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EFFECTS OF ANTIMICROBIAL FEED ADDITIVES ON RUMEN BACTERIA AND IN VITRO LACTIC ACID AND VOLATILE FATTY ACID PRODUCTION

by

Mitchell Brian Taylor

B. S., Kansas State University

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Approved by:

T. G. Nagaraja

T. G. Nagaraja

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INTRODUCTION

The forestomach of ruminants is inhabited by microoganisms that ferment dietary carbohydrates and proteins, producing volatile fatty acids and microbial protein, which in turn serve as the nutrients for the host metabolism. Fermentative digestion in the rumen is beneficial for substances that cannot be digested by the animal's own hydrolytic enzymes. However, microbial fermentation of dietary protein, starch and sugars and products of fermentation such as sugars and amino acids is accompanied by losses in both energy and nitrogen. Therefore, a proper balance between microbial fermentation and hydrolytic digestion is required for optimal production and performance.

In order to maximize the efficiency of feed utilization in ruminants, research has focused towards chemicals which promote adjustments in ruminal fermentation to minimize losses in energy and nitrogen. Such manipulation should not affect the beneficial aspect of microbial digestion such as fiber degradation and microbial protein synthesis from nonprotein nitrogen. Monensin and lasalocid are two antimicrobial compounds that have been widely used to alter rumen fermentation characteristics to improve cattle performance. The success story of monensin and lasalocid has stimulated testing of several antimicrobial compounds.

The objectives of this investigation were:

(1) To determine the sensitivity and resistance of rumen bacterial species to the antimicrobial feed additives.

(2) To evaluate the feed additive potential of the antimicrobial compounds based on in vitro lactic acid inhibition and alterations in volatile fatty acid production.

The following antimicrobial compounds were included in the study: avoparcin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin, RO21-6924/004 and RO22-6447/009.

SENSITIVITY AND RESISTANCE OF RUMEN BACTERIA TO ANTIMICROBIAL FEED ADDITIVES

ABSTRACT

Sensitivity and resistance of rumen bacterial species to avoparcin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin and two new ionphore antibiotics, RO22-6924/004 and RO21-6447/009, were determined. Generally, antimicrobial compounds were inhibitory to gram-positive bacteria and those bacteria that have gram-positive-like cell wall structure. Minimum inhibitory concentration (MIC) ranged from 0.09 to 24.0 µg/ml. Gram-negative bacteria were resistant at the highest concentration tested (48.0 µg/ml). Based on the fermentation products produced, rumen bacteria that produce lactic acid, butyric acid, formic acid or hydrogen were sensitive and bacteria that produce succinic acid or ferment lactic acid were resistant to the antimicrobial compounds. Selenomonas ruminantium was the only major lactic acid-producing bacteria sensitive to all the antimicrobial compounds tested. Avoparcin and tylosin appeared to be less inhibitory (MIC > 6.0 µg/ml) than the other compounds to the two major lactic acid-producing bacteria, Streptococcus bovis and Lactobacillus sp. Ionophore compounds seemed to be more inhibitory (MIC 0.09 - 1.50 µg/ml) than the non-ionophore compounds (MIC 0.75 - 12.0 µg/ml) to the major butyric acid-producing bacteria. Treponema bryantii, an anaerobic rumen spirochete was less sensitive to virginiamycin than to the other antimicrobial compounds. It appears that minimum inhibitory concentration is not a good indicator of the potency of the antimicrobial compounds in altering rumen fermentation characteristics.

INTRODUCTION

Antimicrobial feed additives in animal feeds are used not only for the control and treatment of infectious diseases but also for the enhancement of growth and improvement of feed efficiency. Lasalocid and monensin are ionophore antibiotics, that are used extensively in the cattle industry to improve the efficiency of feed utilization (4, 16). Ionophore antibiotics alter fermentation characteristics resulting in favorable metabolic changes in the rumen (2, 3, 29). Alterations in rumen fermentation are generally attributed to shift in microbial population (6, 10). In vitro studies with pure cultures of rumen bacteria have suggested that hydrogen-, formic-, acetic-, lactic-, and butyric acid-producing bacteria tend to be sensitive, whereas succinic acid-producing and lactic acid-fermenting bacteria tend to be resistant to lasalocid and monensin (6, 10, 17). The wide acceptance of lasalocid and monensin in the cattle industry has lead to investigations of several antimicrobial compounds such as avoparcin (12), laidlomycin (31), lysocellin (R.L. Preston, R.H. Pritchard and G.W. Wolfrom, Lysocellin effects on the gain, feed intake and efficiency of growing-finishing cattle. J. Anim. Sci. 61:493, 1985), narasin (E.L. Potter, C.O. Cooley and L.F. Richardson. Effects of narasin upon the performance of feedlot cattle. J. Anim. Sci. 49:397-398, 1979), salinomycin (21), thiopeptin (15), and virginiamycin (9). Narasin and salinomycin have been shown to be effective in improving feed efficiency at a smaller dosage than either lasalocid or monensin (3). Rumen metabolic changes induced by many of these antimicrobial compounds have been shown to be similar to that of lasalocid and monensin (9, 13, 15, 21, 31). However, the effects of these antimicrobial compounds on specific rumen bacterial species have not been determined. The objective of the investigation was to determine the

sensitivity and resistance of rumen bacteria to avoparcin, narasin, salinomycin, thiopeptin, tylosin, and virginiamycin. Two new ionophore antibiotics RO22-6924/004 and RO21-6447/009 (antibiotic X-14547A produced by Streptomyces antibioticus, 37) were also included in the study.

MATERIALS AND METHODS

Organisms and culture media. The rumen bacterial strains used were Anaerovibrio lipolytica 7553, Bacteroides amylophilus 70, B. ruminicola GA33, B. succinogenes S85, Butyrivibrio fibrisolvens 49, Eubacterium cellulosolvens 5495, E. ruminantium GA 195, Lachnospira multiparus D32, Lactobacillus ruminis RF1, L. vitulinus CL1, Megasphaera elsdenii B159, Ruminococcus albus 7, R. flavefaciens C94, Selenomonas lactilytica PC18, S. ruminantium D, HD1, Streptococcus bovis 7H4, Succinimonas amylolytica B24, Succinivibrio dextrinosolvens 0551, Treponema bryantii and Veillonella alcalescens UW221.

All organisms were grown in prereduced anaerobically-sterilized media with cysteine hydrochloride (0.05%) as the reducing agent. Culture media used were: Brain-heart infusion broth (Baltimore Biologicals Laboratories, Baltimore, MD) for S. bovis; MRS broth for Lactobacilli (8), lactate broth for A. lipolytica, M. elsdenii and V. alcalescens (26) and 40% rumen fluid broth (10) for all other organisms. The anaerobic techniques for preparing and dispensing media were that of Hungate et al. (19) as modified by Holdeman et al. (18).

Antimicrobial compounds. The following compounds were included in the study: avoparcin (American Cyanamid Co., Wayne, NJ), narasin (Elanco Products Co., Indianapolis, IN), salinomycin (A.H. Robins Co., Richmond, VA), thiopeptin

(Fujisawa Pharmaccutical Co., Osaka, Japan), tylosin (Elanco Products Co., Indianapolis, IN), virginiamycin (Smith Kline Animal Health Products, Westchester, PA) and ionophore antibiotics RO22-69/004 and RO21-6447/009 (Hoffmann-LaRoche Co., Nutley, NJ). All antibiotics were dissolved in methanol except avoparcin, which was dissolved in distilled water.

Determination of minimum inhibitory concentration. Sensitivity or resistance of rumen bacterial strains to antibiotics were determined by inoculating cultures into media containing the following concentrations of antibiotics: 0, 0.09, 0.19, 0.38, 0.75, 1.5, 3.0, 6.0, 12.0, 24.0 and 48.0 μg/ml. Three tubes were used for each concentration and the control (0 μg/ml) tube received equivalent amount (10 μl) of methanol. Rubber-stoppered Hungate tubes (Bellco Glass Co., Vineland, NJ) containing 10 ml medium with or without antibiotics were inoculated with 0.1 ml of an 18 h culture grown in the same medium without antibiotic. All incubations were at 39°C for 96 hours. Growth was monitored at 24 h intervals by measuring absorbance at 600 nm in a spectrophotometer (Bausch & Lomb). The minimum inhibitory concentration (MIC) was the lowest antibiotic concentration in which there was no measureable growth. MIC determination of each antibiotic and for each strain was replicated three times.

Effects of antibiotics on growth of resistant bacteria. The rumen bacterial strains that were resistant (MIC > 24.0 μg/ml) to the antibiotics were used. The organisms were grown in 10-ml quantities of culture media containing 0 or 6 μg antibiotic/ml. Three tubes were used for each concentration and each tube was inoculated with 0.1 ml of the inoculum. The inocula were from cultures grown for 18 to 24 h in the same medium without antibiotic. Growth was monitored by

measuring absorbance at 600 nm initially and at 1-hr intervals until maximum absorbance was recorded. The experiment was replicated twice.

RESULTS

Sensitivity of lactic acid-producing rumen bacteria. Generally, the lactic acid-producing rumen bacterial species were sensitive to the antimicrobial compounds (Table 1). The MIC ranged from 0.09 to 12.00 µg/ml concentraton. S. ruminantium was the only major lactic-acid producing bacteria resistant to all the antimicrobial compounds. The highest concentration of the compounds tested was 48.0 µg/ml. Avoparcin and tylosin appeared to be less inhibitory than other antibiotics to S. bovis and Lactobacillus spp.

Sensitivity of major butyric acid-producing rumen bacteria. Rumen bacterial species that produce butyric acid as one of the major fermentation products were sensitive to antibiotics (Table 2). The only exception was Megasphaera elsdenei which was totally resistant to all the antibiotics. Based on MIC, ionophore compounds seemed to be more inhibitory (MIC 0.09 - 1.50 µg/ml) than the non-ionophore antimicrobial compounds (MIC 0.75 to 12.00 µg/ml).

Sensitivity of formic acid- and hydrogen-producing rumen bacteria. Rumen bacterial species that produce formic acid as one of the major fermentation products were sensitive to both ionphore and non-ionophore antimicrobial compounds tested (Table 3). Among the hydrogen-producers only S. ruminantium was resistant (Table 4).

Table 1. Sensitivity of major lactic acid-producing rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Eubacterium cellulosolvens	+[b](0.09)[c]	+(0.75)	+(0.19)	+(0.75)	+(3.00)	+(12.00)	+(1.50)	+(1.50)
Eubacterium ruminantium	+(0.38)	+(1.50)	+(0.75)	+(1.50)	+(0.75)	+(0.75)	+(3.00)	+(1.50)
Lachnospira multiparus	+(0.75)	+(0.75)	+(0.38)	+(0.75)	+(1.50)	+(3.00)	+(3.00)	+(0.75)
Lactobacillus ruminis	+(0.75)	+(12.00)	+(1.50)	+(1.50)	+(3.00)	+(0.09)	+(6.00)	+(1.50)
Lactobacillus vitulinus	+(3.00)	+(12.00)	+(3.00)	+(3.00)	+(6.00)	+(1.50)	+(6.00)	+(1.50)
Selenomonas ruminantium D	-[d]	-	-	-	-	-	-	-
Selenomonas ruminantium HD1	-	-	-	-	-	-	-	-
Streptococcus bovis	+(1.50)	+(1.50)	+(1.50)	+(0.75)	+(12.00)	+(0.09)	+(12.00)	+(3.00)

[a] Sensitivity was determined three times for each strain.
[b] + = Sensitive.
[c] Numbers in parentheses indicate minimum inhibitory concentration (µg/ml).
[d] - = Resistant; the highest concentration of antibiotic tested was 48.0 µg/ml.

Table 2. Sensitivity of major butyric acid-producing rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Butyrivibrio fibrisolvens	+[b](0.38)[c]	+(0.75)	+(0.38)	+(0.75)	+(1.50)	+(6.00)	+(0.75)	+(6.00)
Eubacterium cellulosolvens	+(0.09)	+(0.75)	+(0.19)	+(0.75)	+(3.00)	+(12.00)	+(1.50)	+(1.50)
Eubacterium ruminantium	+(0.38)	+(0.75)	+(0.75)	+(1.50)	+(0.75)	+(0.75)	+(6.00)	+(1.50)
Megasphaera elsdenii	-[d]	-	-	-	-	-	-	-
Selenomonas ruminantium B385	+(1.50)	+(0.75)	+(3.00)	+(1.50)	+(3.00)	+(12.00)	+(6.00)	+(1.50)

[a] Sensitivity was determined three times for each strain.
[b] + = Sensitive.
[c] Numbers in parentheses indicate minimum inhibitory concentration (μg/ml).
[d] - = Resistant; the highest concentration of antibiotic tested was 48.0 μg/ml.

Table 3. Sensitivity of major formic acid-producing rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Eubacterium ruminantium	+(0.38)	+(1.50)	+(0.75)	+(1.50)	+[b](0.75)	+(0.75)	+(3.00)	+(1.50)
Lachnospira multiparus	+(0.75)	+(0.75)	+(0.38)	+(0.75)	+(1.50)	+(3.00)	+(3.00)	+(0.75)
Ruminococcus albus	+(0.75)	+(0.38)	+(0.19)	+(0.38)	+(0.75)	+(0.38)	+(0.38)	+(0.38)
Ruminococcus flavefaciens	+(0.19)	+(0.38)	+(0.75)	+(0.75)	+(0.75)	+(3.00)	+(0.38)	+(0.75)
Treponema bryantii	+(0.75)	+(0.75)	+(0.38)	+(0.38)	+(0.75)	+(3.00)	+(0.38)	+(12.00)

[a]Sensitivity was determined three times for each strain.
[b]+ = Sensitive.
[c]Numbers in parentheses indicate minimum inhibitory concentration (µg/ml).

Table 4. Sensitivity of major hydrogen-producing rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Lachnospira multiparus	+[b](0.75)[c]	+(0.75)	+(0.38)	+(0.75)	+(1.50)	+(3.00)	+(3.00)	+(0.75)
Ruminococcus albus	+(0.75)	+(0.75)	+(0.38)	+(0.75)	+(0.75)	+(0.38)	+(0.38)	+(0.38)
Ruminococcus flavifaciens	+(0.19)	+(0.38)	+(0.75)	+(0.75)	+(0.75)	+(3.00)	+(0.38)	+(0.75)
Selenomonas ruminantium D	-[d]	-	-	-	-	-	-	-

[a] Sensitivity was determined three times for each strain.
[b] + = Sensitive.
[c] Numbers in parentheses indicate minimum inhibitory concentration (µg/ml).
[d] - = Resistant; the highest concentration oof antibiotic tested was 48.0 µg/ml.

Sensitivity of succinic acid-producing rumen bacteria. Bacterides sp. Selenomonas sp. Succinimonas amylolytica and Succinivibrio dextrinosolvens were resistant to all the antimicrobial compounds tested (Table 5). Treponema bryantii, a saccharolytic rumen spirochete that produces acetate:formate:succinate at 1:1:0.5 molar proportion from glucose (32) was sensitive to all the antibiotics.

Sensitivity of lactic acid-fermenting rumen bacteria. Rumen bacterial species that ferment lactic acid were generally resistant to all the antimicrobial compounds (Table 6). However, Anaerovibrio lipolytica was sensitive to narasin and salinomycin at 24 µg/ml concentration and Veillonella alcalescens was inhibited by 24 µg/ml concentration of tylosin and virginiamycin.

Effect of antimicrobial compounds on growth of resistant rumen bacteria. The rate and extent of growth of S. ruminantium, S. lactilytica, s. dextrinosolvens and B. amylophilus were unaffected at 6 µg/ml concentration of antimicrobial compounds (Table 7). A. Lipolytica was not affected by avoparcin and thiopeptin. However, ionophore antibiotics particularly narasin and salinomycin were effective in reducing the growth of A. lipolytica. Tylosin and Virginiamycin also depressed the growth of A. lipolytica. The growth of B. ruminicola was affected by ionophore antibiotics, thiopeptin, tylosin and virginiamycin. Avoparcin had no effect on B. ruminicola. The growth of M. elsdenii was generally not affected by the presence of antimicrobial compounds except for the slight reduction in the presence of salinomycin, thiopeptin and tylosin. Virginiamcyin was the only antibiotic that depressed the growth of S. amylolytica. V. alcalescens was unaffected by avoparcin, thiopeptin and tylosin, but virginiamycin depressed the growth by almost 50% of the control (Table 7).

Table 5. Sensitivity of major succinic acid-producing rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Bacteroides amylophilus	-[b]	-	-	-	-	-	-	-
Bacteroides ruminicola	-	-	-	-	-	-	-	-
Bacteroides succinogenes	-	-	-	-	-	-	-	-
Selenomonas ruminantium D	-	-	-	-	-	-	-	-
Selenomonas ruminantium HD1	-	-	-	-	-	-	-	-
Succinimonas amylolytica	-	-	-	-	-	-	-	-
Succinivibrio dextrinosolvens	-	-	-	-	-	-	-	-
Treponema bryantii	+[c](0.75)[d]	+(0.75)	+(0.38)	+(0.38)	+(0.75)	+(3.00)	+(0.38)	+(12.00)

[a] Sensitivity was determined three times for each strain.
[b] - = Resistant; the highest concentration of antibiotic tested was 48.0 µg/ml.
[c] + = Sensitive.
[d] Numbers in parentheses indicate minimum inhibitory concentration (µg/ml).

Table 6. Sensitivity of lactic acid-fermenting rumen bacteria to antibiotics[a]

Organisms	Ionophore antibiotics				Non-ionophore antibiotics			
	RO22-6924/004	RO21-6447/009	Narasin	Salinomycin	Avoparcin	Thiopeptin	Tylosin	Virginiamycin
Anaerovibrio lipolytica	-[d]	-	+[b](24.00)[c]	+(24.00)	-[d]	-	-	-
Megasphaera elsdenii	-	-	-	-	-	-	-	-
Selenomonas lactilytica	-	-	-	-	-	-	-	-
Selenomonas ruminantium HD_4	-	-	-	-	-	-	-	-
Veillonella alcalescens	-	-	-	-	-	-	+(24.00)	(24.00)

[a] Sensitivity was determined three times for each strain.
[b] + = Sensitive.
[c] Numbers in parentheses indicate minimum inhibitory concentration (µg/ml).
[d] - = Resistant; the highest concentration of antibiotic tested was 48.0 µg/ml.

