c}	6.65^{bc}	6.66	6.61	6.52	6.73^{b}	6.49^{c}	6.54^{bc}
Ammonia-N (mg/dl)	17.0^{b}	15.6^{b}	21.4^{c}	17.7^{b}	19.0^{b}	23.7^{c}	17.8^{b}	18.4^{b}	24.8^{c}

[a]Others include isobutyrate, isovalerate, and valerate.

^{bc}Means in the same row within the same group differ ($P<.05$).

^{bd}Means in the same row within the same group differ ($P<.01$).

^{be}Means in the same row within the same group differ ($P<.001$).

*Means differ ($P<.05$).

on monensin-fed cattle (P<.05) in the post-treatment period only. Ruminal acetate concentration in control cattle decreased (P<.05) with a concurrent increase (P<.001) in butyrate concentration during treatment and post-treatment periods (table 1). Propionate concentration did not change. The change in acetate and butyrate proportion was probably because of high grain diet. Rumen acetate in lasalocid-fed cattle did not change; but butyrate increased (P<.001) in the treatment and post-treatment periods. Propionate concentrations decreased in the treatment (P<.05) and post-treatment periods (P>.01). Rumen acetate in monensin-fed cattle decreased (P<.05) during the treatment period with a concurrent increase in propionate and butyrate. Propionate concentration during post-treatment was lower (P<.05) than the pre-treatment period. There were no significant differences in isobutyrate, isovalerate and valerate in any of the treatment groups. Ruminal ammonia concentration in all groups of cattle did not change during the treatment period but increased (P<.05) during post-treatment.

Among the rumen electrolytes, sodium, potassium, calcium and magnesium decreased (P<.05) during the treatment and post-treatment periods while phosphorous increased in control cattle (table 2). There was no change in rumen chloride concentration. Similar changes were observed in lasalocid- and monensin-fed cattle except for potassium which did not decrease during the treatment and post-treatment periods. Also, sodium in lasalocid-fed cattle did not significantly decrease during the treatment period.

Addition of lasalocid or monensin to the in vitro fermentation with rumen fluid from control, lasalocid- or monensin-fed cattle increased the final pH slightly but not significantly (table 3). Gas production during 6 h incubation decreased with the addition of lasalocid or monensin with all three groups of cattle but only the control cattle during the pre-treatment period was significant (P<.05). Addition of lasalocid or monensin had no effect on total VFA concentration at the end of 6 h incubation with rumen fluid from all three groups of cattle. Acetate proportion

TABLE 2: RUMEN FLUID ELECTROLYTES IN CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Item	Control cattle			Lasalocid-fed cattle			Monensin-fed cattle		
	Pre-treatment	Treatment	Post-treatment	Pre-treatment	Treatment	Post-treatment	Pre-treatment	Treatment	Post-treatment
Dry Matter ($mg \cdot ml^{-1}$)	25.4[a]	34.7[b]	33.8[b]	29.0[a]	32.9[b]	34.8[b]	25.4[a]	34.6[b]	45.0[b]
Sodium ($Meq \cdot l^{-1} mg\ DM^{-1}$)	4.08[a]	3.22[b]	3.03[c]	3.50[a]	2.99[a]	2.60[b]	3.49[a]	2.65[b]	2.10[b]
Potassium ($Meq \cdot l^{-1} mg\ DM^{-1}$)	1.10[a]	.81[b]	.90[b]	1.14	.95	.95	1.09	1.07	.91
Calcium ($Meq \cdot l^{-1} mg\ DM^{-1}$)	.13[a]	.09[b]	.11[ab]	.18[a]	.08[b]	.08[b]	.17[a]	.08[b]	.08[b]
Magnesium ($Meq \cdot l^{-1} mg\ DM^{-1}$)	.47[a]	.09[c]	.16[c]	.64[a]	.12[c]	.13[c]	.46[a]	.18[c]	.12[c]
Phosphorus ($Meq \cdot l^{-1} mg\ DM^{-1}$)	.43[a]	.55[b]	.59[b]	.43[a]	.64[b]	.68[b]	.41[a]	.68[b]	.61[b]
Chloride ($Meq \cdot l^{-1} mg\ DM^{-1}$)	.81	.69	.98	.82	.99	.95	.55	.64	.52

[ab]Means in the same row within the same group differ ($P<.05$).

[ac]Means in the same row within the same group differ ($P<.001$).

TABLE 3: EFFECT OF LASALOCID OR MONENSIN ON IN VITRO FERMENTATION WITH RUMEN FLUID FROM CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Cattle and sampling period	Final pH			Gas production (ml)		
	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml
Control						
Pre-treatment	5.81[a]	5.91[a]	5.91[a]	144.8[a]*	103.5[a]*	103.4[a]*
Treatment	5.48[b]	5.62[b]	5.61[b]	145.5[a]	110.0[a]	109.4[a]
Post-treatment	5.50[b]	5.63[b]	5.59[b]	167.8[b]	159.4[b]	147.0[b]
Lasalocid-fed						
Pre-treatment	5.64[a]	5.75[a]	5.75[a]	123.3[a]	95.4[a]	92.7[a]
Treatment	5.55[b]	5.64[b]	5.64[b]	130.7[a]	113.1[b]	100.8[a]
Post-treatment	5.48[b]	5.59[b]	5.67[b]	179.9[b]	175.3[b]	168.4[b]
Monensin-fed						
Pre-treatment	5.75[a]	5.84[a]	5.85[a]	140.5[b]	105.4[a]	105.7[a]
Treatment	5.46[b]	5.58[b]	5.56[b]	126.0[a]	106.8[a]	108.7[a]
Post-treatment	5.48[b]	5.64[b]	5.56[b]	173.4[b]	161.8[b]	166.8[b]

[ab]Means within the same column from the same cattle differ (P<.001).

*Means differ (P<.05).

decreased with lasalocid- or monensin-treated fermentation but the difference was significant only in the pre-treatment period in control and monensin-fed cattle (table 4). Propionate proportion increased ($P<.001$) with the addition of lasalocid or monensin to the fermentation with rumen fluid from all three groups of cattle during pre-treatment and treatment periods. However, addition of lasalocid or monensin to the post-treatment rumen fluid sample from all three groups of cattle had no effect on propionate production. Butyrate proportion tended to decrease in lasalocid- or monensin-treated fermentation but the difference was not significant (table 5). There was no change in the proportions of isobutyrate, isovalerate and valerate with the addition of lasalocid or monensin during all periods. Addition of lasalocid or monensin to the in vitro fermentation had no effect on final ammonia concentration or microbial cell protein yield during the three periods from all three groups of cattle (table 6).

Discussion

Feeding lasalocid or monensin alters rumen fermentation characteristics to produce less acetate (Dinius et al., 1976; Prange et al., 1978; Gutierrez et al., 1982; Spears and Harvey, 1984), more propionate and less butyrate (Dinius et al., 1976; Utley et al., 1977; Prange et al., 1978; Gutierrez et al., 1982; Shell et al., 1983; Ricke et al., 1984; Spears and Harvey, 1984), and lowered ammonia production and utilization (Hanson and Klopfenstein, 1979; Poos et al., 1979; Thompson and Riley, 1980). In our experiment acetate decreased and propionate increased only in monensin-fed cattle. In lasalocid-fed cattle propionate proportion decreased during the treatment period. Ruminal ammonia concentration was unaffected in lasalocid- or monensin-fed cattle. The lack of response due to lasalocid or monensin feeding appears to be because of tremendous animal variation. Such lack of response has been reported by Hieneman et al. (1978) who indicated that some animals respond erratically to ionophore antibiotics.