Table 7. Effect of antibiotics on the growth of rumen bacteria.[a]

Organisms	Control	Avoparcin[b]	Ionophore RO21-6447/009[b]	Narasin[b]	Salinomycin[b]	Thiopeptin[b]	Tylosin[b]	Virginiamycin[b]
Anaerovibrio lipolytica	100.0 (14.0)[c]	95.6 (18.0)	82.6 (23.0)	60.0 (26.0)	38.2 (20.0)	91.3 (18.0)	52.2 (20.0)	82.6 (21.0)
Bacteroides amylophilus	100.0 (8.0)	93.3 (8.0)	100 (9.0)	93.3 (9.0)	77.3 (9.0)	93.3 (8.0)	93.3 (8.0)	100 (8.0)
Bacteroides ruminicola	100.0 (7.0)	100.0 (7.0)	60.0 (7.5)	60.0 (8.5)	55.0 (8.0)	75.0 (7.0)	35.0 (12.5)	50.0 (9.5)
Megasphaera elsdenii	100.0 (14.0)	86.6 (14.5)	93.3 (14.5)	96.7 (14.0)	86.6 (19.0)	86.6 (15.0)	80.0 (14.5)	96.7 (14.5)
Selenomonas lactilytica	100.0 (6.5)	94.7 (5.0)	94.7 (5.0)	89.5 (5.0)	84.2 (5.0)	94.7 (5.0)	94.7 (5.0)	81.5 (6.1)
Selenomonas ruminantium HD1	100.0 (5.0)	100.0 (6.5)	100.0 (6.5)	100.0 (7.5)	100.0 (7.5)	100.0 (6.5)	100.0 (7.5)	100.0 (6.5)
Succinimonas amylolytica	100.0 (5.0)	100.0 (7.0)	86.4 (7.0)	90.9 (7.0)	90.9 (7.0)	100.0 (7.0)	90.9 (7.0)	68.1 (7.0)
Succinivibrio dextrivosolvens	100.0 (6.0)	110.0 (6.0)	100.0 (6.0)	100.0 (6.0)	125.0 (6.0)	100.0 (6.0)	100.0 (6.0)	100.0 (6.0)
Veillonella alcalescens	100.0 (13.0)	96.1 (13.5)	88.5 (16.0)	84.6 (16.5)	84.6 (16.5)	96.1 (12.5)	92.3 (13.5)	57.7 (21.0)

[a] Percentage of the control.
[b] Concentration was 6.0 µg/ml.
[c] Numbers in parentheses indicate hours required to reach maximum absorbance.

DISCUSSION

Antibacterial spectrum of antimicrobial compounds tested in this study were remarkably similar despite wide differences in chemical structure and mode of action. In general, antimicrobial compounds were inhibitory to gram-positive bacteria such as Eubacterium, Lactobacillus and Streptococcus and to those bacteria that often stain gram negative (Butyrivibrio, Lachnospira and Ruminococcus) but have gram positive-type cell wall structure (7, 28). Gram-negative bacteria such as bacteroides, Megasphaera, Selenomonas, Succinimonas, Succinivibrio and Veillonella were resistant to the antimicrobial compounds. S. ruminantium B385, although stains gram negative was sensitive to all the antimicrobial compounds. Strain B385 differs markedly from other strains of Selenomonas, as it produces butyric acid and not succinic acid as the major fermentation product (5). The cell wall structure of strain B385 has not been determined and it is possible that it may have a gram positive cell wall structure. T. bryantii, an anaerobic rumen spirochete (32) was extremely sensitive to all the antimicrobial compounds, but MIC of virginiamycin was higher (12 μg/ml) than that of the other compounds. This is in contrast to the report that virginiamycin has high antitreponemal activity (38). Virginiamycin is commercially available for the prevention of swine dysentry, caused primarily by T. hyodysenteriae (27).

Although the effects of ionophore antibiotics used in this study on rumen bacteria have not been previously determined, the antibacterial spectrum is in general agreement with the activity of other ionophores, in that gram-positive bacteria are sensitive and gram-negative bacteria are resistant (20, 36). The basic mode of action of ionophore compounds is on transmembrane ion fluxes and the

dissipation of cation and proton gradients, thereby interfering primarily with transport systems and ATP synthesis (3). Sensitivity and resistance pattern of rumen bacteria to avoparcin and tylosin are in agreement with previous reports (1, 14, 33, 35).

Minimum inhibitory concentration of ionophore antibiotics towards various rumen bacterial species were similar except RO21-6447/009, which tended to have higher MIC than others. Among the nonionophore compounds, thiopeptin and virginiamycin appeared to be more inhibitory to lactic acid-producing bacteria than avoparcin and tylosin. The two major lactic acid-producing rumen bacteria, S. bovis and Lactobacillus sp., involved in the onset of lactic acidosis syndrome in cattle (30) were extremely sensitive to RO22-6924/004, narasin, salinomycin, thiopeptin and virginiamycin (MIC 0.09 to 3.0 μg/ml) but were less sensitive to avoparcin and tylosin (MIC 3.0 to 12.0 μg/ml). Muir and Barreto (22) have reported that thiopeptin was more inhibitory to S. bovis than virginiamycin, tylosin, and ionophore antibiotics. Apparently, avoparcin and tylosin are not active against all gram-positive bacteria. Wang et al. (35) reported that S. bovis was sensitive to tylosin only at high concentration (MIC $\geq$ 100 μg/ml). Walton (34) noted that avoparcin was not inhibitory to some gram-positive enteric streptococci and Dutta and Devriese (11) have reported a number of lactobacilli against which avoparcin had no activity. Thiopeptin and salinomycin have been shown to be effective in preventing experimentally induced lactic acidosis in cattle (23-25). The effect of other antimicrobial compounds on lactic acidosis have not been determined.

Sensitivity and resistance pattern of rumen bacteria, grouped on the basis of major fermentation products produced, to the antimicrobial compounds used in this study were similar to that of monensin and lasalocid (6, 10, 17). In general, rumen bacteria that produce lactic acid, butyric acid, formic acid or hydrogen as

the major end product were sensitive and those bacteria that produce succinic acid or ferment lactic acid were resistant. Ruminal changes associated with feeding monensin or lasalocid are generally attributed to shift in bacterial population to produce more propionic, less acetic, butyric, lactic acid and methane (2, 3, 29). Increased propionate production is believed to be due to increased succinate-producing and lactate-fermenting bacteria in the rumen of cattle fed antibiotics (2, 3). Although succinate producers and lactate fermenters were resistant to all the antimicrobial compounds tested, the extent of propionate enhancement obtained with the compounds both in vitro and in vivo was different (13, 21, M.B. Taylor and T.G. Nagaraja, Report on XVII Conference on Rumen Function, 16.17 November 1983). Also, MIC of narasin, salinomycin, RO22-6924/004 to the lactic acid-producing rumen bacteria were very similar to that of lasalocid and monensin. However, salinomycin has been shown to be about 3-fold more potent than lasalocid or monensin in inhibiting lactic acid production (25). Apparently, MIC is not a good indicator of the potency of the antimicrobial compounds in altering rumen fermentation characteristics.

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LACTIC ACID INHIBITION AND ALTERATIONS IN VOLATILE FATTY ACID PRODUCTION BY ANTIMICROBIAL FEED ADDITIVES

ABSTRACT

Batch culture fermentations were used to determine the effect of avoparcin, lasalocid, monensin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin and two new ionophore compounds (RO22-6924/004 and RO21-6447/009) on lactic acid and volatile fatty acid production. Preliminary experiments were conducted with salinomycin to determine the effects of incubation time and rumen fluid inoculum source on lactic acid and VFA production. Maximum inhibition of lactic acid by salinomycin was at 6 h incubation, but 12 h incubation showed a more graded response to antibiotic concentration. Lactic acid inhibition by salinomycin was unaffected by rumen fluid inoculum source. All antimicrobial compounds were effective in inhibiting lactic acid production. Among the ionophores, narasin and salinomycin were more inhibitory than others. Monensin and tylosin in combination was more effective than monensin alone. Maximum alternations in VFA production by salinomycin were obtained in fermentations incubated for 12 h with rumen fluid inoculum from low-grain fed cattle. In general, total VFA concentration was unaffected by antimicrobial compounds except that of RO22-6924/004, tylosin and virginiamycin which caused a reduction at high concentrations. The acetate proportion was not affected by avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin and salinomycin. However, tylosin, monensin and tylosin in combination, thiopeptin and virginiamycin at high concentrations (> 6.0 μg/ml) increased the acetate proportion. All compounds increased the molar proportion of propionate. Tylosin and virginiamycin at high concentrations (> 6.0 μg/ml) decreased the proportion of propionate. Monensin and tylosin combination had no effect on propionate portion. Narasin and salinomycin were the most effective among the compounds tested, in

enhancing propionate production. Ionophore antibiotics were more inhibitory to butyrate production than the nonionophore compounds. Molar proportions of isobutyrate, isovalerate and valerate were generally not affected by the addition of antimicrobial compounds.

Introduction

Antimicrobial feed additives in animal diets are used not only for the control and treatment of infectious diseases but also for the enhancement of growth and improvement of feed efficiency. Lasalocid and monensin are ionophore antibiotics that are used extensively in the cattle industry to improve the efficiency of feed utilization (Brandt, 1982; Goodrich et al., 1985). Lasalocid and monensin alter fermentation characteristics resulting in favorable metabolic changes in the rumen (Bartley and Naragaja, 1982; Bergen and Bates, 1985; Schelling, 1985). Among the favorable alterations in rumen fermentation, enhanced propionate production at the expense of acetate and butyrate, inhibition of lactic acid production, decreased methane and reduced proteolysis and deamination are well documented (Bartley and Nagaraja, 1982; Bergen and Bates, 1984; Schelling, 1984). Because of the wide acceptance of lasalocid and monensin, several antimicrobial compounds such as avoparcin (Johnson et al., 1979; Dyer et al., 1980) laidlomycin (Spires and Algeo, 1983), lysocellin (Wolfrom, 1983; Preston et al., 1985), narasin (Dinusson et al., 1979; Potter et al., 1979), salinomycin (Merchen and Berger, 1985), thiopeptin (Gill et al., 1979) and virginiamycin (DeMeyer and Van Neval, 1985) are being investigated as possible feed additives. Rumen metabolic changes induced by several of the potential feed additives have been shown to be similar to that of lasalocid and monensin (Gill et al., 1979; Froetschel et al., 1983;

Spires and Algeo, 1983; Merchen and Berger, 1985). However, information on relative efficacy of the antimicrobial compounds in altering rumen fermentation characteristics is not available. The purpose of this investigation was to quantitatively evaluate the ability of antimicrobial compounds to alter rumen fermentation characteristics. The evaluation was based on in vitro inhibition of lactic acid and alterations in VFA production.

Materials and Methods

Antimicrobial compounds. The following compounds were used in the in vitro evaluation: avoparcin[1], lasalocid[2], monensin[3], narasin[3], tylosin[3], salinomycin[4], thiopeptin[5] and virginiamycin[6] Also, two new ionophore antibiotics[2], R022-6924/004 and RO21-6487/009 (antibiotic X-14547A produced by Streptomyces antibioticus; Westley et al., 1978) were also included in the evaluation. All antibiotics were dissolved in methanol except avoparcin, which was dissolved in distilled water.

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[1]American Cyanamid Co., Wayne, NJ.

[2]Hoffmann-LaRoche Inc., Nutley, NJ.

[3]Elanco Products Co., Indianapolis, IN.

[4]A.H. Robins Co., Richmond, VA.

[5]Fuji-Sawa Pharmaceutical Co., Osaka, Japan.

[6]Smith Kline Animal Health Products, Westchester, PA).

Effect on lactic acid production. Batch culture fermentations with glucose as the substrate were set up to determine the effect of antimicrobial compounds on L(+) and D(-) lactic acid production. Preliminary experiments were conducted to determine the effects of incubation time (3, 6, 9 and 12 h) and rumen fluid inoculum source (cattle fed alfalfa hay to grain diet at 80:20, 50:50 and 20:80 ratio) on L(+) and D(-) lactic acid production from glucose fermentation. Only salinomycin was used in the preliminary experiments.

To determine the effect of incubation time, rumen fluid from a rumen-fistulated steer fed a diet of alfalfa hay (IFN 1-00-063) (80%) and grain (20%) was used. The composition of the grain portion of the diet was 49.2% rolled corn (IFN 4-02-931), 49.2% cracked sorghum grain (IFN 4-08-139), 1.0% dicalcium phosphate (IFN 6-01-080), .25% trace mineral salts (IFN 6-04-152) and .1% vitamins A and D mixture. Rumen fluid was obtained 4 h postfeeding and strained through four layers of cheese cloth. Fifteen ml strained rumen fluid and 5 ml mineral buffer (pH 6.8, Dennis et al., 1981a) were incubated with 3.0 g glucose in 50 ml polyethylene centrifuge tubes. Salinomycin, dissolved in methanol was added at 0, .38, .75, 1.5, 3.0, 6.0, 12.0 μg/ml incubation mixture. Control tubes (0 μg/ml) received an equivalent amount of methanol. Each tube was flushed with CO_2 gas, stoppered with rubber stoppers equipped with bunsen valves and incubated at 39 C. Fermentations were set up in duplicate. Sample aliquots were removed at 3, 6, 9 and 12 h and pH recorded. Samples were deproteinized with 8% perchloric acid and centrifuged at 48,400 x g for 20 min and the cell-free supernatant was analyzed for L (+) and D (-) lactic acid concentrations (Dennis et al., 1981b). The experiment was replicated three times.

To determine the effect of rumen fluid inoculum source on lactic acid production, rumen fluid was obtained from three steers fed a diet of alfalfa hay

and grain at 80:20, 50:50 and 20:80 ratio, respectively. The composition of grain diet was as before. Batch culture fermentations were set up as before and samples collected at the end of 12 h incubation were used to record pH and measure L (+) and D (-) lactic acid concentrations. The experiment was replicated three times.

The effects of avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin, salinomycin, tylosin, thiopeptin, virginiamycin and monensin and tylosin combination (3:1) on L (+) and D (-) lactic acid production were determined. Rumen fluid inoculum was from a steer fed alfalfa hay and grain (80:20) diet and incubation time was 12 h. Antibiotics were added at 0, .09, .19, .38, .75, 1.5, 3.0, 6.0, 12.0 and 24.0 µg/ml. Fermentations were set up in duplicates and final pH, L (+) and D (-) lactic acids were determined as before. Each antibiotic was tested at least three times with rumen fluid collected on different days.

In order to quantitate the effects of antimicrobial compounds on L(+) and D(-) lactic acids, IC50 (Inhibitory concentration) defined as concentration (µg/ml) required to inhibit L(+) and D(-) lactic acids by 50% of the control (0 µg/ml) was calculated.

Effects on VFA production. Batch culture fermentations with a mixture of carbohydrate, protein and B vitamins as substrate were set up to determine the effects of antimicrobial compounds on VFA production. Preliminary experiments were conducted to determine the effects of incubation time (6, 12, 18, and 24 h) and rumen fluid inoculum source (cattle fed alfalfa hay and grain diet at 80:20, 50:50 and 20:80 ratio) on VFA production. Only salinomycin was used in the preliminary experiments.

To determine the effect of incubation time, rumen fluid from a rumen-fistulated steer fed a diet of alfalfa hay and grain (50:50) was used. The

composition of the grain portion of the diet was as before. Rumen fluid from collected 4 to 5 h postfeeding and strained through four layers of cheesecloth. Fifteen ml strained rumen fluid and 15 ml mineral buffer (pH 6.8) were incubated with a substrate mixture consisting of glucose, xylose, cellobiose, maltose, urea (50 mg each), casein hydrolyzate (100 mg) and B vitamins (300 µl; Scheifinger et al., 1975) in 50 ml- polyethylene centrifuge tubes. Salinomycin, dissolved in methanol was added at 0, .76, 1.5, 3.0, 6.0, 12.0, 24.0 and 48.0 µg/ml incubation mixture. Control tubes (0 µg/ml) received an equivalent amount of methanol. Each tube was flushed with CO_2 gas, stoppered with rubber stoppers equipped with bunsen valves and incubated at 39 C. Fermentations were set up in duplicates for each concentration. Sample aliquots were removed at 6, 12, 18 and 24 h and pH recorded. Samples were then acidified with 6N HCl and analyzed for VFA in a gas chromatograph (Bartley et al., 1979). The experiment was replicated three times.

To determine the effect of rumen fluid inoculum source on VFA production, rumen fluid was collected from three steers fed a diet of alfalfa hay and grain at 80:20, 50:50 and 20:80 ratio, respectively. The composition of the grain portion of the diet was as before. Batch culture fermentations were set up as before and samples were collected at the end of 12 h incubation to record pH and analyze for VFA concentration. The experiment was replicated three times.

The effects of avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin, salinomycin, tylosin, thiopeptin, virginiamycin and monensin and tylosin combination (3:1) on VFA production were determined. Rumen fluid inoculum was from a steer fed alfalfa hay and grain (50:50) diet and incubation time was for 12 h. Antimicrobial compounds were added at 0, .75, 1.5, 3.0, 6.0, 12.0, 24.0 µg/ml. Fermentations were set up in duplicate and VFA concentration

measured as before. Each antibiotic was tested at least three times with rumen fluid collected on different days.

In order to quantitate the effects of antimicrobial compounds on VFA production an EC25 (Enhancement concentration) defined as the concentration (μg/ml) required to enhance propionate production by 25% of the control (0 μg/ml) and IC25 (Inhibitory concentration) defined as the concentration (μg/ml) required to reduce acetate:propionate ratio or butyrate by 25% of the control (0 μg/ml) were calculated.

Statistical analyses. Experiments dealing with incubation time and diet effects (rumen fluid inoculum source) were analyzed by General Linear Models procedure of Statistical Analysis Systems (SAS, 1982) using a randomized block design, with replication as the blocking factor and a two-way treatment structure utilizing all the incubation times or diets and antibiotic concentrations combinations. The data on the effects of various antimicrobial compounds on lactic acid and VFA productions were analyzed by GLM procedure of SAS, using a randomized complete block design and a one-way treatment structure with antibiotic concentration as the treatment variable and replication as the blocking variable. Least square means were tested by least significant differences only after F tests were significant.

Results

Effects of incubation time and salinomycin concentration on lactic acid production. Total lactic acid concentration increased progressively ($P<.05$) with a concurrent decrease in pH with increased incubation time in both control (no

antibiotic) and salinomycin-treated fermentations (table 1). Both L(+) and D(-) lactic acid concentrations were highest at 12 h incubation. The extent of L(+) lactic acid increase over incubation time was much higher than that of D(-) lactic acid. D(-) lactic acid accounted for 55% of the total in the control at 3 h but declined to 6-10% with further incubation. Salinomycin-treated fermentations had higher final pH and lower L(-) and D(-) lactic acid concentrations than the control (table 1). However, the proportion of D(-) lactic acid was higher in the salinomycin-treated fermentation than the control. Maximal inhibition of lactic acid by salinomycin was at 6 h incubation (table 2). The 12 h incubation showed a more graded response to the salinomycin concentration than did the other incubation times. Accordingly, IC50 for L(+) and total lactic acid was lowest at 6 h incubation (table 2).

Effects of rumen fluid inoculum source and salinomycin concentration on lactic acid production. Total and L(+) lactic acid concentrations at the end of 12 h incubation in the control were lowest in the fermentation with rumen fluid inoculum from a high-grain (80%) fed steer and highest in the fermentation with rumen fluid inoculum from cattle fed a low-grain diet (table 3). Difference in final pH were reflective of lactic acid concentration. Apparently D(-) lactic acid concentration was unaffected by the rumen fluid inoculum source. However, the proportion of D(-) lactic acid was higher in the control fermentations with rumen fluid from high-grain fed cattle than in the fermentations with rumen fluid from low-grain fed cattle (21.9 vs 10.4%). Addition of salinomycin to the fermentation resulted in higher pH and lower L(+), D(-) and total lactic acid concentrations than the control with no antibiotic. Apparently, the extent of inhibition of L(+) and total lactic acid was unaffected by the rumen fluid inoculum source (table 3).

TABLE 1. EFFECTS OF INCUBATION TIME AND SALINOMYCIN CONCENTRATION ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Incubation time and antibiotic concentration µg/ml	pH	Lactic acid Total	L(+)	D(-)	D(-) %	Percent inhibition Total	L(+)	D(-)
		mg/ml						
3 h								
0	6.22[c]	.14[c]	.06[c]	.08[c]	55.1	—	—	—
.38	6.33[bc]	.06[bc]	.03[c]	.03[c]	50.0	56.0	86.0	68.0
.75	6.36[bc]	.05[bc]	.02[c]	.03[c]	60.0	62.4	56.3	68.1
1.50	6.32[bc]	.06[bc]	.02[c]	.04[c]	66.0	57.5	54.4	60.5
3.00	6.34[bc]	.06[bc]	.02[c]	.04[c]	66.0	55.8	54.0	59.4
6.00	6.38[bc]	.09[bc]	.03[c]	.06[c]	66.0	35.7	46.3	36.9
12.00	6.33[bc]	.09[bc]	.03[c]	.06[c]	66.0	35.7	44.0	23.4
6 h								
0	5.10[d]	2.09[d]	1.96[d]	.13[c]	6.8	—	—	—
.38	5.71[bd]	.57[bd]	.51[bd]	.06[c]	14.5	71.5	73.0	53.3
.75	5.91[bd]	.19[bd]	.14[bd]	.05[c]	28.9	90.1	92.4	63.5
1.50	5.94[bd]	.13[bc]	.08[c]	.05[c]	33.9	92.7	95.4	63.8
3.00	5.94[bd]	.16[bc]	.09[bc]	.07[c]	38.3	91.2	94.7	51.8
6.00	5.98[bd]	.16[bc]	.08[bc]	.08[c]	50.0	91.0	95.3	39.1
12.00	6.00[bd]	.18[bc]	.07[bc]	.11[c]	58.9	90.3	92.3	14.9
9 h								
0	4.54[e]	4.18[e]	3.88[e]	.30[d]	7.4	—	—	—
.38	5.04[be]	1.99[be]	1.69[be]	.30[d]	18.2	53.2	58.1	0.0
.75	5.29[be]	.73[be]	.57[be]	.16[c]	22.2	82.0	84.8	46.0
1.50	5.30[be]	.58[bd]	.44[bd]	.14[c]	23.1	85.6	88.0	55.6
3.00	5.32[be]	.53[bd]	.38[bc]	.15[c]	29.4	86.9	90.1	51.2
6.00	5.34[be]	.62[bd]	.31[bc]	.31[d]	50.0	84.3	91.8	-3.3
12.00	5.31[be]	.50[bc]	.30[bc]	.20[c]	38	87.8	92.3	37.6
12 h								
0	4.33[e]	5.65[f]	5.04[f]	.61[e]	10.8	—	—	
.38	4.80[be]	2.59[bf]	2.06[bf]	.53[e]	20.6	53.1	57.7	15.2
.75	4.92[bf]	1.57[bf]	1.14[bf]	.43[d]	28.5	72.5	77.3	30.2
1.50	4.98[bf]	1.24[be]	.75[bd]	.49[d]	38.8	78.0	84.8	20.9
3.00	5.01[bf]	1.08[be]	.64[bd]	.44[d]	38.5	81.3	87.3	31.4
6.00	5.07[bd]	.95[be]	.57[be]	.38[bd]	40.2	83.3	88.5	37.1
12.00	5.08[be]	.81[bd]	.43[bc]	.38[bd]	46.4	85.3	90.9	37.1
SE	.10	.24	.21	.07				

[a]Least square means and standard error.

[b]Different from 0 µg/ml concentration within each incubation time (P<.05).

[c,d,e]Column means within each concentration bearing the same superscript do not differ (P<.05).

TABLE 2. EFFECT OF INCUBATION TIME ON LACTIC ACID INHIBITION BY SALINOMYCIN

Incubaton time, h	IC50,[a] µg/ml		
	Total lactic	L(+) Lactic	D(-) Lactic
3	.34	.52	.28
6	.27	.26	.36
9	.36	.33	1.06
12	.36	.33	>12.00

[a]IC50 is the salinomycin concentration required to inhibit lactic acid by 50% of the control.

TABLE 3. EFFECTS OF RUMEN FLUID INOCULUM SOURCE AND SALINOMYCIN CONCENTRATION ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION

Rumen fluid inoculum source and antibiotic Concentration µg/ml	pH	Lactic acid Total	L(+)	D(-)	D(-) %	Percent inhibition Total	L(+)	D(-)
		mg/ml						
20% grain diet								
0	4.31[c]	5.60[c]	5.02[c]	.59[c]	10.4	--	--	--
.38	4.77[bc]	1.82[bc]	1.28[bc]	.54[c]	30.8	67.5	74.7	8.5
.75	4.89[bc]	1.23[bc]	.82[bc]	.41[c]	34.5	78.3	84.0	26.4
1.50	4.90[bc]	1.10[bc]	.74[bc]	.36[bc]	33.8	80.3	85.4	34.2
3.00	4.94[bc]	1.12[bc]	.73[bc]	.40[bc]	35.8	79.8	85.5	27.2
6.00	5.00[bc]	.95[bc]	.60[bc]	.35[bc]	36.7	83.0	88.1	34.5
12.00	5.07[bc]	.82[bc]	.46[bc]	.35[bc]	46.3	85.2	90.8	32.8
50% grain diet								
0	4.22[d]	4.63[d]	3.85[d]	.79[c]	1.68	--	--	--
.38	4.69[bc]	1.78[bc]	1.12[bc]	.66[c]	38.6	62.3	71.5	15.5
.75	4.70[bc]	1.36[bc]	.78[bc]	.58[c]	44.3	71.0	80.1	24.5
1.50	4.89[bc]	.99[bc]	.53[bcd]	.46[bc]	46.4	78.9	86.4	41.7
3.00	4.87[bc]	1.00[bc]	.52[bcd]	.49[bc]	48.5	78.5	86.7	37.4
6.00	4.90[bc]	.93[bc]	.49[bc]	.44[bc]	47.3	80.0	87.3	43.7
12.00	4.95[bc]	.77[bc]	.38[bc]	.39[bc]	51.0	83.5	90.3	49.2
80% grain diet								
0	4.47[cd]	3.60[e]	2.80[e]	.78[c]	21.9	--	--	--
.38	5.22[bd]	1.13[bd]	.79[bcd]	.34[bd]	31.2	68.8	72.6	54.9
.75	5.29[bd]	.75[bd]	.50[bc]	.25[bd]	33.9	78.6	81.9	65.0
1.50	5.30[bd]	.45[bd]	.27[bcd]	.18[bd]	40.6	87.1	90.2	76.0
3.00	5.30[bd]	.40[bd]	.25[bd]	.15[bd]	41.7	88.5	90.9	79.3
6.00	5.32[bd]	.38[bd]	.23[bc]	.14[bd]	35.8	88.7	91.0	80.4
12.00	5.36[bd]	.28[bd]	.17[bd]	.11[bd]	39.3	91.8	93.8	84.5
SE	.07	.16	.13	.05				

[a]Least square means and standard error (SE).
[b]Different from 0 µg/ml concentration within each diet (P<.05).
[c,d,e]Column means within each concentration bearing the same superscript do not differ (P<.05).

However, the extent of D(-) lactic acid inhibition by salinomycin was higher in fermentations with rumen fluid inoculum from cattle fed a high-grain diet than that of the fermentations with rumen fluid inocula from cattle fed a medium- or low-grain diet (table 5). Accordingly IC50 of salinomycin for L(+) and total lactic acid was unaffected by the rumen fluid inoculum source and IC50 for D(-) lactic acid was lowest in fermentations containing rumen fluid from high-grain fed cattle (table 4).

Effects of antimicrobial compounds on lactic acid production. Fermentation of glucose with rumen fluid from cattle fed a low-grain diet resulted in low pH (< 4.5) and high lactic acid concentration at the end of 12 h incubation. L(+) lactic acid was the predominant isomer and D(-) isomer usually accounted for 10-20% of the total lactic acid. Fermentations treated with antimicrobial compounds had higher final pH and lower L(+), D(-) and total lactic acid concentration than the control with no antibiotic (tables 5-15). In all instances, the extent of L(+) and total lactic acid inhibition appeared to be dose dependent at low antibiotic concentrations (.09 to 1.5 μg/ml). All antimicrobial compounds except thiopeptin (table 12) increased D(-) lactic acid production at low concentrations but were inhibitory at higher concentrations. Also, the extent of D(-) lactic inhibition was lower than that of the inhibition of L(+) lactic acid. Consequently, the proportion of D(-) lactic acid in antibiotic-treated fermentation were higher than that of the control. Maximal inhibition of D(-) lactic acid was observed with thiopeptin and virginiamycin-treated fermentations (tables 16 and 17).

Among ionophore antibiotics narasin and salinomycin were most effective and RO21-6447/009 was least effective in inhibiting lactic acid production. Based on IC50 for total lactic acid, lasalocid appeared to be more inhibitory than

TABLE 4. EFFECT OF RUMEN FLUID INOCULUM SOURCE ON LACTIC ACID INHIBITION BY SALINOMYCIN

Rumen fluid inoculum source	IC50,[a] µg/ml		
	Total lactic	L(+) Lactic	D(-) Lactic
20% Grain diet	.28	.25	>12.00
50% Grain diet	.31	.27	>12.00
80% Grain diet	.28	.25	.35

[a]IC50 is the salinomycin concentration required to inhibit lactic acid by 50% of the control.

TABLE 5. EFFECT OF AVOPARCIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
			mg/ml		%			
0	4.29	4.54	3.66	.88	19.9	—	--	—
.09	4.31	4.18	3.35	.83	20.1	6.8	7.0	3.1
.19	4.36	4.06	3.08	.98	24.4	10.4	13.8	-26.8
.38	4.42	3.66[c]	2.61[b]	1.05	29.2	19.5	27.0	-40.0
.75	4.55[c]	3.09[d]	2.09[c]	1.00	32.7	30.0	38.3	-19.1
1.50	4.84[d]	2.76[d]	1.15[d]	.61	34.4	60.9	67.4	20.9
3.00	4.85[d]	1.63[d]	1.05[d]	.58[b]	35.4	64.0	70.7	23.0
6.00	4.88[d]	1.58[d]	1.02[d]	.56[b]	35.6	65.0	71.5	25.3
12.00	4.87[d]	1.62[d]	1.04[d]	.58[b]	35.9	63.1	70.1	22.1
24.00	4.96[d]	1.36[d]	.88[d]	.48[c]	35.4	68.7	74.3	37.2
SE	.06	.33	.31	.10				

[a]Least square means and standard error (SE).

[b], [c], [d]Different from 0 µg/ml concentration ($P<.05$, .01, .001)

TABLE 6. EFFECT OF IONOPHORE RO 22-6924/004 ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		——	mg/ml	——	%			
0	4.23	4.91	4.27	.64	13.0	-	-	-
.9	4.30	4.68	3.92	.76	16.2	4.7	8.2	-18.8
.19	4.32	4.13[b]	3.01[d]	1.12[b]	27.1	15.9	29.5	-75.0
.38	4.46[b]	3.42[d]	2.29[d]	1.13[b]	33.0	30.3	46.4	-76.6
.75	4.73[b]	2.07[d]	1.37[d]	.70	33.8	57.8	67.9	-9.4
1.50	4.77[c]	1.80[d]	1.16[d]	.64	35.6	63.3	72.8	0.0
3.00	4.81[d]	1.82[d]	1.17[d]	.65	35.7	62.9	72.6	-1.6
6.00	4.80[d]	1.77[d]	1.13[d]	.64	36.2	64.0	73.5	0.0
12.00	4.89[d]	1.57[d]	.98[d]	.59	37.6	68.0	77.1	7.8
24.00	4.97[d]	1.26[d]	.80[d]	.46	36.5	74.3	81.3	28.1
SE	.06	.17	.12	.13				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration ($P<.05$, .01, .001)

TABLE 7. EFFECT OF IONOPHORE RO21-6447/009 ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		———	mg/ml	———	%			
0	4.40	4.73	3.69	1.04	21.9	—	—	—
.09	4.39	4.54	3.47	1.07	23.8	4.0	6.3	-3.7
.19	4.39	4.69	3.59	1.10	23.7	.9	3.0	-6.6
.38	4.40	4.78	3.59	1.19[b]	24.9	-1.1	3.0	-15.0
.75	4.47[b]	4.39	3.16[c]	1.23[b]	28.0	7.4	14.6	-18.5
1.50	4.68[d]	2.57[d]	1.62[d]	.95	37.1	56.5	7.9	45.9
3.00	4.74[d]	2.06[d]	1.29[d]	.77[c]	37.5	56.2	65.0	24.8
6.00	4.70[d]	2.31[d]	1.43[d]	.88[b]	38.0	51.1	61.1	15.2
12.00	4.71[d]	2.46[d]	1.51[d]	.94	38.4	48.1	59.0	8.9
24.00	4.72[d]	2.50[d]	1.54[d]	.96	38.5	47.1	58.3	6.8
SE	.02	.15	.13	.05				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration (P<.05, .01, .001)

TABLE 8. EFFECT OF LASALOCID ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		mg/ml			%			
0	4.28	4.79	3.99	.80	16.7	-	-	-
.09	4.40	3.77[c]	2.79[d]	.98	26.0	21.3	30.1	-15.0
.19	4.47	3.53[c]	2.42[d]	1.11	31.4	25.1	39.4	-38.8
.38	4.64[b]	2.82[d]	1.95[d]	.87	30.9	41.1	51.1	-8.8
.75	4.86[d]	1.91[d]	1.23[d]	.68	35.6	60.1	69.2	15.0
1.50	4.82[d]	1.85[d]	1.00[d]	.76	41.1	61.3	72.7	5.0
3.00	4.97[d]	1.60[d]	1.04[d]	.56	35.0	66.6	73.9	30.0
6.00	5.03[d]	1.35[d]	.84[d]	.51	37.8	71.0	79.0	48.8
12.00	5.16[d]	1.08[d]	.73[d]	.35[b]	32.4	77.5	81.7	56.3
24.00	5.24[d]	.82[d]	.43[d]	.29[c]	47.6	82.9	89.2	63.8
SE	.06	.17	.12	.13				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration ($P<.05$, .01, .001)

TABLE 9. EFFECT OF MONENSIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		———	mg/ml	———	%			
0	4.30	4.68	3.81	.88	18.8	-	-	-
.09	4.41	3.54[c]	2.50[c]	1.04	21.4	24.4	34.4	-18.2
.19	4.50	3.13[d]	2.04[d]	1.09	34.8	33.1	46.5	-23.9
.38	4.71[d]	2.66[d]	1.71[d]	.95	35.7	43.2	55.1	-8.0
.75	4.81[d]	2.38[d]	1.63[d]	.75	31.5	49.1	57.2	14.8
1.50	4.82[d]	1.88[d]	1.15[d]	.73	38.8	59.8	69.8	17.1
3.00	4.93[d]	1.52[d]	.97[d]	.55[b]	36.2	67.5	74.5	37.5
6.00	5.05[d]	1.43[d]	.92[d]	.51[b]	35.7	69.4	75.9	42.1
12.00	5.07[d]	1.26[d]	.75[d]	.51[b]	40.5	73.1	80.3	42.1
24.00	5.10[d]	1.13[d]	.68[d]	.45[c]	39.8	75.9	82.2	48.9
SE	.07	.23	.18	.08				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration ($P<.05, .01, .001$)

TABLE 10. EFFECT OF NARASIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		——	mg/ml	——	%			
0	4.25	5.87	4.74	1.13	19.2	—	—	—
.09	4.37	4.88[b]	3.59[c]	1.29	27.3	16.5	25.0	-25.0
.19	4.54[b]	3.43[d]	2.32[d]	1.11	33.2	41.5	52.0	-14.0
.38	4.82[d]	2.17[d]	1.32[d]	.85	39.1	63.0	72.3	12.2
.75	5.02[d]	1.42[d]	.86[d]	.56[b]	38.8	75.9	81.5	47.0
1.50	5.03[d]	1.27[d]	.78[d]	.49[c]	38.5	78.4	83.4	50.8
3.00	5.02[d]	1.25[d]	.76[d]	.49[c]	39.3	78.7	83.9	50.8
6.00	5.05[d]	1.13[d]	.69[d]	.44[c]	38.9	80.6	85.7	51.8
12.00	5.11[d]	.94[d]	.58[d]	.36[c]	38.5	84.0	88.1	60.3
24.00	5.19[d]	.69[d]	.41[d]	.28[d]	41.5	88.3	91.6	70.1
SE	.07	.25	.22	.16				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration (P<.05, .01, .001)

TABLE 11. EFFECT OF SALINOMYCIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		——	mg/ml	——	%			
0	4.46	4.06	3.57	.49	12.1	--	--	--
.09	4.48	3.57	2.75[d]	.82[b]	22.7	9.79	20.5	-82.5
.19	4.66	3.29[c]	2.37[d]	.92[c]	27.5	16.9	31.1	-98.7
.38	4.95[b]	1.83[d]	1.20[d]	.63	34.0	54.3	65.8	-36.5
.75	5.23[d]	.98[d]	.64[d]	.34	32.8	82.1	30.4	76.4
1.50	5.25[d]	.94[d]	.64[d]	.30	31.1	76.9	82.1	37.5
3.00	5.19[d]	.93[d]	.62[d]	.31	31.4	77.3	82.6	32.2
6.00	5.18[d]	1.01[d]	.68[d]	.33	32.5	74.6	80.5	25.1
12.00	4.95[b]	.87[d]	.60[d]	.27	31.6	77.7	82.7	32.5
24.00	4.95[b]	.75[d]	.50[d]	.25	34.2	80.3	85.2	35.2
SE	.13	.18	.14	.08				

[a]Least square means and standard error (SE).