TABLE 4: EFFECT OF LASALOCID OR MONENSIN ON IN VITRO FERMENTATION WITH RUMEN FLUID FROM CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Cattle and sampling period	Total VFA (μ mole/ml)			Acetate (moles/100 moles)			Propionate (moles/100 moles)		
	Control 0 μg/ml	Lasalocid 15 μg/ml	Monensin 15 μg/ml	Control 0 μg/ml	Lasalocid 15 μg/ml	Monensin 15 μg/ml	Control 0 μg/ml	Lasalocid 15 μg/ml	Monensin 15 μg/ml
Control									
Pre-treatment	57.02^{d}	54.50^{d}	54.72^{d}	58.6^{ad}	54.1^{bd}	53.3^{bd}	25.1^{ad}	32.4^{cd}	33.7^{cd}
Treatment	68.33^{e}	65.59^{e}	66.77^{e}	54.3^{e}	50.1^{e}	50.0^{e}	22.1^{ae}	28.8^{ce}	30.1^{ce}
Post-treatment	68.27^{e}	69.29^{e}	68.04^{e}	55.5^{e}	54.2^{d}	53.0^{d}	21.5^{e}	23.6^{e}	25.6^{e}
Lasalocid-fed									
Pre-treatment	61.06^{d}	58.76^{d}	59.94^{d}	56.4^{de}	52.7^{d}	52.2^{d}	25.5^{ad}	32.0^{cd}	32.5^{cd}
Treatment	65.55^{e}	64.87^{e}	64.64^{e}	55.5^{d}	51.7^{d}	51.4^{d}	23.5^{ae}	28.4^{ce}	30.0^{ce}
Post-treatment	66.06^{e}	68.48^{e}	66.36^{e}	57.5^{e}	56.7^{e}	55.7^{e}	20.2^{e}	21.4^{e}	23.1^{e}
Monensin-fed									
Pre-treatment	60.69^{d}	57.27^{d}	58.28^{d}	58.9^{ad}	54.4^{bd}	53.9^{bd}	24.5^{a}	32.1^{cd}	33.0^{cd}
Treatment	70.28^{e}	73.02^{e}	73.57^{e}	52.9^{e}	49.1^{e}	49.5^{e}	25.5^{ad}	30.9^{ce}	30.5^{cd}
Post-treatment	66.63^{e}	66.31^{e}	67.59^{e}	58.2^{d}	57.3^{e}	56.7^{e}	20.0^{e}	22.4^{e}	22.5^{e}

^{ab}Means in the same row differ (P<.05).

^{ac}Means in the same row differ (P<.001).

^{de}Means in the same column within the same group differ (P<.05).

TABLE 5: EFFECT OF LASALOCID OR MONENSIN ON IN VITRO FERMENTATION WITH RUMEN FLUID FROM CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Cattle and sampling period	Butyrate (moles/ 100 moles)			Others (moles/ 100 moles)[a]		
	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml
Control						
Pre-treatment	12.1[b]	10.5[b]	10.0[b]	4.4	3.4	3.1
Treatment	17.6[c]	15.8[c]	15.2[c]	6.3	5.9	4.9
Post-treatment	18.2[c]	17.5[c]	17.0[c]	5.1	5.0	4.6
Lasalocid-fed						
Pre-treatment	12.7[b]	11.1[b]	10.8[b]	5.8	4.8	4.6
Treatment	14.5[c]	14.0[c]	13.4[c]	6.6	6.2	5.3
Post-treatment	17.0[c]	16.8[c]	16.5[c]	5.5	5.3	4.9
Monensin-fed						
Pre-treatment	12.2[b]	10.3[b]	10.2[b]	4.6	3.3	3.2
Treatment	15.0[c]	13.9[c]	13.9[c]	6.9	6.4	6.3
Post-treatment	15.8[c]	14.4[c]	14.9[c]	6.3	6.2	6.1

[a]Others include isobutyrate, isovalerate and valerate.

[bc]Means within the same column from the same cattle differ ($P<.001$).

TABLE 6: EFFECT OF LASALOCID OR MONENSIN ON IN VITRO FERMENTATION WITH RUMEN FLUID FROM CONTROL AND LASALOCID- OR MONENSIN-FED CATTLE

Cattle and sampling period	Ammonia-N (mg/dl)			Microbial Protein[a]		
	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml	Control 0 µg/ml	Lasalocid 15 µg/ml	Monensin 15 µg/ml
Control						
Pre-treatment	8.3[b]	8.1[b]	7.2[b]	85.0[b]	76.9[b]	78.1[b]
Treatment	7.6[b]	8.1[b]	8.2[b]	96.9[e]	90.0[e]	90.0[e]
Post-treatment	10.6[c]	14.0[c]	11.6[c]	96.9[e]	94.4[e]	95.0[e]
Lasalocid-fed						
Pre-treatment	9.6	10.2[b]	9.0[b]	87.5[b]	78.1[b]	79.4[b]
Treatment	9.1	11.0[b]	9.8[b]	104.4[e]	97.5[e]	98.1[e]
Post-treatment	9.8	14.6[c]	13.2[c]	120.0[e]	111.9[e]	116.3[e]
Monensin-fed						
Pre-treatment	8.8	9.2[b]	8.7[b]	90.0[b]	80.0[b]	83.8[b]
Treatment	10.8	13.9[c]	14.2[c]	123.1[e]	110.6[e]	114.4[e]
Post-treatment	11.0	16.5[d]	15.1[d]	117.5[e]	106.9[e]	116.9[e]

[a]Expressed as mg protein/g substrate.