[b], [c], [d]Different from 0 µg/ml concentration (P<.05, .01, .001)

TABLE 12. EFFECT OF THIOPEPTIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		mg/ml			%			
0	4.44	5.09	4.09	1.00	19.7	--	--	--
.09	4.58	2.93[d]	2.17[d]	.76[c]	29.5	42.2	47.0	24.0
.19	4.71[b]	1.55[d]	1.06[d]	.49[d]	38.2	69.7	74.5	51.0
.38	5.01[d]	.92[d]	.60[d]	.32[d]	35.7	81.9	85.4	68.0
.75	5.10[d]	.91[d]	.59[d]	.32[d]	35.1	82.2	85.6	68.0
1.50	5.13[d]	.83[d]	.55[d]	.28[d]	33.4	83.8	86.8	72.0
3.00	5.10[d]	.75[d]	.46[d]	.29[d]	40.8	85.2	88.8	70.0
6.00	5.10[d]	.65[d]	.40[d]	.25[d]	39.8	87.3	90.4	75.0
12.00	5.14[d]	.74[d]	.47[d]	.27[d]	34.7	85.4	88.5	73.0
24.00	5.09[d]	.56[d]	.36[d]	.20[d]	37.8	89.1	91.4	80.0
SE	.08	.29	.27	.06				

[a]Least square means and standard error (SE).

[b], [c], [d]Different from 0 µg/ml concentration ($P<.05$, .01, .001)

TABLE 13. EFFECT OF TYLOSIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		mg/ml			%			
0	4.36	4.70	3.77	.93	19.7	--	--	--
.09	4.36	4.43	3.40	1.03	23.6	3.5	7.7	-11.7
.19	4.60[b]	3.36[c]	2.40[d]	0.96	29.8	29.1	36.5	-5.7
.38	4.84[d]	1.87[d]	1.27[d]	.60[b]	31.1	59.9	65.6	36.3
.75	4.91[d]	1.69[d]	1.14[d]	.55[c]	31.7	62.9	68.3	41.9
1.50	5.00[d]	1.56[d]	1.09[d]	.47[d]	34.5	64.5	69.9	47.9
3.00	5.17[d]	.79[d]	0.47[d]	.32[d]	46.6	82.9	87.0	64.0
6.00	5.29[d]	.46[d]	0.20[d]	.26[d]	60.4	90.4	95.1	69.4
12.00	5.31[d]	.45[d]	0.15[d]	.30[d]	68.7	90.4	96.0	65.1
24.00	5.36[d]	.38[d]	0.11[d]	.28[d]	74.7	91.9	97.4	67.3
SE	.08	.36	.26	.09				

[a]Least square means and standard error (SE).

[b], [c], [d]Different from 0 µg/ml concentration (P<.05, .01, .001)

TABLE 14. EFFECT OF VIRGINIAMYCIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		——	mg/ml	——	%			
0	4.23	5.01	4.14	.87	17.2	--	--	--
.09	4.31	4.20[b]	3.12[d]	1.08	25.9	15.1	24.9	-31.7
.19	4.67[d]	2.09[d]	1.45[d]	.64	29.8	58.1	65.2	19.9
.38	4.94[d]	.83[d]	.63[d]	.20[d]	26.5	83.3	85.0	74.1
.75	4.94[d]	.94[d]	.65[d]	.29[d]	32.1	81.1	84.6	62.3
1.50	5.00[d]	.85[d]	.55[d]	.30[d]	37.3	82.9	87.1	57.1
3.00	5.08[d]	.64[d]	.41[d]	.23[d]	40.1	87.0	90.3	66.3
6.00	5.20[d]	.46[d]	.27[d]	.19[d]	45.5	90.8	93.7	72.5
12.00	5.65[d]	.40[d]	.17[d]	.23[d]	52.5	91.8	95.9	67.4
24.00	5.94[d]	.36[d]	.20[d]	.16[d]	44.7	93.0	95.1	82.6
SE	.05	.19	.11	.12				

[a]Least square means and standard error (SE).

[b, c, d]Different from 0 µg/ml concentration ($P<.05, .01, .001$).

TABLE 15. EFFECT OF MONENSIN AND TYLOSIN ON LACTIC ACID CONCENTRATION FROM IN VITRO FERMENTATION OF GLUCOSE[a,b]

Antibiotic concentration µg/ml	Final pH	Lactic acid				Percent Inhibition		
		Total	L(+)	D(-)	D(-)	Total	L(+)	D(-)
		——	mg/ml	——	%			
0	4.29	5.44	4.51	.93	16.8	—	—	—
.09	4.38	4.94	4.04	.90	18.4	8.7	10.3	3.5
.19	4.40	4.38[c]	3.27[d]	1.21[c]	26.7	17.7	27.5	-34.4
.38	4.88[e]	2.01[e]	1.27[e]	.74	34.6	63.7	71.7	26.0
.75	4.97[e]	1.54[e]	1.01[e]	.53[c]	32.3	72.4	77.6	46.7
1.50	5.15[e]	1.11[e]	.68[e]	.43[e]	37.1	80.3	84.7	60.2
3.00	5.10[e]	1.05[e]	.65[e]	.40[e]	37.0	81.4	85.4	63.1
6.00	5.14[e]	.95[e]	.54[e]	.41[e]	39.0	83.2	87.9	60.1
12.00	5.26[e]	.60[e]	.29[e]	.31[e]	51.7	89.4	93.5	66.4
24.00	5.35[e]	.38[e]	.10[e]	.28[e]	78.9	93.1	97.6	68.9
SE	.08	.24	.23	.09				

[a]Monensin and tylosin were added at 3:1 ratio.

[b]Least square means and standard error (SE).

[c, d, e]Different from 0 µg/ml concentration ($P<.05$, .01, .001)

TABLE 16. LACTIC ACID INHIBITION BY ANTIMICROBIAL FEED ADDITIVES

Antibiotics	Total Lactic Acid			L(+) Lactic Acid			D(-) Lactic Acid		
	IC50[a] µg/ml	Maximum inhibition %	Concentration[b] µg/ml	IC50[a] µg/ml	Maximum inhibition %	Concentration[b] µg/ml	IC50[a] µg/ml	Maximum inhibition %	Concentration[b] µg/ml
Avoparcin	1.24	68.7	24.00	1.05	74.3	24.00	>24.00	37.2	24.00
RO22-6924/004	.65	74.3	24.00	.44	81.3	24.00	>24.00	28.1	24.00
RO21-6447/009	2.10	56.2	3.00	1.38	65.0	3.00	>24.00	24.8	3.00
Lasalocid	.58	82.9	24.00	.36	89.2	24.00	6.96	63.8	24.00
Monensin	.81	75.9	24.00	.27	80.3	24.00	>24.00	48.9	24.00
Narasin	.27	88.3	24.00	.17	91.6	24.00	1.34	70.1	24.00
Salinomycin	.36	80.3	24.00	.29	85.2	24.00	>24.00	37.5	1.50
Thiopeptin	.12	89.1	24.00	.10	91.4	24.00	.18	80.0	24.00
Tylosin	.32	91.9	24.00	.28	97.4	24.00	1.70	69.4	24.00
Virginiamycin	.16	93.0	24.00	.15	95.9	12.00	.30	82.6	24.00
Monensin + Tylosin	.32	93.1	24.00	.29	97.6	24.00	.93	68.9	24.00

[a]Concentration required to inhibit lactic acid by 50% of the control.

[b]Concentration at which maximum inhibition was observed.

TABLE 17. EFFECTS OF INCUBATION TIME AND SALINOMYCIN CONCENTRATION ON IN VITRO VOLATILE FATTY ACIDS PRODUCTION[a]

Incubation time and salinomycin concentration µg/ml	Final pH	Total VFA mM	Molar proportion (moles/100 moles)						Acetate: propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate: propionate ratio reduction
			Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
6 h												
0	6.32^{c}	95.9^{c}	61.4^{c}	21.8^{c}	1.2^{c}	12.6^{c}	1.7^{c}	1.4^{c}	2.82^{c}	0.0	0.0	0.0
.75	6.26^{c}	102.0^{c}	61.1^{c}	22.8^{c}	1.0^{c}	12.1^{c}	1.5^{c}	1.3^{c}	2.68^{c}	4.8	3.9	4.9
1.50	6.28^{c}	97.3^{c}	61.5^{c}	23.3^{c}	1.0^{c}	11.4^{bc}	1.4^{c}	1.3^{c}	2.64^{bc}	7.2	9.0	6.6
3.00	6.25^{c}	99.3^{c}	61.6^{c}	23.7^{bc}	.8^{c}	11.1^{bcd}	1.4^{c}	1.3^{c}	2.60^{c}	9.1	11.6	7.9
6.00	6.20^{bc}	99.2^{c}	61.9^{c}	23.4^{c}	.9^{c}	11.7^{cd}	1.4^{c}	1.3^{c}	2.63^{bc}	7.4	7.6	6.8
12.00	6.17^{bc}	98.2^{c}	61.5^{c}	23.3^{c}	.9^{c}	11.7^{ce}	1.4^{c}	1.2^{c}	2.64^{bc}	7.0	7.6	6.4
24.00	6.13^{bc}	95.8^{c}	62.3^{c}	22.8^{c}	.6^{c}	11.6^{bc}	1.4^{c}	1.2^{c}	2.73^{c}	5.0	7.7	3.1
48.00	6.08^{bc}	97.0^{c}	62.7^{c}	22.2^{c}	.8^{c}	11.4^{c}	1.4^{c}	1.6^{c}	2.83^{c}	2.0	7.5	-0.8
12 h												
0	6.43^{d}	118.9^{d}	58.9^{d}	23.6^{d}	1.1^{c}	13.3^{cd}	1.9^{c}	1.3^{c}	2.51^{b}	0.0	0.0	0.0
.75	6.37^{d}	122.3^{d}	57.8^{d}	26.0^{bd}	1.0^{c}	12.3^{c}	1.6^{c}	1.3^{c}	2.23^{bd}	10.5	7.5	11.0
1.50	6.35^{bd}	121.8^{d}	58.0^{d}	26.9^{bd}	.9^{c}	11.7^{bc}	1.5^{c}	1.2^{c}	2.16^{bd}	14.0	12.1	13.9
3.00	6.31^{bd}	122.5^{d}	57.0^{bd}	29.1^{bd}	.9^{c}	11.0^{bc}	1.6^{c}	1.0^{c}	1.96^{bd}	23.3	17.0	21.9
6.00	6.32^{bd}	117.0^{d}	56.2^{bd}	30.3^{bd}	.8^{c}	10.8^{bc}	1.4^{c}	1.0^{c}	1.86^{bd}	28.4	18.0	25.9
12.00	6.28^{bd}	111.9^{d}	55.8^{bd}	30.6^{bd}	.6^{c}	10.6^{bc}	1.4^{c}	1.0^{c}	1.83^{bd}	29.8	20.0	26.9
24.00	6.23^{bd}	118.2^{d}	55.8^{bd}	30.7^{bd}	.7^{c}	10.5^{bd}	1.3^{c}	1.0^{c}	1.82^{bd}	30.5	20.9	27.2
48.00	6.17^{bd}	114.6^{d}	56.8^{bd}	29.3^{bd}	.7^{c}	10.8^{bc}	1.3^{c}	1.1^{d}	1.94^{b}	24.5	18.2	22.3
18 h												
0	6.47^{d}	126.2^{d}	58.3^{de}	23.2^{cd}	1.3^{c}	13.5^{cd}	2.3^{d}	1.5^{c}	2.53^{d}	0.0	0.0	0.0
.75	6.46^{d}	132.0^{de}	57.9^{d}	24.4^{c}	1.1^{c}	12.9^{cd}	2.2^{d}	1.5^{c}	2.39^{d}	5.2	4.5	5.5
1.50	6.42^{d}	127.8^{d}	57.2^{d}	26.1^{bd}	1.1^{c}	12.3^{bcd}	2.1^{d}	1.3^{c}	2.19^{bd}	12.5	8.0	13.4
3.00	6.37^{d}	126.8^{d}	55.0^{bd}	28.3^{bde}	.9^{c}	12.1^{bcd}	2.0^{d}	1.2^{c}	1.96^{bd}	22.0	10.0	22.5
6.00	6.34^{bd}	127.2^{d}	55.3^{bd}	28.5^{be}	.8^{c}	12.4^{d}	2.0^{d}	1.2^{c}	1.94^{bd}	22.8	7.2	23.3
12.00	6.36^{bde}	130.0^{e}	52.7^{be}	29.2^{be}	1.0^{d}	13.7^{d}	2.9^{d}	1.5^{d}	1.88^{bd}	25.9	1.8	26.0
24.00	6.30^{bd}	128.5^{d}	53.1^{be}	29.9^{bd}	1.2^{d}	11.7^{bc}	2.6^{d}	1.5^{d}	1.78^{bd}	29.2	13.5	29.5
48.00	6.27^{bd}	130.4^{e}	51.9^{be}	31.1^{be}	1.2^{d}	11.4^{bc}	2.8^{d}	1.6^{c}	1.68^{be}	34.1	15.7	33.5
24 h												
0	6.48^{d}	140.1^{e}	57.0^{e}	22.5^{cd}	1.5^{c}	14.0^{d}	3.0^{e}	2.0^{d}	2.53^{d}	0.0	0.0	0.0
.75	6.46^{d}	138.0^{e}	55.1^{be}	24.1^{c}	1.7^{d}	13.4^{d}	3.3^{e}	2.4^{bd}	2.30^{bd}	6.7	4.1	9.1
1.50	6.47^{d}	141.7^{e}	54.9^{be}	25.3^{bd}	1.9^{d}	12.9^{bc}	3.3^{e}	2.2^{d}	2.18^{bd}	12.0	11.8	13.9
3.00	6.44^{d}	141.3^{e}	54.0^{be}	26.5^{be}	1.9^{d}	12.2^{bcd}	3.3^{e}	2.1^{d}	2.04^{bd}	17.4	13.4	19.2
6.00	6.45^{e}	142.9^{e}	52.8^{be}	27.5^{be}	2.0^{d}	12.1^{bd}	3.3^{e}	2.3^{d}	1.92^{bd}	22.0	13.9	24.0
12.00	6.40^{d}	142.9^{d}	51.9^{be}	28.6^{be}	2.0^{e}	11.8^{bc}	3.3^{e}	2.4^{be}	1.82^{bd}	26.6	15.8	28.1
24.00	6.39^{be}	146.0^{e}	51.0^{bl}	29.4^{bd}	2.0^{e}	11.6^{bc}	3.3^{e}	2.6^{be}	1.74^{bd}	30.6	17.1	31.3
48.00	6.39^{be}	145.5^{l}	49.9^{bl}	30.7^{bde}	2.1^{e}	11.1^{bc}	3.3^{e}	2.9^{be}	1.63^{e}	36.2	20.9	35.6
SE	.03	3.7	.6	.6	.1	.6	.1	.1	.1	2.0	2.9	2.0

[a]Least square means and standard error (SE)

[b]Different from 0µg/ml within each incubation time (P<.05)

[c,d,e,f]Column means bearing the same superscript within each concentration do not differ (P<.05)

monensin (table 16), although IC50 for L(+) lactic acid was slightly lower for monensin than lasalocid. Among the nonionophore compounds avoparcin was the least effective compound in inhibiting lactic acid. Thiopeptin, virginiamycin and tylosin were extremely effective in inhibiting lactic acid production. The extent of inhibition of total lactic acid was almost 90 to 95% at the highest concentration (24.0 µg/ml) with thiopeptin, tylosin and virginiamycin. Among the compounds tested, thiopeptin and virginiamycin seemed to be the most effective inhibitors of lactic acid production from glucose fermentation. Monensin and tylosin combination (3:1) were more effective than monensin alone but not different from tylosin alone in inhibiting lactic acid production (table 6).

Effects of incubation time and salinomycin concentration on VFA production

Total VFA concentration increased progressively ($P<.05$) with increased incubation time in both control (no antibiotic) and salinomycin-treated fermentations (table 17). The molar proportion of acetate in the control fermentation was higher ($P<.05$) at 6 h incubation than at 12, 18 or 24 h incubation. Incubation time had no effect on the molar proportions of propionate and isobutyrate and acetate:propionate ratio in the control fermentation. However, molar proportions of butyrate and valerate at 24 h were higher than that of the 18, 12 and 6 h incubations ($P<.05$) and that of isovalerate was higher at 24 and 18 h ($P<.05$) than at 12 and 6 h incubations (table 17). Addition of salinomycin to the fermentation had no effect on the total VFA concentration at all incubation time. However, final pH of the fermentation mixture tended to be lower in salinomycin-treated fermentations than the control. Molar proportions of VFA except that of isobutyrate and butyrate were unaffected by the addition of salinomycin at 6 h incubation. Salinomycin-treated fermentations incubated for 12 h or longer generally had lower

proportions of acetate and butyrate and higher proportion of propionate than the control. Also molar proportions of isobutyrate, isovalerate and valerate were not affected by salinomycin except for slight increase in isobutyrate and isovalerate at 24 h incubation. Salinomycin-treated fermentations incubated for 12 h or longer had lower acetate:propionate ratio at all concentrations than the control (table 17). The extent of propionate enhancement, butyrate and acetate:propionate ratio inhibition in fermentations incubated for 12 h or longer by salinomycin was similar (table 17). However, EC25 for propionate was lowest at 12 h incubation (table 18).

Effects of rumen fluid inoculum source and salinomycin concentration on VFA production. Final pH of the fermentation mixture was unaffected by the rumen inoculum source, although total VFA concentration tended to be higher with rumen fluid inoculum collected from high-grain fed than medium- or low-grain fed cattle (table 19). Also, rumen fluid inoculum source had no effect on the molar proportions of individual VFA in the control fermentations. Addition of salinomycin had no effect on total VFA concentration. However, final pH of the salinomycin-treated fermentation tended to be lower than that of the control. Salinomycin decreased the molar proportion of acetate only at 24 and 48.0 µg/ml concentrations in fermentations with rumen fluid inoculum from cattle fed high-grain diet. Molar proportion of propionate increased in fermentations treated with salinomycin. Accordingly, acetate:propionate ratio was lower in salinomycin-treated fermentations than the control. The extent of propionate enhancement and acetate:propionate ratio reduction was highest in fermentations with rumen fluid inoculum from cattle fed low-grain diet and lowest in fermentations with rumen fluid inoculum from cattle fed high-grain diet (table 19). EC25 for propionate in fermentations with rumen fluid from low-grain fed cattle was lower than in the

TABLE 18. EFFECT OF INCUBATION TIME ON PROPIONATE ENHANCEMENT BUTYRATE INHIBITION AND ACETATE-PROPIONATE RATIO REDUCTION BY SALINOMYCIN.

Incubation time, h	Propionate enhancement EC25,[a] µg/ml	Butyrate inhibition IC25,[b] µg/ml	Acetate-propionate ratio reduction IC25,[b] µg/ml
6	>48.00	>48.00	>48.00
12	4.00	>48.00	5.46
18	10.26	>48.00	9.78
24	9.91	>48.00	7.46

[a]Salinomycin concentration required to enhance propionate by 25% of the control

[b]Salinomycin concentration required to reduce butyrate or acetate-propionate ratio by 25% of the control.