[bc]Means within the same column from the same cattle differ (P<.05).

[bd]Means within the same column from the same cattle differ (P<.01).

[be]Means within the same column from the same cattle differ (P<.001).

Ruminal electrolyte concentrations were similar to those reported by Bennink et al. (1978) except for higher chloride concentration. Ruminal soluble phosphorus concentration increased slightly during the treatment whereas calcium, sodium, magnesium and potassium concentrations decreased slightly. This is in contrast with Starnes et al. (1984) who reported that soluble ruminal calcium, magnesium, and potassium concentrations decreased and phosphorous and sodium remained unchanged when antibiotics were administered. Starnes et al. (1984) also reported no differences in ruminal mineral concentrations or osmolality between lasalocid and monensin, but both had lower values compared to the control.

Dinius et al. (1976), Herod et al. (1979) and Poos et al. (1979) have indicated possible microbial adaptation to monensin. Ricke et al. (1984) reported no evidence of adaptive rumen effects. If the altered fermentation patterns produced are due only to a bacterial population shift resistant to lasalocid or monensin, this must be accomplished in 6 hours. Dawson and Boling (1983) reported that it took 7 d to develop a resistant population while decreases in acetate and butyrate and increases in propionate were measured immediately. Dawson and Boling (1983) also reported at 10 d post treatment the VFA had returned to the pre-treatment values but the bacterial population took 18 d to return to pre-treatment values. This suggests a resistant population does not necessarily alter rumen fermentation, but rather the effect of ionophore antibiotics may be due to altered metabolic pathways (Bergen and Bates, 1984). It also suggests the antibiotics must be fed continually to obtain a response. By testing in in vitro fermentation with lasalocid and monensin with the rumen fluid from antibiotic treated cattle, any adaptive rumen effects should be observed. Decreased acetate, butyrate, and gas production and increased ammonia concentration were measured in vitro during the treatment period. However, the differences were not significant. Propionate proportion increased with the addition of lasalocid or monensin during pre-treatment and treatment periods. Propionate enhancement with the addition of lasalocid or

monensin to rumen fluid from lasalocid- or monensin-fed cattle is suggestive of lack of a microbial population shift in the rumen. But Dawson and Boling (1983) have reported increased numbers of monensin-resistant population in the rumen of cattle fed monensin. Therefore, it appears that a microbial shift alone does not produce more propionate and continuous feeding of lasalocid or monensin is required to elicit the propionate enhancement. However, because such a response was not observed for other measurements such as decreased acetate, butyrate and ammonia concentration, the evidence is not conclusive.

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A LITERATURE REVIEW: LASALOCID® AND MONENSIN MODE OF ACTION IN THE RUMEN

Antibiotics serve the livestock industry in various ways. Many are used as therapeutic agents to treat bacterial, fungal, and viral infections as well as parasitic diseases. In addition, certain antibiotics are used to enhance growth and improve feed efficiency. Antibiotic feed supplements have been extensively used in every major livestock-producing country. Such wide acceptance of antibiotics is attributed to their established benefits of increasing growth rate, improving feed conversion, and reducing mortality and morbidity from clinical or subclinical infections. Antibiotics commonly used in livestock production as dietary additives include bacitracin, chlorotetracycline, oxytetracyline, erythromycin, penicillin, streptomycin, tylosin and virginiamycin. Recently a new group of antibiotics has received a lot of attention and wide acceptance. They are called ionophores due to their ability to carry ions across lipid barriers including artificial and biological membranes (Pressman, 1973). Ionophores catalyze transport by 1) enveloping an ion at a membrane interphase with a consequent dehydration of the ion; 2) diffusing across the membrane as a cation complex; 3) releasing the ion which undergoes concomitant rehydration at the opposite interphase; and 4) diffusing back uncomplexed to the original interphase to complete the catalytic cycle. Ionophores thus function as mobile carriers for cations in membranes (Pressman, 1973). They select ions by a combined function of the energy required for desolvation of the ion and the liganding energy obtained on complexation (Pressman, 1976).

Presently there are two main ionophores, lasalocid and monensin (figures 1 and 2). Lasalocid is produced by Streptomyces lasaliensis and it is a divalent ionophore because of its preference for divalent cations such as ca^{2+}, rather than monovalent ions like K^{1+} or Na^{1+}. Monensin is produced by Streptomyces cinnamonensis and its preference is for monovalent cations such as Na^{1+} (Westley, 1982).

Figure 1. Molecular structure of lasalocid (X-537A) (6-{7R-{5S-ethyl-5-(5R-ethyltetrahydro-5-hydroxy-6S-methyl-2H-pyran-2R-yl)tetrahydro-3S-methyl-2S-furanyl}-4S-hydroxy-3R,5S-dimethyl-6-oxononyl}-2-hydroxy-3-methylbenzoic acid).

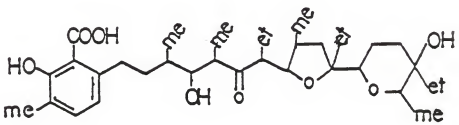
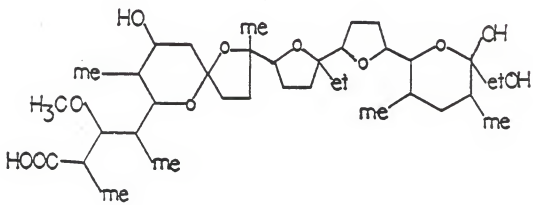


Figure 2. Molecular structure of monensin (2-{5-ethyltetrahydro-5-{tetrahydro-3-methyl-5-{tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl}-2-furyl}-2-furyl}-9-hydroxy-8methoxy-$\alpha,\gamma,2,8$-tetramethyl-1,6-dioxaspiro{4.5}decane-7-butyric acid).



They were originally marketed for their effectiveness in controlling coccidiosis in chicken (Shumard and Callender, 1967; Mitrovic and Schildknecht, 1974). They are also effective against coccisiosis in calves (Fitzgerald and Mansfield, 1973; Stromberg et al., 1981). Recently they have shown to be beneficial by increasing the efficiency of livestock production by decreasing the amount of feed consumed per pound of gain.

Effect on Animal Production

Typical animal response to lasalocid or monensin is little or no effect on average daily gain while decreasing feed consumption (Raun et al., 1976; Hanson and Klopfenstein, 1979; Bartley et al., 1979; Thonney et al., 1981; Berger et al., 1981). This is observed regardless whether the diet is concentrate (Raun et al., 1976; Utley et al., 1977; Boling et al., 1977; Thompson and Riley, 1980; Muntifering et al., 1981) or roughage such as alfalfa (Bartley et al., 1979), silage (Gill et al., 1976; Pendlum et al., 1980; Guttierrez et al., 1982), hay (Turner et al., 1977) or pasture (Oliver, 1975; Boling et al., 1977; Utley et al., 1978; Males et al., 1979; Spears and Harvey, 1984). Although feed efficiency increases, dry matter digestibility occasionally increases (Joyner et al., 1979; Wedegaertner and Johnson, 1983) but usually there is no effect (Dinius et al., 1976; Utley et al., 1977; Berger et al., 1981; Thornton and Owens, 1981; Gutierrez et al., 1982). This indicates there must be an increase in metabolizable energy or an alteration in the use of metabolizable energy and(or) a decrease in maintenance requirements to allow the animal to retain more of the energy consumed. Thorton and Owens (1981) reported an increase in metabolizable energy. Wedegaertner and Johnson (1983) reported the same results along with an increase in heat production which was proportional to the increased metabolizable energy without an effect on maintenance. Even though there are differences in the way energy is utilized, it apparently has no effect on

the carcass measurements or the proportions of fat, lean, and bone in the edible portion of the carcass (Potter et al., 1976; Berger et al., 1981).