TABLE 19. EFFECTS OF RUMEN FLUID INOCULUM SOURCE AND SALINOMYCIN CONCENTRATION ON IN VITRO VOLATILE FATTY ACIDS PRODUCTION[a]

Rumen fluid inoculum source and salinomycin concentration µg/ml	Final pH	Total VFA mM	Molar proportion (moles/100 moles)						Acetate: propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate: propionate ratio reduction
			Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
20% Grain Diet												
0	6.54	106.6^{c}	55.9	25.0	1.0^{cd}	14.4^{bc}	1.9^{c}	1.9	2.24	-	-	-
.75	6.53	114.0^{c}	56.4	27.7	.8^{cd}	11.7^{bc}	1.8^{c}	1.7^{cd}	2.04	10.7	8.9	8.7
1.50	6.53	113.0^{c}	56.6^{c}	29.5^{bcd}	.7^{c}	10.2^{bc}	1.6^{c}	1.4^{cd}	1.92	18.2	29.3	14.0
3.00	6.52	112.1^{c}	55.5	31.1^{bcd}	.6^{c}	10.3^{bc}	1.4^{c}	1.1^{c}	1.79^{b}	24.7	28.7	19.9
6.00	6.50	111.9^{c}	55.3	31.7^{bcd}	.7^{c}	9.6^{bc}	1.5^{c}	1.3^{c}	1.75^{b}	26.8	33.7	21.7
12.00	6.46	110.1^{c}	54.5^{c}	32.7^{bcd}	.6^{c}	9.6^{bc}	1.4^{c}	1.2^{c}	1.67^{b}	31.1	34.1	25.6
24.00	6.42	107.4^{c}	54.2^{c}	32.9^{bcd}	.6^{c}	9.7^{bc}	1.3^{c}	1.2^{c}	1.65^{b}	31.9	33.3	26.4
48.00	6.39	101.8^{c}	56.7^{c}	30.7^{bc}	.6^{c}	9.8^{bc}	1.2^{c}	1.1^{c}	1.86^{b}	22.8	32.8	17.1
50% Grain Diet												
0	6.44	109.7^{c}	55.9	27.1	.7^{c}	13.4^{c}	1.5^{d}	1.4	2.07	-	-	-
.75	6.41	109.5^{c}	56.2	29.1^{bc}	.6^{c}	11.7^{c}	1.3^{d}	1.2^{c}	1.93	7.4	13.2	6.4
1.50	6.40	109.2^{c}	54.3^{cd}	32.6^{bc}	.4^{c}	10.5^{bc}	1.2^{c}	1.1^{c}	1.66^{b}	20.3	22.1	19.8
3.00	6.39	110.5^{c}	53.2	34.7^{bc}	.4^{c}	10.0^{bc}	1.0^{c}	1.0^{c}	1.53^{b}	28.0	25.1	26.1
6.00	6.35	105.4^{c}	53.0	34.6^{bc}	.4^{c}	10.0^{bc}	1.0^{c}	1.0^{c}	1.53^{b}	27.7	23.9	26.1
12.00	6.32	109.2^{c}	52.6^{cd}	35.0^{bc}	.4^{c}	9.8^{bc}	1.1^{c}	1.1^{c}	1.51^{b}	29.2	27.5	27.1
24.00	6.33	104.9^{c}	52.3^{cd}	35.4^{bc}	.3^{c}	9.8^{bc}	1.0^{c}	1.2^{c}	1.49^{b}	30.4	26.8	28.2
48.00	6.26	104.1^{c}	53.0^{c}	34.1^{bd}	.3^{c}	10.2^{bc}	1.0^{c}	1.3^{c}	1.56^{b}	26.0	23.9	24.3
80% Grain Diet												
0	6.44	135.5^{d}	54.0	25.5	1.2^{d}	14.8	2.6^{e}	1.9	2.16	-	-	-
.75	6.41	136.0^{d}	53.6	25.9	1.2^{d}	14.6^{d}	2.7^{e}	2.0^{d}	2.10	1.7	1.2	2.5
1.50	6.39	141.1^{d}	52.4^{d}	27.3^{cd}	1.5^{d}	14.0^{d}	2.7^{e}	2.0^{d}	1.96	7.2	5.2	9.4
3.00	6.39	137.1^{d}	52.0	28.0^{cd}	1.1^{d}	14.0^{d}	2.7^{e}	2.1^{d}	1.89	10.0	5.1	12.5
6.00	6.38	134.9^{d}	51.8	28.2^{d}	1.3^{d}	13.8^{d}	2.7^{e}	2.0^{d}	1.87	10.8	6.3	13.4
12.00	6.38	141.7^{d}	50.2^{bd}	29.1^{bd}	1.4^{d}	14.0^{d}	3.0^{e}	2.3^{d}	1.76^{b}	14.8	4.9	18.5
24.00	6.35	142.8^{d}	48.9^{bd}	30.1^{bcd}	1.4^{d}	14.0^{d}	3.0^{e}	2.7^{d}	1.68^{b}	17.8	5.5	22.9
48.00	6.35	139.5^{d}	47.1^{bd}	31.3^{bcd}	1.4^{d}	14.1^{d}	3.0^{e}	3.1^{d}	1.54^{b}	23.0	4.2	29.1
SE	.10	6.1	1.3	1.2	.2	.7	.2	.3	.11	2.5	2.4	2.6

[a]Least square means and standard error (SE).

[b]Different from 0µg/ml within each diet (P<.05).

[c,d,e]Column means bearing the same superscript within each concentration do not differ (P<.05).

fermentation with rumen fluid from cattle fed 50% grain diet (table 20). However, IC25 for acetate:propionate ratio was similar in fermentations with rumen fluid from cattle fed low-grain or medium-grain diet. Salinomycin addition lowered ($P<.05$) the molar proportion of butyrate in fermentations with rumen fluid inocula from cattle fed low- or medium-grain diet but not in fermentations with rumen fluid from high-grain fed cattle (table 20). Molar proportions of isobutyrate, isovalerate and valerate were unaffected by either rumen fluid inoculum source or salinomycin.

Effects of antimicrobial compounds on VFA production. Total VFA concentration was unaffected by the addition of the antimicrobial compounds (tables 21 to 31) except that of RO22-6924/004, tylosin and virginiamycin which caused a reduction ($P<.05$) at 24.0 µg/ml concentration (tables 22, 29 and 30). Occassionally, total VFA concentrations were higher in antibiotic treated fermentations than the control. The acetate proportion was not affected by avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin and salinomycin. However, tylosin, monensin and tylosin combination, thiopeptin and virginiamycin increased ($P<.05$) the molar proportion of acetate at 6 µg/ml or higher concentrations (tables 28 to 31). Avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin, salinomycin and thiopeptin increased the molar proportion of propionate. In most instances the increase was significant at concentrations greater than .75 µg/ml. Tylosin and virginiamycin at low concentrations (.75-3.0 µg/ml) increased ($P<.05$) and at high concentrations (> 6.0 µg/ml) decreased ($P<.05$) the molar proportion of propionate (tables 29 and 30). Monensin and tylosin combination had no effect on propionate proportion (table 31). Narasin and salinomycin appeared to be the most effective among the

TABLE 20. EFFECT OF RUMEN FLUID INOCULUM SOURCE ON PROPIONATE ENHANCEMENT, BUTYRATE INHIBITION AND ACETATE-PROPIONATE RATIO REDUCTION BY SALINOMYCIN.

Rumen Fluid inoculum source	Propionate enhancement EC25,[a] µg/ml	Butyrate Inhibition IC25,[b] µg/ml	Acetate-Propionate ratio reduction IC25,[b] µg/ml
20% grain diet	3.43	1.34	11.27
50% grain diet	2.42	2.95	2.74
80% grain diet	>48.00	>48.00	32.00

[a]Salinomycin concentration required to enhance propionate by 25% of the control

[b]Salinomycin concentration required to reduce butyrate or acetate-propionate ratio by 25% of the control

TABLE 21. EFFECT OF AVOPARCIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	96.18	55.8	24.5	1.0	14.9	1.9	2.0	2.28	0	0	0
.75	97.25	55.5	25.6	1.0	14.5	1.8	1.7	2.17	4.2	2.7	3.3
1.50	94.99	54.9	27.5	1.0	13.6	1.7	1.6	2.00	11.8	8.9	9.1
3.00	96.33	55.1	29.3[b]	1.9	11.9[b]	1.6	1.4	1.88	18.9	20.1	14.7
6.00	93.71	55.3	29.4[b]	1.9	11.5[b]	1.6	1.4	1.88	19.4	22.0	14.7
12.00	96.23	54.3	30.7[c]	1.9	11.4[c]	1.5	1.4	1.77	26.3	22.9	21.2
24.00	94.52	53.6	30.4[c]	1.8	12.3[c]	1.5	1.4	1.76	24.8	17.1	21.6
SE	2.20	1.0	1.0	-	.6	-	-	.3	4.0	-	4.2

[a] Least square means and standard error (SE).

[b,c] Different from 0 µg/ml concentration (P<.01, .001).

TABLE 22. EFFECT OF RO22-6924/004 ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	101.45	52.2	29.6	.8	14.3	1.7	2.8	1.79	0	0	0
.75	99.66	53.1	32.5[c]	.7	10.4[d]	1.5	1.8[b]	1.65	10.7	26.0	7.6
1.50	104.55	52.4	34.3[d]	.7	9.5[d]	1.5	1.6[c]	1.54[c]	17.0	32.6	13.6
3.00	101.85	50.9	36.0[d]	.7	9.2[d]	1.5	1.6[c]	1.42[c]	22.7	34.1	20.2
6.00	99.45	50.3	36.7[d]	.6	9.6[d]	1.3	1.6[c]	1.37[c]	26.1	34.0	23.1
12.00	96.91	49.9	37.5[d]	.6	9.1[d]	1.3	1.6[c]	1.27[c]	27.7	35.8	28.2
24.00	92.58[c]	50.3	37.4[d]	.6	9.0[d]	1.3	1.6[c]	1.31[c]	27.7	36.4	26.2
SE	1.91	1.1	.6	.1	.6	.1	.3	.10	2.7	2.7	2.1

[a] Least square means and standard error (SE).

[b,c,d] Different from 0 µg/ml concentration (P<.05, .01, .001).

TABLE 23. EFFECT OF IONOPHORE RO21-6447/009 ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	111.74	55.5	27.9	.7	12.8	1.6	1.9	2.07	0	0	0
.75	117.86	56.3	31.0[b]	.7	9.2[c]	1.5	1.6	1.91	11.1	30.9	11.1
1.50	121.63	56.4	33.4[c]	.5	7.4[c]	1.2	1.3	1.78	19.7	43.6	19.7
3.00	118.11	54.9	34.8[c]	.5	7.4[c]	1.1	1.2	1.75	25.0	44.3	25.0
6.00	121.48	54.5	36.1[c]	.3	7.4[c]	1.1	1.0	1.58	30.1	44.4	30.1
12.00	110.77	55.6	35.7[c]	.3	6.4[c]	1.0	1.0	1.62	28.8	52.4	28.8
24.00	114.40	53.9	37.4[c]	.3	6.4[c]	1.1	1.1	1.56	35.4	51.6	35.4
SE	4.30	.8	.8	-	.4	-	-	.2	-	-	4.2

[a] Least square means and standard error (SE).

[b,c] Different from 0µg/ml concentration (P<.01, .001).

TABLE 24. EFFECT OF LASALOCID ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	117.46	54.1	27.2	1.0	13.1	2.2	2.5	1.99	0	0	0
.75	122.92	55.0	29.6	.9	10.3[b]	2.0	2.3	1.86	9.1	20.7	6.2
1.50	114.44	54.2	32.9[b]	.8	9.0[b]	1.6	1.7	1.65	21.1	31.0	16.3
3.00	117.70	54.0	34.1[b]	.4	8.1[b]	1.6	1.5	1.68	25.1	36.5	19.6
6.00	118.83	53.8	34.4[b]	.6	7.9[b]	1.7	1.6	1.56	26.5	38.9	20.9
12.00	120.50	52.7	36.1[b]	.5	7.7[b]	1.5	1.6	1.50	32.8	40.5	25.9
24.00	112.91	52.0	37.3[b]	.2	7.6[b]	1.3	1.5	1.39[b]	37.4	41.3	28.9
SE	4.10	.7	.9	-	.4	-	-	.2	-	-	4.8

[a] Least square means and standard error (SE).

[b] Different from 0µg/ml concentration (P<.001).

TABLE 25. EFFECT OF MONENSIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	136.12	55.7	27.4	.7	11.9	2.1	2.3	2.01	0	0	0
.75	135.59	55.0	30.0	.4	10.7[b]	2.0	2.2	1.83	9.0	10.0	9.2
1.50	120.53	54.4	32.4[c]	.4	9.5[d]	1.7	1.7	1.68	18.5	20.7	17.0
3.00	125.46	55.3	32.5[c]	.4	8.7[d]	1.6	1.5	1.70	18.5	26.7	15.5
6.00	134.30	54.3	33.4[d]	.6	8.9[d]	1.7	1.6	1.63	22.1	25.8	19.9
12.00	126.38	53.6	34.4[d]	.5	9.5[d]	1.5	1.4	1.56	25.6	28.4	23.5
24.00	111.00	55.6	34.3[d]	.1	7.4[d]	1.3	1.4	1.62	25.3	37.7	20.3
SE	9.29	.9	.9	-	.3	-	-	.2	4.0	-	3.7

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P<.05, .01, .001).

TABLE 26. EFFECT OF NARASIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	105.42	50.4	29.2	1.0	14.7	2.2	2.5	1.73	0	0	0
.75	109.86	51.3	34.2[b]	.8	10.0[b]	1.9	1.9	1.50	17.1	32.0	13.3
1.50	115.86	50.6	35.6[b]	.8	9.5[b]	1.7	1.8	1.42	21.9	35.4	17.9
3.00	103.63	50.4	36.8[b]	.7	9.3[b]	1.7	1.5	1.37	26.0	36.7	20.8
6.00	107.61	50.3	37.1[b]	.7	8.9[b]	1.6	1.6	1.36	27.1	40.0	21.4
12.00	103.82	49.9	38.5[b]	.5	8.2[b]	1.4	1.5	1.30	31.8	44.2	24.9
24.00	107.65	49.3	38.4[b]	.5	8.4[b]	1.4	1.6	1.29	31.5	42.9	25.4
SE	2.48	1.6	.6	-	.3	-	-	.20	-	-	4.8

[a]Least square means and standard error (SE).

[b]Different from 0µg/ml concentration (P< .001).

TABLE 27. EFFECT OF SALINOMYCIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	105.40	58.2	23.9	.7	13.3	2.0	1.9	2.44	0	0	0
.75	112.87	60.1	27.1[c]	.5	9.3[d]	1.5	1.4	2.22	13.8	31.7	8.7
1.50	110.47	59.4	28.9[d]	.6	8.6[d]	1.4	1.2	2.06	21.1	37.1	15.0
3.00	121.19[b]	58.4	30.7[d]	.4	7.9[d]	1.3	1.1	1.90	28.5	41.2	22.1
6.00	116.34	57.8	31.8[d]	.4	7.6[d]	1.3	1.1	1.82[b]	33.3	43.6	25.5
12.00	117.36[b]	57.3	32.4[d]	.5	7.6[d]	1.2	1.2	1.77[b]	35.9	44.2	26.5
24.00	113.43	56.9	31.9[d]	.5	8.3[d]	1.2	1.4	1.78[b]	33.9	39.8	25.7
SE	3.81	1.0	.6	-	.5	-	-	.20	-	-	4.8

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P< .05, .01, .001).

TABLE 28. EFFECT OF THIOPEPTIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	101.71	54.5	24.3	1.1	15.6	2.1	2.4	2.24	0	0	0
.75	107.05	56.0	28.5[d]	.8	11.0[d]	2.0	1.7	1.95	17.9	29.5	12.0
1.50	111.29	56.0	28.6[d]	1.2	10.8[d]	2.0	1.7	1.96	18.1	30.5	12.3
3.00	114.02[b]	56.5	28.2[d]	.9	10.8[d]	1.9	1.7	2.00	16.6	30.4	10.3
6.00	117.77[b]	57.2[b]	27.3[c]	.9	11.0[d]	2.0	1.7	2.10	13.3	29.4	6.4
12.00	118.50[b]	57.0[b]	27.9[c]	.9	10.9[d]	1.9	1.5	2.04	15.1	30.3	8.5
24.00	109.01	57.6[b]	27.2[c]	.9	10.9[d]	1.9	1.6	2.12	12.9	30.1	5.4
SE	4.06	.7	.6	-	.6	-	-	.24	-	-	3.7

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P< .05, .01, .001).

TABLE 29. EFFECT OF TYLOSIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	127.59	56.4	24.1	.9	14.2	2.1	2.3	2.34	0	0	0
.75	125.85	56.1	27.9[d]	.9	11.4[c]	2.0	1.8	2.01	16.4	18.4	13.8
1.50	133.11	57.9	26.8[c]	.9	10.9[d]	1.9	1.7	2.16	11.8	21.7	7.7
3.00	131.79	58.2	24.9[c]	.9	11.9[b]	2.1	2.0	2.34	3.8	11.9	0.1
6.00	124.74	61.7[d]	22.2[b]	1.0	11.4[c]	2.0	1.7	2.28	-7.5	17.6	-19.0
12.00	120.30	64.2[d]	19.3[d]	1.0	11.5[c]	2.2	1.7	3.33[c]	-19.4	17.4	-42.1
24.00	115.93[b]	66.1[d]	17.7[d]	1.0	11.4[c]	2.1	1.7	3.74[d]	-26.5	18.1	-60.2
SE	2.85	.8	.5	-	.6	-	-	.3	-	-	4.2

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P<.05, .01, .001).

TABLE 30. EFFECT OF VIRGINIAMYCIN ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[a]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	97.92	54.8	26.1	1.0	14.2	1.9	2.1	2.10	0	0	0
.75	102.48	55.5	30.4[c]	.9	10.3[d]	1.6	1.5	1.83	16.5	28.7	12.7
1.50	105.87[b]	55.3	29.7[b]	1.0	10.5[d]	1.8	1.6	1.86	14.0	26.4	10.9
3.00	103.45	56.4	28.6	1.0	10.6	1.8	1.6	1.97	9.5	25.5	4.9
6.00	102.58	59.8[d]	24.7	.9	10.9[d]	2.1	1.6	2.42	-5.4	23.1	-15.7
12.00	94.12	62.7[d]	21.3[d]	1.0	11.3[d]	2.0	1.7	2.94[b]	-18.5	19.7	-41.5
24.00	85.96[d]	63.2[d]	19.5[d]	1.1	12.4[c]	2.1	1.8	3.24[c]	-25.5	12.0	-55.7
SE	2.33	.6	.9	-	.3	-	-	.24	-	-	4.2

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P< .05, .01, .001).