Effects on Rumen Microbes and Ruminal Fermentation

This improved feed efficiency is because of altered rumen fermentation. The characteristic alterations of ruminal fermentation are the depression of acetate (Dinius et al., 1976; Raun et al., 1976; Richardson et al., 1976; Boling et al., 1977; Prange et al., 1978; Bartley et al., 1979; Gutierrez et al., 1982; Spears and Harvey, 1984) and butyrate (Dinius et al., 1976; Raun et al., 1976; Boling et al., 1977; Utley et al., 1977; Prange et al., 1978; Fuller and Johnson, 1983; Shell et al., 1983; Spears and Harvey, 1984) with concurrent increases in propionate (Dinius et al., 1976; Raun et al., 1976; Richardson et al., 1976; Utley et al., 1977; Boling et al., 1977, Prange et al., 1978; Bartley et al., 1979; Fuller and Johnson, 1981; Gutierrez et al., 1982; Shell et al., 1983; Spears and Harvey, 1984; Ricke et al., 1984) without affecting the total volatile fatty acid production (Richardson et al., 1976; Dinius et al., 1976; Bartley et al., 1979; Fuller and Johnson, 1981). In vitro studies of rumen isolates have shown that bacteria which are hydrogen-, formate-, acetate-, butyrate- and lactate-producing bacteria tend to be inhibited; whereas succinate- and propionate-producing and lactate-utilizing bacteria are resistant or rapidly develop resistance (Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). The alteration in cell permeability causes these bacteria to be sensitive to lasalocid and monensin. This suggests a resistant ruminal bacterial population is being selected and will alter ruminal fermentation patterns.

A propionate enhanced fermentation is more energetically efficient and theoretically reduces the losses associated with acetate and butyrate production (Hungate, 1966). Increased propionate may also lower the heat increment (Blaxter, 1962; Smith, 1971), spare amino acids normally used for gluconeogenesis (Leng et

al., 1967; Reilly and Ford, 1971) and stimulate body protein synthesis (Eskeland et al., 1974). This potential increase is a theoretical savings of 5.6% of the gross energy consumed by the animal (Richardson et al., 1976).

Inhibition of Methane Production

Another fermentation loss that is reduced by lasalocid and monensin is the production of methane. Although there may be a toxic effect by ionophore antibiotics on methanogens (Chen and Wolin, 1979), the reduction of methanogenesis is apparently due to inhibition of formate and hydrogen production. Formate is broken down to hydrogen and carbon dioxide which gives methanogens carbon and hydrogen for methane production (Van Nevel and Demeyer, 1977).

Methanogenesis is reduced in in vitro fermentation with either forage or grain substrate (Van Nevel and Demeyer, 1977; Chen and Wolin, 1979; Slyter, 1979; Bartley et al., 1979; Chalupa et al., 1980; Fuller and Johnson, 1981). In vivo experiments with concentrate diets have indicated monensin decreases methanogenesis (Joyner et al., 1979; Thornton and Owens, 1981; Wedegaertner and Johnson, 1983). Forage diets, however, have resulted in considerably less or no effects of monensin on methanogenesis (Garrett and Johnson, 1983). Slyter (1979) reported a relationship between methanogenesis and acetate:propionate ratio. In the absence of methanogenesis, the decrease in the ratio of acetic and propionic acid was entirely the result of increased propionate, whereas with methanogenesis the decrease in the ratio was the result of a combination of decreased acetic and increased propionic acid.

Influence on Nitrogen Metabolism

The increase in feed efficiency observed with lasalocid and monensin is usually greater than that which can be attributed to a propionate enhanced

fermentation. Hanson and Klopfenstein (1979) reported the feed efficiency of a diet containing 10.5% crude protein plus monensin was almost equal to a control diet containing 12.5% crude protein. Others have reported the same effect under various conditions (Gill et al., 1977; Dartt et al., 1978; Mies et al., 1979). This may be due to a protein sparing effect since proteolysis is decreased (Van Nevel and Demeyer, 1977; Poos et al., 1979; Whetstone et al., 1981). This protein sparing effect has been attributed to an increase in dietary nitrogen reaching the abomasum coupled with a decrease in bacterial nitrogen passing to the lower tract (Poos et al., 1979; Muntifering et al., 1981). Decreased ruminal proteolysis occurs while nonammonia, nonmicrobial nitrogen and total peptides apparently increase (Whetstone et al., 1981). This indicates an inhibition of microbial deamination of amino acids (Van Nevel and Demeyer, 1977; Schelling et al., 1978) because ammonia concentration decreases (Hanson and Klopfenstein, 1979; Poos et al., 1979; Muntifering et al., 1980; Thompson and Riley, 1980).

The ruminal ammonia concentration at any given time is the result of hydrolysis of non protein nitrogen, degradation of protein, assimilation by bacteria, passage to the lower digestive tract, absorption from the retriculorumen and diffusion from blood into the rumen (Chalupa, 1972; Owens and Bergen, 1983). Rumen microbial protein synthesis requires an adequate supply of nitrogen to achieve maximum efficiency (Stern, 1982). If the nitrogen level is not adequate, uncoupled fermentation may occur and this will result in fermentation without useful ATP production (McMeriman et al., 1976; Buttery, 1977). In other words, the rate of fermentation is not necessarily dependent upon the rate of microbial growth (Stern, 1982). From the in vitro studies Van Nevel and Demeyer (1977) conducted, they suggested that monensin uncouples growth from fermentation. In contrast, if the nitrogen level is excessive, energy may be the limiting factor for efficient utilization of nitrogen. The highest yields of cells are obtained when the

mean time spent in ruminal fermentation is such that the bacteria pass onwards as soon as their development is complete (Stern, 1982). Otherwise, bacteria use substrate for maintenance or for storage as carbohydrate or lipid and the efficiency of production of bacterial protein falls (Stern, 1982). Allen and Harrison (1979) reported that feeding monensin to sheep decreased rumen dilution rate with a concomitant decrease in microbial protein synthesis. This may be one reason for decreased microbial cell protein synthesis (Whetstone et al., 1981; Bartely et al., 1979) since it has been reported that monensin does not affect the assimilation of ammonia into microbial protein (Dinius, 1978). Lemenager et al. (1978) have also reported a decrease in dilution rate but that was disputed by Rogers and Davis (1982). They contended that drastically reduced feed consumption would lower water intake and reduce saliva flow which could likely be observed as reduced dilution rate of rumen fluid. The results of their study showed that monensin does not decrease dilution rate, but when sodium bicarbonate, which increases the dilution rate, is added with monensin, they counteract each other and an acetate fermentation is enhanced.

Although the trends usually are the same with lasalocid and monensin, recent studies (Ricke, et al., 1984) do show a difference with lasalocid having higher absorbed nitrogen and higher ruminal ammonia concentration compared to the control while monensin was lower. This agrees with Paterson et al. (1983) who showed a 6 to 13% improvement in nitrogen digestibility with lasalocid. It must be noted that the former study was with sheep and the latter with cattle.

Influence on Mineral Metabolism

Recent studies have shown lasalocid and monensin also influence mineral metabolism in vivo. Starnes et al. (1984) reported whole rumen fluid concentrations of calcium, phosphorus, sodium and zinc were not altered. The concentrations of

magnesium, potassium and calcium in the soluble fraction decreased while the magnesium and concentrations in whole rumen fluid were slightly decreased. Both ionophores lowered rumen osmolality and increased apparent absorption of only magnesium, phosphorus, and sodium. Serum mineral concentrations were similar for all treatments except zinc and copper increased. This is in contrast with Spears and Harvey (1984) who reported serum concentration of magnesium and potassium to be lower when animals were fed lasalocid on pasture.

Microbial Adaptation

Several studies have reported evidence that adaptation to monensin may occur in the rumen. Since lasalocid behaves like monensin (Bartley et al., 1979; Fuller and Johnson, 1981), it is reasonable to assume that adaptation would occur to lasalocid as well although there are no published reports. Cellulose digestion in vitro is reduced by monensin when inoculum was obtained from animals not consuming monensin in their diets (Simpson et al., 1976). However, the depression of cellulolytic activity was overcome when animals had received monensin for three weeks (Dinius et al., 1976). Poos et al. (1979) observed that by 40-46 days, the depressing effect on nitrogen metabolism was overcome. It is interesting that when steers were fed monensin 148 days, the characteristic propionate enhancement was still observed (Richardson et al., 1976). Bartley (unpublished data) has shown that when using rumen fluid from animals adapted to lasalocid or monensin for 21 days, there is no change in microbial cell protein synthesis, gas production or concentration of ruminal acids. Ricke et al. (1984) has reported no direct or adaptive rumen effects on in situ cellulose or nitrogen disappearance with either lasalocid or monensin.