TABLE 31. EFFECT OF MONENSIN AND TYLOSIN (3:1) ON IN VITRO VOLATILE FATTY ACID (VFA) PRODUCTION[ab]

Antibiotic concentrations µg/ml	Total VFA mM	Molar proportion (moles/100 moles)						Acetate-propionate ratio	Percent propionate enhancement	Percent butyrate inhibition	Percent acetate-propionate ratio reduction
		Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate				
0	90.60	50.5	25.7	1.1	17.6	2.4	2.8	1.97	0	0	0
.75	92.62	50.2	27.0	1.0	14.9[d]	2.3	2.6	1.86	4.1	14.9	3.1
1.50	90.87	51.8	27.7	.9	15.2[c]	2.1	2.5	1.87	7.1	13.0	3.0
3.00	93.84	52.5[c]	27.5	.8	14.8[d]	2.1	2.4	1.91	6.6	15.1	1.2
6.00	90.73	52.9[c]	26.9	.8	15.2[c]	1.8	2.1	1.97	4.5	13.0	-1.4
12.00	85.34	54.3[d]	26.1	.7	16.0	1.7	2.3	2.08	1.5	8.5	-7.1
24.00	84.66	54.3[c]	24.3	.8	16.1	1.7	1.9	2.28	-5.0	8.1	-16.7
SE	2.30	.6	.7	-	.6	-	-	.28	-	-	4.2

[a] Least square means and standard error (SE).

[b,c,d] Different from 0µg/ml concentration (P<.05, .01, .001).

compounds tested and lasalocid was more effective than monensin in enhancing propionate. Acetate:propionate ratio tended to be lower in antibiotic-tested fermentations than the control. Tylosin and virginiamycin increased ($P<.05$) acetate:propionate ratio at high concentrations (24 or 48.0 μg/ml). Based on IC25 for acetate:propionate ratio, salinomycin appeared to be the most potent of all the antibiotics. The molar proportion of butyrate was reduced ($P<.05$) by all the antimicrobial compounds even at the lowest concentration tested (.75 μg/ml). In general, ionophore antibiotics were more inhibitory to butyrate production than the nonionophore antibiotics (table 32). Molar proportions of isobutyrate, isovalerate and valerate were not affected by the addition of antimicrobial compounds.

Discussion

Because of the wide acceptance of lasalocid and monensin in the cattle industry, several antimicrobial compounds are being investigated as possible feed additives (Chalupa, 1984). Among the ionophore antibiotics, RO22-6924/004, narasin and salinomycin have shown excellent promise and are reportedly more potent than lasalocid or monensin for improving feed efficiency and rate of gain of feedlot cattle (Dinusson et al., 1979; Potter et al., 1979; Thonney et al., 1983; Merchen and Berger, 1985). Avoparcin, a glycopeptide antibiotic (McGahren et al., 1980) has been shown to be similar to monensin in its effect on the performance of finishing cattle (Johnson et al., 1979; Dyer et al., 1980; Cuthbert et al., 1984). Thiopetin, a sulfur containing peptide (Miyairi et al., 1972) is an effective inhibitor of S. bovis (Muir and Barreto, 1979) and therefore, effective in controlling lactic acidosis in cattle (Muir et al., 1980). Thiopeptin supplementation of a high-concentrate diet (11 ppm) increased weight gain and feed efficiency (Gill et al., 1979). Improved

TABLE 32. PROPIONATE ENHANCEMENT, ACETATE:PROPIONATE RATIO REDUCTION AND BUTYRATE INHIBITION BY ANTIBIOTICS

Antibiotics	Propionate			Acetate:Propionate ratio			Butyrate		
	EC25[a] µg/ml	Maximum enhancement %	Concentration[d] µg/ml	IC25[b] µg/ml	Maximum reduction %	Concentration[d] µg/ml	IC25[c] µg/ml	Maximum inhibition %	Concentration[d] µg/ml
Avoparcin	11.66	25.3	12.00	>24.00	22.8	24.00	>24.00	23.5	12.00
RO22-6924/004	4.97	27.7	12.00	7.93	28.2	12.00	.72	36.4	24.00
RO21-6447/009	3.19	28.7	24.00	22.15	25.6	24.00	.67	50.0	12.00
Lasalocid	2.86	37.1	24.00	12.86	30.2	24.00	1.10	42.0	24.00
Monensin	11.03	25.2	24.00	>24.00	22.4	12.00	2.58	37.8	24.00
Narasin	2.63	31.8	12.00	12.80	25.4	24.00	.59	44.2	12.00
Salinomycin	2.31	35.6	12.00	5.64	27.5	12.00	.62	42.9	12.00
Thiopeptin	>24.00	17.7	1.50	>24.00	13.0	.75	.66	30.8	1.50
Tylosin	-	15.8	.75	-	14.1	.75	-	23.2	1.50
Virginiamycin	-	16.5	.75	-	12.9	.75	.68	27.5	.75
Monensin + Tylosin	-	7.8	1.50	-	5.6	.75	-	15.9	3.00

[a]EC25 is the antibiotic concentration required to enhance propionate by 25% of the control.

[b]IC25 is the antibiotic concentration required to reduce acetate:propionate ratio by 25% of the control.

[c]IC25 is the antibiotic concentration required to inhibit butyrate by 25% of the control.

[d]Concentration at which maximum enhancement or inhibition occurred.

performance of tylosin fed cattle is generally attributed to its ability to prevent liver abscesses (Potter et al., 1985). Virginiamycin, a growth-promoting antibiotic used in pigs has also been shown to have growth-promotion effect in cattle (Demeyer and VanNevel, 1985).

Improvement in efficiency of feed utilization in lasalocid- or monensin-fed cattle is attributed to favorable alterations in rumen fermentation characteristics (Bartley and Nagaraja, 1982; Bergen and Bates, 1984; Schelling, 1984). The mechanisms by which lasalocid and monensin alter fermentation characteristics are believed to be due to shift in the rumen microbial community. Bacteria that produce lactic, butyric, formic and H_2 are very sensitive, whereas, succinate producing and lactate-fermenting bacteria are resistant, thereby, resulting in a bacterial community that produces less lactic, less butyric, less methane and more propionic acid (Chen and Wolin, 1979; Dennis et al., 1981b).

Rumen fermentation changes induced by many of the antimicrobial compounds have been shown to be similar to that of monensin and lasalocid in that acetate:propionate ratio is decreased, lactic acid production is inhibited, methanogenesis is depressed and ruminal degredation of protein and amino acids is lowered (Dyer et al., 1979; Muir et al., 1980; Froetschel et al., 1983; DeMeyer and Van Nevel, 1985; Merchen and Berger, 1985; Nagaraja et al., 1985). Also, sensitivity and resistance of rumen bacteria to the various antimicrobial compounds used in the study were very similar to lasalocid and monensin in that gram positive bacteria are resistant and gram negative bacteria are sensitive (Akkad and Hobson, 1966; Wang et al., 1966; Fulghum et al., 1968; Watanabe et al., 1981; Stewart et al., 1983, Taylor and Nagaraja, manuscript submitted for publication). Avoparcin and tylosin have been shown to be less inhibitory to the two major gram positive lactic acid producing bacteria (Streptococcus bovis and lactobacillus sp.) (Walton,

1978; Dutta and DeVriese, 1981; Taylor and Nagaraja manuscript submitted for publication). However, both avoparcin and tylosin were very effective in inhibiting lactic acid production from glucose fermentation in the in vitro system. In fact, tylosin was more effective in inhibiting lactic acid production than the ionophore antibiotics and the latter are more inhibitory to S. bovis and lactobacilli than the former. This is difficult to explain but it could be speculated that tylosin in a mixed culture fermentation may get metabolised and the metabolite may be more inhibitory to the lactic acid producing bacteria than the parent compound. Among the ionophore compounds narasin and salinomycin were more effective in inhibiting lactic acid production than lasalocid and monensin. This is in agreement with our previous report that salinomycin at .22 mg/kg body weight was as effective as lasalocid or monensin at .66 mg/kg in preventing experimentally induced lactic acidosis in cattle (Nagaraja et al., 1985).

Narasin and salinomycin also were more potent in enhancing propionate production and reducing acetate:propionate ratio than lasalocid and monensin. A similar difference in efficacy has also been observed in feedlot performance studies. The optimum dosage for narasin and salinomycin is about 3-fold less than that of lasalocid and monensin (Dinusson et al., 1979; Potter et al., 1979; Merchen and Berger, 1985). It is interesting that tylosin's effect on VFA production was antagonistic to that of monensin. Tylosin lowered propionate production and increased acetate production. Consequently, combination of monensin and tylosin (3:1) had no effect on acetate:propionate ratio at the recommended dosage level (assuming 6 µg/ml represents ruminal concentration of monensin and tylosin in cattle fed at 30 g and 10 g/ton, respectively). However, Potter et al. (1985) based on the summary of 14 feedlot trials demonstrated additive effects of monensin and

tylosin. Liver abscess control, along with improvements in daily gain and feed efficiency were obtained by the addition of tylosin to monensin-containing diets.

In conclusion, in vitro quantitative evaluation, based on propionate enhancement, acetate:propionate ratio reduction and lactic acid inhibition may be used as a screening method to predict the feed additive potential of the antimicrobial compounds.

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ANTIMICROBIAL FEED ADDITIVES

INTRODUCTION

Antimicrobial agents are used not only for the control and treatment of bacterial and parasitic diseases, but also for the enhancement of body growth and the improvement of feed efficiency. The discovery in the late 1940's that feeding chlortetracycline to chicks enhanced their growth rate marked the beginning of the large scale use of antibiotics in animal feeds. Antibiotics as feed additives have been used commercially in animal feed for improving weight gain and feed efficiency for almost thirty-five years (Silver and Mercer, 1978). In the United States alone, more than one million kg. of antibiotics are used annually as diet supplements (Hays, 1978). Forty percent of the antibiotics sold are for non-medicinal use. This wide acceptance of feed additives can be attributed to their ability to improve growth and feed efficiency, and reduce mortality and morbidity from clinical and subclinical infections.

Some of the more commonly used antibiotics in livestock production include bacitracin, bambermycin, chlortetracycline, erythromycin, hygromycin, lasalocid, lincomycin, monensin, neomycin, novobiocin, nystatin, oxytetracycline, penicillin, streptomycin, tylosin, and virginiamycin (table 1). Other antibiotics which experimentally show promise await further testing and possible approval.

MECHANISM OF ACTION

The mechanism in which antibiotics improve growth is not fully understood. At least three general modes of action have been postulated (Hayes, 1978). A) a metabolic effect, in which the antibiotics directly affect the rate or pattern of the metaboolic processes of the animal. B) a nutrient-sparing effect in which the antibiotics reduce the dietary requirement for certain nutrients by stimulating the growth of desirable organisms that synthesize vitamins or amino acids, by

TABLE I. ANTIMICROBIAL AGENTS APPROVED FOR USE IN FOOD ANIMALS

Antibiotic	Trade name	Approved usage			
		cattle	sheep	swine	chicken
Bacitracin methylene disalicylate	BMD[a] Fortracin[a] Solutracin[a]	M		F,G,M	E,F,G,M
Bacitracin zinc	Albac[a] Bacifern[c] Zinc-Bacitracin[c]	F,G		F,G	F,G
Bambermycins	Flavomycin[d]			F,G	C,F,G
Chlortetracycline	Aureomycin[e] CTC[f] CLTC[g] Chlorate[h] Pfichlor[i]	F,G,M	F,G,M	F,G,M	C,F,G,M
Erythromycin	Gallimycin[j]	F,G		F,G	E,F,G,M
Hygromycin B	Hygromix[k]			A	A
Lasalocid	Avatec[l] Bovatec[l]	F,G	C		C
Lincomycin	Lincomix[m]			M	C,F,G,M
Monensin	Coban[k], Rumensin[k]	F,G			
Neomycin	Neomycin sulfate[l] Neomix[m]	M	M	M	M
Novobiocin	Albamix[m]				M
Nystatin	Myco-20[n]				M
Oxytetracycline	Terramycin[i] OXTC[g]	F,G,M	F,G,M	F,G,M	E,C,F, G,M
Penicillin	Penicillin P-100[i] Procaine Penicillin[n]			F,G	F,G,M
Salinomycin	Biocox[o]				C
Streptomycin	Strepcillin F-25[c]			F,G,M	E,F,G,M
Tylosin	Tylan[k]	M		F,G,M	F,G,M
Virginiamycin	Stafac[g]			F,G,M	F,G

A = Anthelmintic; C = Coccidiostat; E = Egg production; F = Feed efficiency; G = Growth; M = Medicinal (antibacterial or antifungal)

[a]A.L. Laboratories, Inc., Englewood Cliffs, NJ; [b]Internaational Minerals and Chemical Corp., Terre Haute, IN; [c]Salsbury Laboratories, Inc., Charles city, IA; [d]American Hoechst Corp., Somerville, NJ; [e]American Cyanamid Co., Wayne, NJ; [f]SDS Biotech Corp., Painesville, OH; [g]Smith Kline Animal Health Products, Westchester, PA; [h]Hess and Clark, Inc., Ashland, OH; [i]Pfizer Inc., New York, NY; [j]Ceva Laboratories, Inc., Overland Park, KS; [k]Elanco Products Co., Indianapolis, IN; [l]Hoffmann-LaRoche Inc., Nutley, NJ; [m]Upjohn Co., Kalamazoo, MI; [n]E.R. Squibb and Sons, Princeton, NJ; [o]Agri-Bio Corp., Gainesville, GA.

depressing the organisms that compete with the host for nutrients, by increasing the availability of nutrients via chelation mechanisms, or by improving the absorptive capacity of the intestinal tract. C) A disease control effect by suppressing organisms causing clinical or subclinical disease, by inhibition of multiplication of organisms that produce toxins which reduce performance but result in no obvious disease symptoms.

A. Metabolic effect. There is evidence that metabolic reactions in the host are influenced by antibiotics. Brody et al. (1954) found that tetracyclines inhibit fatty acid oxidation in the mitochondria. Braude and Johnson (1953) discovered the use of chlortetracycline affected water and nitrogen excretion, and correlated this to the metabolic rate of pigs. Hash et al. (1964) demonstrated the inhibition of protein synthesis in the presence of tetracycline. Although it is apparent that some alterations in metabolism do occur, it is unlikely that the metabolic effects alone could account for the growth promotion in animals.

B. Nutrient sparing effect. It is recognized that certain organisms synthesize vitamins and amino acids which are essential to the host animal, while other organisms compete with the host for these same essential nutrients. Anderson et al. (1952) discovered an increase in the numbers of intestinal coliforms other than Escherichia coli when animals were fed penicillin. These organisms synthesize nutrients which are dietary essentials for the host. Therefore, a diet that is deficient in vitamins or nutrients may be partly corrected by microbial synthesis following a shift in microbial population induced by antibiotics, resulting in improved gain and/or efficiency.

Lucas (1957), Stokstad (1954) and others have indicated a greater response to antibiotics when included in an inadequate diet. This enhanced response may

also be attributed to a reduction in the population of organisms which compete with the host for essential nutrients, particularly those nutrients close to or below the animals requirements. Lactobacilli require amino acids in proportions similar to that of a growing pig (Kellogg et al., 1964). Antibiotics most effective in reducing the population of Lactobacilli in the intestinal tract are the most effective growth promotors in growing pigs (Kellogg et al., 1966).

An increase in the absorptive capacities of the gut of the host animal also may aid in growth promotion. Braude et al. (1955) discovered a thinner gut wall in pigs fed antibiotic than those receiving no antibiotic. Rusoff et al. (1954) and Coates (1953) reported similar findings in calves and chicks. A thinner intestinal wall implies potential for improved absorption with a resulting increase in the utilization of nutrients. This was exemplified by Catron et al. (1953) who reported an increase in the rate of glucose absorption in animals fed diets containing antibiotics.

C. Disease control effect. Early in the history of antibiotic usage, it was discovered that the general well being of the animal was inversly proportional to the response one received from antibiotic addition. Speer et al. (1950) noted that healthy, well nourished pigs did not respond to antibiotics when housed in clean and disinfected pens. Studies indicate a greater response to antibiotics in contaminated environments than those housed in clean environments. Hays and Speer (1960) found that pigs in a clean environment had a 33% improvement in gain and a 10.5% improvement in feed efficiency when supplemented with spiramcyin. Pigs in an uncleaned building were also fed spiramycin with a resulting 75% increase in growth rate and a 37% improvement in feed conversion. Similar results were obtained for chlortetracycline and oxytetracycline. The addition of antibiotics to feed acted to reduce the buildup of nonspecific infection in the

uncleaned pens, resulting in a greater improvement in performance with respect to the clean and disinfected pens.

ANTIMICROIBAL FEED ADDITIVES FOR RUMINANTS

A major distinction between ruminant and nonruminant animals is the possession by ruminants of a modified forestomach or rumen, specifically adapted to the microbial digestion of feed. The rumen is inhabited by many microorganisms that carry out fermentation of complex polysaccharides and proteins, producing volatile fatty acids and microbial protein which in turn serve as the nutrients for the metabolism of the host. Fermentative digestion by microorganisms in the rumen is advantageous for substances that cannot be digested by the animals own hydrolytic enzymes. However, microbial fermentation of dietary protein, starch and sugars and products of fermentation such as sugars and amino acids is accompanied by losses in both energy and animal nitrogen. Therefore, for optimal production a proper balance between microbial fermentation and hydrolytic digestion is desirable.

In order to maximize the efficiency of feed utilization in ruminants, research in the past decade has been focussed towards chemicals which promote adjustments in ruminal fermentation to decrease losses in energy and nitrogen and improve animal performance (Chalupa, 1977). Such manipulation should not affect the beneficial aspect of microbial digestion such as fiber degradation and microbial protein synthesis from nonprotein nitrogen. Modifying rumen microorganisms can alter efficiency of animal performance in several ways (Owens et al., 1984). Chemical agents may: (1) Alter the levels of normal metabolites (2) Prevent accumulation of abnormal compounds (3) Inhibit catabolism of specific nutrients.

Most chemicals were initially identified as adjustors of specific fermentation pathways such as propionate, methane, or lactate production, amino

acid utilization or urea hydrolysis, but later it became apparent that control of selected fermentative pathways was not as specific as originally hypothesized (Chalupa, 1979).

The most widely researched chemical is a polyether antibiotic called monensin which has been shown to alter rumen fermentation to improve cattle performance. Monensin was first approved as a feed additive for feedlot cattle in 1976, and by 1978 over 80% ofthe cattle in feedlots were being fed monensin. Such rapid acceptance led to the introduction of another polyether antibiotic called lasalocid sodium in 1982. The monensin and lasalocid success story has stimulated testing of other ionophore and nonionophore antibiotics which alter rumen fermentation. Currently the following antibiotics have received attention as potential feed additives for cattle: actaplanin, avoparcin, laidlomycin, lysocellin, narasin, salinomycin, thiopeptin, and virginiamycin (table 2).