Effect on Nutritional Diseases

Additional benefits from lasalocid and monensin are the prevention of coccidiosis as previously discussed. Monensin is somewhat effective against horn and face flies (Herald et al., 1982) and prevents acute bovine pulmonary edema and emphysema which results from the conversion of L-tryptophan to 3-methylindole in the rumen (Hammond et al., 1978). Both are effective against lactic acidosis (Nagaraja et al., 1981) because they both inhibit the major lactate-producing bacteria streptococcus and lactobacillus (Dennis et al., 1981).

Lasalocid and monensin are also moderately effective against both legume and grain bloat (Sakauchi and Hoshino, 1981; Bartley et al., 1983). Lasalocid tends to be more effective against grain bloat whereas monensin tends to be more effective on legume bloat (Bartley et al., 1983).

Ionophore antibiotics are beneficial to the cattle industry. However, they are not without their risks. If improper mixing results in overdosing cattle, both lasalocid and monensin can be toxic. At their recommended feeding level of 30 g per ton or .97 mg per kg of body weight, both are safe. Lasalocid has been safely fed at 150 g per ton (Bovatec Technical Manual). The oral LD_{50} of monensin is 21.9 $mg \cdot kg^{-1}$ body weight (Beck and Harries, 1979) although toxicity symptoms occur at lower levels (Collins and McCrea, 1978; Beck and Harries, 1979). The oral LD_{50} for lasalocid is approximately 70 $mg \cdot kg^{-1}$ body weight (Galitzer, 1984) indicating it is a safer drug.

Ionophores antibiotics have great potential in the cattle industry. They not only improve feed efficiency, but they improve cattle health. These antibiotics are unique in that even if bacteria do adapt and develop resistance, they apparently maintain their effect. This could indicate they probably will be effective for a long time.

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EFFECTS OF IONOPHORE ANTIBIOTICS ON RUMEN FERMENTATION

by

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ABSTRACT

The effects of monensin sodium, lasalocid sodium and a new ionophore antibiotic, RO22-6924/004 on rumen fermentation were tested in vitro. The substrate was ground corn with soybean meal or corn starch with urea. Antibiotic concentrations tested were 0, 15, 30, 60 and 120 $\mu g \cdot ml^{-1}$. All three antibiotics decreased gas production, microbial cell protein yield, acetate concentration and butyrate concentration and increased propionate concentration. Ammonia concentration decreased with ground corn and soybean meal but increased with starch and urea. Results suggest RO22-6924/004 is more potent than either lasalocid or monensin.

The effects of the ionophore antibiotics on in vitro ammonia metabolism were further tested. The substrate was similar to the previous experiment. Antibiotics were added at 0, 15 and 60 $\mu g \cdot ml^{-1}$ and samples for ammonia determination were collected at .5, 1.0, 2.0, 4.0 and 6.0 hours. Antibiotics reduced the ammonia concentration with ground corn and soybean meal while they increased with starch and urea. The increase appeared to be due to decreased ammonia utilization. Lasalocid and monensin were then tested in vitro at 0 or 15 $\mu g \cdot ml^{-1}$ with rumen fluid inoculum from cattle fed lasalocid, monensin or no antibiotic (control). Addition of lasalocid or monensin to inoculum from control cattle decreased ammonia concentration from SBM and increased with urea. Addition of antibiotics to inoculum from monensin-fed cattle had no effect, but monensin addition to lasalocid-fed cattle decreased ammonia concentration with SBM.

Three groups of cattle, control, lasalocid-fed and monensin-fed, were used to determine if rumen microbes become adapted to long term feeding of lasalocid or monensin. No differences were measured in ruminal pH, total or individual VFA, ammonia or electrolyte concentration between the groups except monensin-fed cattle had higher propionate than control cattle. Lasalocid and monensin were tested (0 or 15 $\mu g \cdot ml^{-1}$) in vitro with rumen fluid inoculum from each group. In antibiotic-treated fermentation, final pH and ammonia concentration increased slightly and gas production, microbial cell protein synthesis, acetate and butyrate concentrations decreased slightly. Only propionate proportion consistently increased. Results suggest that a microbial population shift alone does not account for propionate enhancement and continuous feeding of lasalocid or monensin is required to elicit the altered fermentation response.