MONENSIN

Monensin is a monocarboxylic acid polyether ionophore (Fig 1) produced by Streptomyces cinnamonensis (Haney and Hoehm, 1968). It was the first polyether antibiotic to be structurally defined (Agtarap and Chamberlin, 1967). The cation selectivity has been determined to be $Na^+ >> K^+ > Rb^+ > Li^+ > Cs^+$ (Westley, 1982). The antibiotic possesses broad spectrum anticoccidial activity in chickens and is marketed as a coccidiostat under the trade name Coban^R (Elanco Products Co., Indianapolis, IN). Monensin is highly effective against gram positive bacteria, but exhibits no activity against gram negative bacteria (Watanabe et al., 1981; Westley, 1982). It is believed that feeding monensin alters rumen microbial population causing a beneficial shift in fermentation, thus enhancing the feed efficiency (Bergen and Bates, 1984). Monensin, marketed under the trade name

TABLE 2. ANTIMICROBIAL FEED ADDITIVES UNDER INVESTIGATION FOR USE IN CATTLE

Antibiotics	Producing organism	Chemistry	Company
Actaplanin	Actinoplanes missouriensis	Glycopeptide	Eli Lilly Co., Indianapolis, IN
Avoparcin[a]	Streptomyces candidus	Glycopeptide	American Cyanamid Co., Wayne, NJ
Laidlomycin	Streptomyces eurocidicus var asterocidicus	Polyether ionophore	Syntex, Palo Alto, CA
Lysocellin	Streptomyces longwoodensis	Polyether ionophore	International Minerals Corp., Terre Haute, IN
Narasin	Streptomyces aureofaciens	Polyether ionophore	Eli Lilly Co., Indianapolis, IN
Salinomycin[b]	Streptomyces albus	Polyether ionophore	A.H. Robins Co., Richmond, VA
Thiopeptin	Streptomyces tateyamensis	Peptide	Fujisawa Pharmaceutical Co., Osaka, Japan
Virginiamycin[c]	Streptomyces virginiae	Macrocyclic lactone peptolide	Smith Kline Animal Health Products, Westchester, PA

[a]Approved (trade name Avotan) for use as a growth promotor in animals in Europe

[b]Approved (trade name Bio-Cox) for use as a corcidiostat in chickens

[c]Approved (trade name Stafac) for use as a growth promotor in chickens and swine.

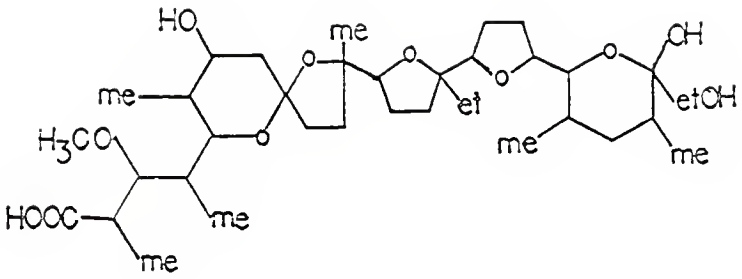

Figure 1. Structure of Monensin

Rumensin^{R} (Elanco Products Co., Indianapolis, IN) has been used extensively in feedlot cattle since 1975 with a great deal of success.

Influence on cattle performance. Since its use in feedlot diets began, monensin has gained wide acceptance in the cattle feeding industry. Numerous experiments have been conducted to evaluate the influence of monensin on performance of feedlot cattle. A summary of 228 trials involving 11,274 head of cattle fed control or monensin-containing diets has been compiled (Goodrich et al., 1984). Cattle fed monensin gained 1.6% faster, consumed 6.4% less feed, and required 7.5% less feed/100 kg gain than cattle fed control diets (table 3). Carcass characteristics were not significantly influenced by monensin. Responses of cattle to monensin and implants were additive. When fed to beef cows, monensin reduced amounts of feed required to maintain cow weight. The factors that may modify these responses are not fully understood, but several synergistic modes of action have been postulated.

Influence on feed intake. The influence of monensin on feed intake is well documented (Goodrich et al., 1984; Schelling, 1984). The feed intake depression due to monensin when high grain diets are fed averages 10.7% over a wide range of conditions (Anonymous, 1975a). This average includes the initial depression of as much as 16% when cattle are first exposed to monensin. Realistic feed intake depressions for high grain-fed cattle adapted to monensin would likely be 5-6% (Owens, 1980). The depression for moderate level roughage diets appears to be about 3% (Anonymous, 1975a). Gains are not depressed with high or moderate levels of highly fermentable carbohydrates, and an improvement in feed conversion results. Cattle receiving monensin under pasture conditions may be consuming up to

TABLE 3. EFFECT OF MONENSIN ON CATTLE PERFORMANCE[a]

Item	Control	Monensin[b]	Percent change
No. of cattle	5,696	5,578	
Initial weight, kg	284	283	
Final weight, kg	430	432	
Daily gain, kg	1.09	1.10	1.6
Daily feed intake, kg	8.27	7.73	-6.4
Feed/kg gain, kg DM	8.09	7.43	-7.5

Summary of 228 trials, Goodrich et al., 1984. Mean monensin dose was 246 mg per head.

15% more forage (Pond and Ellis, 1981), with an increase in the rate of gain up to 17% (Anonymous, 1975b).

Effect of rumen bacteria. The basic mode of action of monensin and other ionophores is to modify the movement of ions across biological membranes. The primary result is the entry of sodium into the cells (Smith and Rosengart, 1978). Other secondary cellular ion changes may also occur (Schanne et al., 1979).

These ion changes cause an alteration in rumen fermentation in part by shifting the microbial flora. Chen and Wolin (1979) have studied the effects of monensin on the growth of methanogenic and rumen saccharolytic bacteria. Ruminococcus albus, R. flavefaciens, and Butyrivibrio fibrisolvens, the major formate and hydrogen producers, were inhibited by 2.5 μg/ml of monensin. Bacteroides succinogenes and B. ruminicola were resistent to monensin at 20 μg/ml, but growth was delayed. Selenomonas ruminantium was resistant to 40 μg/ml of monensin. Among the methane bacteria, monensin inhibited Methanobacterium MOH, M. formicium and M. barkeri, but Methanobrevibacter ruminantium was resistant to monensin. It was proposed that monensin may act in the rumen by suppressing the population of gram-positive bacteria which ferment carbohydrates to hydrogen and formate, or to acetate and butyrate, and selecting for antibiotic resistant gram-negative bacteria which produce succinate or produce propionate from succinate.

Similar findings have been reported by other workers. Henderson et al. (1981) reported B. ruminicola, S. ruminantium, Anaerovibria lipolytica and Megasphaera elsdenii, succinate- and propionate-producing rumen bacteria, were not affected by monensin up to 10 μg/ml. M. ruminantium was slightly inhibited by monensin, with B. fibrisolvens, R. albus and Streptococcus bovis being inhibited to different extents at concentrations ranging from 0.1 to 10 μg/ml. B. succinogenes showed a delayed growth in the presence of monensin.

Dennis et al (1981a) reported monensin inhibited B. fibrisolvens, Eubacterium cellulosolvens, E. ruminantium, Lachnospira multiparus, Lactobacillus ruminis, L. vitulinus, R. albus, R. flavefaciens, and S. bovis the major lactate producing rumen bacteria. B. succinogenes, B. ruminicola, S. ruminantium, Succinimonas amylolytica and Succinivibrio dextrinosolvens, which produce succinate as a major end product were not inhibited by monensin. Anaerovibrio lipolytica, S. ruminantium, M. elsdenii and Veillonella alcalescens the major lactate utilizers, were not inhibited by monensin. These results suggest that monensin acts in the rumen by selecting for succinate-producing and lactate-fermentating bacteria, and inhibiting those bacteria which produce lactate. This selection could in part lead to the decreased acetate to propionate ratio. Methane production in the rumen could be depressed through the inhibition of hydrogen and formate producers, eg., R. albus, R. flavefaciens, and L. multiparus. Dawson and Boling (1984) reported that inhibition of B. ruminicola by monensin was greatly influenced by the mineral content of the medium. All strains of B. ruminicola tested were more sensitive to monensin in medium containing low concentrations of potassium. Similar effects were observed with R. albus, R. flavefaciens, B. fibrisolvens and B. succinogenes.

There is very little information on the effects of monensin on the composition of the microbial population in the rumen. Brulla and Bryant (1980) have shown increased proportions of Bacteroides species and a decreased proportion of monensin-sensitive Butyrivibrio fibrisolvens in the rumen of cattle fed monensin. Dawson and Boling (1983) monitored total and monensin-resistant anaerobic bacterial populations in the rumens of steers fed monensin containing (33 mg/kg) and unmedicated diets. The mean percentage of anaerobic population resistant to monensin (10 µg/ml) was greater in animals fed monensin supplemented diet for 33 days than in those receiving the control diet. However, the greater

proportions of monensin-resistant organisms were not necessarily associated with altered fermentation patterns. It was suggested that the selection of monensin-resistant microbial groups within the rumen cannot completely account for changes in ruminal fermentations.

Effect on rumen protozoa. Because monensin is a coccidiostat (Ruff, 1982) it is likely that protozoal population in the rumen is affected. Dinius et al. (1976) reported that monensin did not change protozoal numbers or types in growing cattle fed orchard grass. Richardson et al. (1978) found that protozoal populations in the rumen were reduced by feeding monensin to cattle on a high-grain diet but not on a high-roughage diet. Reduction in rumen protozoal population has been reported to be greater in cattle on urea than on a natural protein as dietary nitrogen source (Poos et al., 1979). Such a dietary influence on the effect of monensin on protozoa is suggestive of differing sensitivity of protozoal types to the antibiotic. Dennis et al. (1981b) have reported entodiniomorphs particularly Ophryoscolex, Entodinium and Diplodinium were more sensitive to monensin than the holotrichs.

Several rumen fermentation changes have been well documented which more directly cause the animal performance response normally observed when the ruminants are fed monensin.

Decreased acetate:propionate ratio. Early research with monensin clearly demonstrated a decrease in the acetate to propionate ratio (Richardson et al., 1976). This ratio change presumes propionate is produced at the expense of acetate (Van Maanen et al., 1978) and butyrate (Richardson et al., 1976). This ratio shift is viewed as favorable for ruminants because propionate production by rumen fermentation appears to be more efficient than that of acetate (Hungate, 1966;

Chalupa, 1977). A second possible factor is evidence of propionate being utilized more efficiently than acetate by the tissue (Smith, 1971). A third possible advantage of propionate is its flexibility as an energy source. Propionate has the potential to be used for gluconeogenesis or be directly oxidized by the citric acid cycle. This additional substrate for glycolysis may provide energetic advantages to the ruminant by generating more reduced coenzyme outside the mitochondrial membrane (Schelling, 1984).

The advantageous shift in the propionate to acetate ratio can partially be attributed to definite changes in the microbial population and/or microbial metabolism in response to monensin. Monensin has been found to inhibit acetate producing bacteria, but not succinate-producing and propionate-producing bacteria (Chen and Wolin, 1979; Dennis et al., 1981a). The net effect would be a decrease in acetate production with a corresponding increase in propionate production.

Control of lactic acidosis. Research indicates that monensin may aid in the prevention of lactic acidosis. Lactic acidosis in cattle stems from an increase in lactic acid in rumen fluid and blood following overconsumption of highly fermentable carbohydrates such as grains (Dunlop and Hammond, 1965; MacKenzie, 1967; Dunlop, 1972). Cattle are most susceptible to acidosis following abrupt shifts in diet from roughage to concentrate, or when stress temporarily reduces feed intake so that abnormally high intake results (Elam, 1976). The result is an accumulation of lactic acid in rumen fluid and blood, which produces clinical signs of acidosis such as diarrhea, anorexia, dullness, hyperventilation, dehydration and death.

Rumen and blood lactic acid levels increase in unadapted cattle on high concentrate diets because Streptococcus bovis initially proliferates and produces lactic acid. The resulting drop in pH enhances for the growth of Lactobacilli

species (Dunlop, 1972). Therefore, a logical approach to controlling acidosis is to prevent proliferation of S. bovis. Dennis et al. (1981a) have demonstrated the inhibition of S. bovis and Lactobacilli sp. by monensin. The net result would be a reduction in lactic acid production in the rumen of cattle, particularly during the shift from roughage to high grain diets. In vitro fermentations of various sugars and ground grains with rumen fluid from either hay- or grain-fed cattle indicate monensin effectively reduced lactate production (Dennis et al., 1981c). Nagaraja et al. (1981, 1982) reported prevention of glucose- or corn-induced lactic acidosis in cattle by monensin. These observations indicate monensin may improve feed efficiency by preventing clinical and subclinical cases of lactic acidosis in cattle stressed with carboydrate.

Decreased proteolysis and deamination. Monensin may have a protein sparing effect, possibly sparing amino acids normally used for gluconeogenesis (Leng et al., 1967; Reilly and Ford, 1971). Numerous studies have shown monensin significantly reduces ruminal degradation of dietary protein (Schelling et al., 1977; Van Nevel and Demeyer, 1977; Poos et al., 1979; Chalupa, 1980). Schelling et al. (1977) have aslo demonstrated that monensin decreased the rate of free amino acid degradation in rumen fluid. Monensin has been shown to decrease bacterial N reaching the abomasum of adapted steers, and increase the dietary protein reaching the abomasum (Poos et al., 1979). This supports a protein-sparing effect of monensin by increasing ruminal escape of dietary protein.

Depressed methane production. Another mode of action is depressed methane production. In vitro studies indicate a decrease in microbial methane production with monensin (Bartley et al., 1979; Chalupa, 1980). This has also been demonstrated in vivo (Joyner et al., 1979; Thornton and Owens, 1981; Benz and

Johnson, 1982). Monensin has been reported to select against hydrogen-producing and formate-producing rumen bacteria thereby decreasing the precursors required for methanogenesis (Chen and Wolin, 1979; Dennis et al., 1981a). Monensin has been shown to decrease the metabolism of formate to carbon dioxide and hydrogen, resulting in a decrease in methane production (Van Nevel and Demeyer, 1977). Russell and Martin (1984) advanced a hypothesis that depression of methane production in the presence of monensin was dependent on hydrogenase activity on $NADH_2$ coupled to amino acid fermentation by rumen microorganisms. The depression in methane production appears to be small, therefore resulting in only a slight improvement in the efficiency of production.

Alterations in digestibility. Several reports indicate an influence of monensin on digestibility, but results are variable. Simpson (1978) found that monensin decreased cellulose digestibility in cattle unadapted to monensin. Animals with a 21-day adaptation time to monensin had no effect on cellulose digestibility (Dinius et al., 1976). No alteration in acid detergent fiber digestibility of forage occurred when cattle were adapted to monensin for two weeks (Benz and Johnson, 1982). Several studies indicate increases in dry matter digestibility due to monensin (Dinius et al., 1976; Poos et al., 1979; Thornton and Owens, 1981). It appears that monensin, in general, results in a slight improvement in digestibility. There appears to be many factors such as rate of passage, rumen fill, feed intake, and diet which can influence the digestibility of the feed or feed component in question. Research at this time indicates monensin may decrease rumen turnover rate and increase rumen fill (Lemenager et al., 1978), but more research in this area is warranted.

Bloat prevention. Bloat is a disorder in ruminants with an unclear etiology. Cattle have an intrinsic susceptibility to bloat that is determined genetically (Clarke and Reid, 1974). Bloat can be characterized as either feedlot bloat (grain bloat) or legume bloat. Both types are characterized by excessive foaming of ruminal digesta. Feedlot bloat occurs in cattle fed large quantities of grain and small quantities of roughage (Bartley et al., 1975). In legume bloat, although ruminal microorganisms contribute to foaming, the primary foaming agents are derived from plants (Clarke and Reid, 1974).

Bartley et al. (1982) observed that monensin reduced legume bloat in cattle by about 66%. Monensin also reduced grain bloat by 64%. Sakauchi and Hoshino (1981), have reported the anticoccidial action of ionophores tends to decrease total protozoa in rumen contents. Monensin may therefore have a bloat depressing effect by depressing putative slime production by protozoa.

Prevention of acute bovine pulmonary edema and emphysema. Acute bovine pulmonary edema and emphysema (ABPE) or fog fever is a naturally occuring disease of adult cattle characterized by sudden onset of acute respiratory distress soon after a change to lush forage (Breeze and Carlson, 1982). ABPE is caused by the formation, absorption and lung metabolism of 3-methyl indole (3MI) (Carlson et al., 1975). L-tryptophan, a naturally occuring amino acid, is converted to indolepyruvic acid which is then converted to indoleacetic acid in the rumen. Indolepyruvic acid is then decarboxylated to 3MI, possibly by Lactobacilli sp. (Yokohoma et al., 1977; Hammond et al., 1978).

A logical approach to control of ABPE would be to inhibit 3MI formation by altering rumen fermentation. Studies have demonstrated that monensin can effectively reduce 3MI production and prevent the clinical signs and pulmonary lesions of ABPE (Hammond et al., 1978; Hammond and Carlson, 1980). In vitro

studies (Honeyfield et al., 1985) have demonstrated a lower conversion of tryptophan to 3MI from monensin treated cows compared with untreated cows. Conversion of tryptophan to indole was also lower. Monensin also reduced in vivo 3MI production. These results indicate monensin reduced clinical cases of ABPE.

LASALOCID

Lasalocid is a carboxylic acid polyether ionophore (Fig 2) produced by Streptomyces lasaliensis (Berger et al., 1951). Although structurally similar to monensin, it differs in ion selectivity. Lasalocid is capable of transporting divalent cations, in addition to monovalent cations. The cation selectivity has been determined to be $Ba^{+} >> Cs^{+} > Rb^{+}, K^{+} > Na^{+}, Ca^{2+}, Mg^{2+} > Li^{+}$ (Westley, 1982). Lasalocid is also highly effective against gram-positive bacteria, including Mycobacterium, but exhibits no activity against gram negative bacteria (Westley, 1977). Lasalocid possesses broad spectrum anticoccidial activity (Edgar and Flanagan, 1974) in chickens and is marketed under the trade name of Avatec^{R} (Hoffmann-LaRoche Inc., Nutley, NJ).

Lasalocid is also effective in improving feed efficiency of ruminants. It is approved under the trade name Bovatec^{R} (Hoffmann-La Roche Inc., Nutley, NJ) to improve feed efficiency and rate of gain. It is also approved for use as a coccidiostat in sheep. Number of trials have been conducted to compare the efficacy of lasalocid with that of monensin in improving feed efficiency in cattle. Recently a 17-trial study comparing lasalocid to monensin, when both feed additives were fed at 30 g/ton was compiled by Stuart (1984). Lasalocid improved feed conversion by 7.2% over the control treatment and 3.4% above the monensin treatment. Feed intake was similar for lasalocid- and monensin-containing feeds which were approximately 2% below the control feed intake. Lasalocid increased average daily gain by 5.3% above control and 4.2% above monensin (table 4).

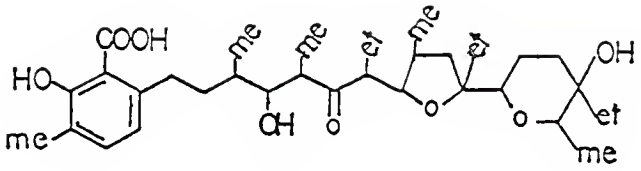

Figure 2. Structure of Lasalocid

TABLE 4. COMPARATIVE EFFECTS OF LASALOCID AND MONENSIN ON PERFORMANCE IN FEEDLOT CATTLE[a]

Treatment	Average daily gain (kg)	Average daily feed intake (kg)	Feed conversion (feed/gain)
Control	1.29	10.06[b]	7.94[b]
Lasalocid	1.36	9.87[c]	7.37[c]
Percent improvement over control	+5.3		+7.2
Percent improvement over monensin	+4.2		+3.4
Monensin	1.30	9.82[c]	7.63[d]
Percent improvement over control	+1.1		+3.9

[a]Stuart, 1984.

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

The enhancement of feed efficiency is attributed to induced rumen fermentation changes similar to those produced by monensin. The rumen fermentation alterations which have been documented are:

1. Shift in the proportions of volatile fatty acids without affecting the total production. Increased propionate and decreased acetate and butyrate (Davis, 1978; Bartley et al., 1979; Fuller and Johnson 1981; Thonney et al., 1981; Nocereni et al., 1985).
2. Decreased methane production (Bartley et al., 1979; Fuller and Johnson, 1981; Russell and Martin, 1984).
3. Decreased lactic acid production from carbohydrates (Dennis et al., 1981c) and prevention of experimentally induced lactic acidosis (Nagaraja et al., 1981, 1982).
4. Protein sparing effect by inhibiting proteolysis and deamination (Bartley et al., 1979; Fuller and Johnson, 1981).
5. Selective inhibition of rumen bacteria (Dennis et al., 1981a).
6. Inhibition of rumen protozoa (Dennis et al., 1981b).
7. Prevention of frothy bloat in high-grain feed cattle (Bartley et al., 1982).
8. Reduction of 3-methylindole formation from tryptophan and control of acute bovine pulmonary edema and emphysema (Nocereni et al., 1985).

Despite many similarities, the differences between the two antibiotics appear to be in the degree of rumen fermentation changes induced. Bartley et al. (1979) have reported that the extent of reduction in acetate-propionate ratio was greater with lasalocid than monensin in a batch culture fermentation. Similar difference was observed in VFA production in a continuous fermentation system (Fuller and Johnson, 1981). Methane inhibition was greater with lasalocid than monensin (Bartley et al., 1979). However, Fuller and Johnson (1981) and Russell and

Martin (1984) found no difference in the extent of methane inhibition between the two antibiotics. Fuller and Johnson (1981) reported that lasalocid addition to both high-grain and roughage substrates in a continuous fermentor resulted in 17 to 18% relative reduction in the fraction of nitrogen digested while monensin depresed N digestibility by an average of 16% with high-grain substrate and by 24% with roughage substrate.

Lasalocid has been reported to be much more effective than monensin in inhibiting lactic acid production in vitro (Dennis et al., 1981c) as well as in the prevention of experimentally induced lactic acidosis (Nagaraja et al., 1981, 1982). Dennis et al. (1981a) determined that most of the lactate producing rumen bacteria were sensitive to both lasalocid and monensin. Lasalocid inhibited all strains of S. bovis tested, while one strain (124) was resistant to monensin. None of the lactate-utilizing bacteria was sensitive to lasalocid or monensin. Therefore, it appears the ability of lasalocid to prevent lactic acidosis to a greater degree than monensin must be related to factors other than bacterial sensitivity.

The effect of lasalocid on frothy bloat has been determined (Bartley et al., 1982). Lasalocid reduced legume bloat by 26% and grain bloat by 92%. The effectiveness of lasalocid has been attributed to its unique ability to inhibit the growth of all important strains of S. bovis, a causitive organism in feedlot bloat (Bartley et al., 1975).

Lasalocid has also been shown to prevent acute bovine pulmonary emphysema and edema (ABPE) (Nocerini et al., 1985). In vitro conversion of tryptophan to 3-methylindole and indole by rumen fluid was sharply reduced. Lasalocid effectively reduced ruminal conversion of tryptophan to 3-methylindole and prevented the development of tryptophan induced ABPE in cattle.

SALINOMYCIN

Salinomycin is a monocarboxylic acid polyether ionophore (Fig 3) produced by Streptomyces albus (Kinashi et al., 1973). The cation selectivity has been determined to be K+ > Na+ > Cs+ >> Ca++ (Westley, 1982). The antibiotic possesses broad spectrum anticoccidial activity in chickens (Chappel and Babcock, 1979) and is marketed as a coccidiostat under the trade name Bio-cox^{R}. (Feed Additive Compendium, 1985). Like lasalocid and monensin, the antibiotic is highly effective against gram-positive bacteria including Mycobacterium, but exhibits no activity against gram-negative bacteria (Miyazaki et al., 1974; Liu, 1982).

Influence on cattle performance. Because of the similarity of salinomycin to lasalocid and monensin, studies have been conducted to evaluate its potential as a feed additive. McClure et al. (1980) conducted a 126-d finishing trial with 48 yearling steers and heifers fed a 20% roughage diet supplemented with 0, 16.5, 33.0 and 50.0 g salinomycin per metric ton. Average daily gains were 1.30, 1.57, 1.47 and 1.33 kg and feed intakes were 11.0, 11.0, 10.4 and 10.2 $kg \cdot d^{-1}$ for the cattle fed diets containing 0, 16.5, 33.0 and 50.0 g salinomycin, respectively. Feed efficiency was improved by 21% for the cattle fed salinomycin at 16.5 and 33.0 g/ton and 10% for those fed 50.0 g/ton. Owens et al. (1982) fed salinomycin at 0, 5.5, 11, 22 and 33 ppm of an 89% whole shelled corn and 5% cottonseed hull diet to 140 finishing steers for 110 d. Averaged across salinomycin levels, gain was increased 9.4% and feed efficiency increased 7.8%. Feed intake was increased by a mean of 1% by salinomycin. At the optimum drug level of 11 ppm, gain and feed efficiency were increased by 12.9 and 9.5%. Turgeon et al. (1982) tested salinomycin at 0, 5.5, 11, 16.5 and 22 ppm in cattle fed 79.3% high moisture corn, 10% corn silage and 10.7% supplemented diet. Average daily gain was 1.25, 1.22, 1.20, 1.19 and 1.23 and feed conversion was 7.10, 6.88, 7.04, 7.12 and 6.84 for

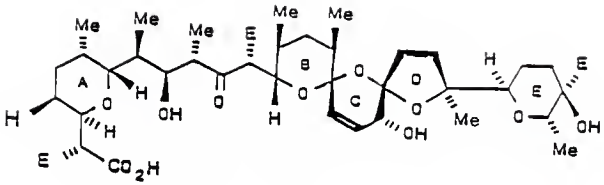

Figure 3. Structure of Salinomycin

cattle fed 0, 5.5, 11, 16.5 and 22 ppm salinomycin, respectively. Ferrell et al. (1983) fed salinomycin at 0.5, 10, 15 and 20 g per ton of a ground corn based diet to 140 finishing steers and heifers. Averaged across salinomycin levels, gain was increased by 2.6% and feed efficiecy was improved by 7.8%. Salinomycin at 15 g/ton produced the optimum level of performance with an increase in rate of gain and feed efficiency of 8.9% and 10.9%, respectively. Zinn and Axe (1983a) conducted a 187-d growing finishing trial involving 150 large frame Okie calves to determine the efficacy of salinomycin fed 0.5, 10, 15 and 20 g/ton airy dry feed. Rate of gain was not influenced significantly however, feed efficiency was by an average of 5.9% at 10 and 20 g/ton salinomycin supplementation.

In a trial involving 105 Angus x Hereford steers (Merchen and Berger, 1985), salinomycin was tested at 0, 5.5, 11, 22 or 33 ppm. Cattle were fed a diet containing 60% corn silage at the beginning of the feeding period with a gradual reduction to 15% corn silage: 85% grain over the first 21 d of a 134-d trial. Salinomycin level had a quadratic effect ($P<.05$) on daily weight gain and resulted in a quadratic ($P<.05$) decrease in feed intake with an average improvement of 10.3% in feed efficiency (table 5).

The effect of salinomycin on performance of cattle grazed on pasture (Barclay et al., 1984; Bagley et al., 1984) and fed corn silage (Turgeon et al., 1983) have been studied. Barclay et al. (1984) evaluated salinomycin levels of 0, 50, 100 and 150 $mg \cdot hd^{-1} \cdot d^{-1}$ in a grazing trial involving 96 yearling steers. Salinomycin was provided in a .9 kg of ground corn per head fed once daily. Daily gain for the 0, 50, 100 and 150 mg treatments was .35, .38, .46 and .41 kg, respectively. Bagley et al. (1984) fed salinomycin at 0, 50, 100 and 150 $mg \cdot hd^{-1} \cdot d^{-1}$ in .9 kg ground corn to yearling crossbred steers grazed on bermudagrass pastures. Daily gains, total gains and final weights were greater for steers fed salinomycin compared to the control diet. The ionophore increased gains by 44 to 53% over

TABLE 5. EFFECT OF SALINOMYCIN ON CATTLE PERFORMANCE

	Control	Salinomycin g/ton 5.0	10.0	15.0	20.0	30.0	Reference
Average daily gain, kg	1.41	1.53	1.59	-	1.52	1.51	Owens et al., 1982
Feed/gain, kg	6.53[a]	6.10[b]	5.91[b]		6.05[b]	6.04[b]	
Average daily gain, kg	1.25	1.22	1.20	1.19	1.23	-	Turgeon et al., 1983
Feed/gain, kg	7.10	6.88	7.04	7.12	6.84	-	
Average daily gain, kg	1.40	1.50	1.50	1.40	1.30	-	Ferrell et al., 1983
Feed/gain, kg	7.29[a]	6.92[c]	6.91[c]	7.01[bc]	7.22[ab]	-	
Average daily gain, kg	1.16	1.11	1.20	1.11	1.15	-	Zinn and Axe, 1983b
Feed/gain, kg	5.75[a]	5.83[a]	5.41[b]	5.58[b]	5.45[b]	-	
Average daily gain, kg	1.27	1.37	1.34	-	1.34	1.27	Merchen and Berger, 1985
Feed/gain, kg	6.79	6.13	6.10	-	5.89	6.23	

[a,b,c]Means in a row with different superscripts differ (P<.05).

control with shifts in ruminal VFA levels (table 6). Turgeon et al. (1983) fed salinomycin at 0, 5.5, 11, 22 and 33 ppm to cattle fed 88% corn silage, 12% soybean meal based supplemented diet. As the dietary level of salinomycin increased, steers gained faster, consumed less feed and became more efficient. Average daily gain was 1.21, 1.21, 1.27, 1.31 and 1.28 kg/d and feed coversion was 8.88, 8.66, 7.87, 7.83, 7.55 for steers fed salinomycin at 0, 5.5, 11, 22, and 33 ppm, respectively.

Comparative effects of salinomycin with lasalocid or monensin on animal performance. Animal performance trials involving dose titration studies have suggested that optimum dosage of salinomycin was 10-15 g/ton of feed (Owens et al., 1982; Ferrell et al., 1983; Turgeon et al., 1983; Zinn and Axe, 1983b; Bagley et al., 1984; Barclay et al., 1984; Merchen and Berger, 1985). It appears that salinomcyin is about two or three times as potent as lasalocid or monensin.

Heinemann (1984) conducted a 112-d feedlot trial involving 108 yearling steers to compare the effects of monensin (300 $mg \cdot d^{-1} \cdot hd^{-1}$), lasalocid (300 $mg \cdot d^{-1} \cdot hd^{-1}$) and salinomycin (100 $mg \cdot d^{-1} \cdot hd^{-1}$) on animal performance and incidence of liver abscesses. The steers were fed 85% grain (17% cracked barley, 83% dry rolled corn) and 15% alfalfa cubes. Steers fed monensin gained slower than lasalocid or salinomycin-fed steers. Steers fed salinomycin gained more efficiently (6.31 kg feed/kg gain) than the controls (6.86) and those fed lasalocid (6.94) and monensin (6.78). Incidence of liver abscesses tended to be lower for steers fed monensin.

TABLE 6. EFFECT OF SALINOMYCIN ON ANIMAL PERFORMANCE AND RUMEN VOLATILE FATTY ACID (VFA) CONCENTRATION IN GRAZING BEEF STEERS[a,b]

Item	Salinomycin ($mg \cdot hd^{-1} \cdot d^{-1}$)				SE
	0	50	100	150	
No. of steers	10	10	10	10	
Average daily gain, kg	.49	.71	.73	.75	.12
Final weight, kg	277	310	317	318	8.6
Propionate, %	16.1	17.1	18.5	19.8	.48
Total VFA, mM	101.2	95.7	94.0	91.0	6.8
Acetate:propionate	4.37	4.23	3.86	3.56	.12

[a]Bagley et al., 1984.

[b]Grazed on bermuda grass pasture.

Martin et al. (1984) conducted a study with yearling steers fed whole shelled corn supplemented with salinomycin (10 g/ton of feed), lasalocid (30 g/ton of feed) or monensin (25 g/ton of feed) plus tylosin (90 mg/head daily). Body weight gains and feed efficiency were greatest for steers fed salinomycin. Performance of steers on monensin-tylosin combinations was similar to that of lasalcoid-fed steers (table 7).

Merchen and Berger (1985) conducted a trial involving 126 Charolais cross steers to compare salinomcyin with that of monensin. The steers were on a diet of 90% concentrate (ground corn) and 10% oat silage. Salinomycin was fed at 5.5, 11, 16.5 adn 22 ppm and monensin at 22 ppm for 111 days. Addition of either ionophore to the diet had no effect on feed intake. Steers fed 5.5 ppm of salinomycin and 22 ppm monensin had more rapid rates of gain than did steers fed the control diet. Steers fed higher levels of salinomycin had rates of gain intermediate to these groups. Addition of either ionophore to the diet had no effect on feed intake. No significant improvements in feed efficiency were noted among treatments.

Effect on rumen fermentation and nutrient digestibility. Fontenot et al. (1980) conducted in vitro experiment to study the effect of 0, 3, 30 or 300 ppm salinomycin with incubation times of 6, 12, 18 and 24 h on VFA level and dry matter digestion. Molar proportion of propionic acid increased and of acetic acid decreased with each increase in level of salinomycin at all incubation times. The effect was smallest at 6 hours. Dry matter digestibility increased at 3 ppm level and decreased at the higher levels. In a subsequent in vitro experiment in which levels of salinomycin varied from 0 to 55 ppm, molar proportions of propionic acid increased and of acetic acid decreased with salinomycin level. In a 61-d experiment conducted with 30 yearling cattle individually fed an 80% concentrate

TABLE 7. STEER PERFORMANCE WITH VARIOUS IONOPHORES AND ANTIBIOTICS

Item	Control	Salinomycin (10 g/ton)	Lasalocid (30 g/ton)	Monensin (25 g/ton) + Tylosin (90 $mg \cdot d^{-1} \cdot hd^{-1}$)
No. of steers	32	32	32	31
Initial weight, kg	342.7	345.0	345.5	349.1
Final weight, kg	482.3^{c}	512.7^{b}	490.5^{c}	498.2^{bc}
Daily gain, kg	1.12^{c}	1.38	1.17	1.32
Daily feed intake, kg	8.67^{c}	9.44^{b}	8.57^{c}	8.86^{bc}
Feed/gain	7.55	6.86	7.37	7.35

[a]Adapted from Martin et al., 1984.

[b,c]Means in a row with different superscripts differ ($P<.05$).

[d]Composition of the diet (%): whole shelled corn 88.14, corn silage 4.00, soybean meal 3.71, cottonseed meal 2.00, limestone 1.00, urea .45, molasses .38, salt .30 and premix .02 (11.8% crude protein).

diet supplemented with 0, 5.5, 16.5, 33.0 and 50 g salinomycin per metric ton, average molar proportion of propionic acid was 32%, 36, 34, 43 and 41%, respectively. Corresponding decreases in molar proportion of acetic, butyric, and isovaleric acids were recorded (Fontenot et al., 1980).

Webb et al. (1980) conducted metabolism steers in 18 yearling steers fed 80% concentrate diet supplemented with salinomycin at 0, .4 and 0.8 $mg \cdot kg^{-1}$ body weight. Crude protein digestibility increased and crude fiber digestibility decreased linearly as salinomycin intake increased. Salinomycin had no affect on nitrogen retention or rumen pH, but decreased rumen ammonia-N levels. Molar proportions of rumen propionate increased and acetate decreased with salinomycin intake. There was a linear decline in butyrate and isovalerate and linear increase in valerate as salinomycin intake increased. A linear decline in methane production was also observed with increased salinomycin intake. Similar shifts in VFA proportions have been reported by others (McClure et al., 1980; Bagley et al., 1984).

Zinn and Axe (1983b) used four steer calves with cannulas in the rumen and proximal duodenum in a 4 x 4 latin square design to test four levels of salinomycin (0, 5, 10 and 15 g/ton air dry feed). Steers were fed a 90% concentrate diet. Total tract digestion of organic matter, fiber, and protein was not significantly altered by salinomycin supplementation. However, ruminal digestion of organic matter was reduced 6.2%. Neither feed protein degradation in the rumen nor microbial efficiency (g microbial N/kg organic matter fermented) was significantly altered. Differences in ruminal molar concentrations of acetate, propionate and butyrate were non-significant. However, molar ratios of acetate and propionate tended to be reduced at the 10 and 15 g/ton levels of salinomycin supplementation. In a sheep digestion trial (Merchen and Berger, 1985), addition of salinomycin at 5.5, 11 or 22 ppm to 60% concentrate diets had no effect on apparent digestibility

of dry matter, organic matter, neutral detergent fiber, acid detergent fiber or starch in comparison with control diets. Apparent nitrogen digestibility was increased in sheep fed salinomycin. Salinomycin did not affect total volatile fatty acid concentrations in the rumen but resulted in a linear increase in molar proportion of propionate and a linear decrease in molar proportions of acetate, butyrate and in acetate: propionate ratios (table 8). The shifts in VFA proportions were fully expressed within 4 d after salinomycin feeding (Merchen and Berger, 1985).

Salinomycin has been tested (Bartley et al., 1982) to determine its effectiveness in preventing bloat. Salinomycin did not control bloat, thus demonstrating that not all ionophores are equally effective in controlling feedlot bloat.

NARASIN

Narasin is a polyether monocarboxylic ionophore produced by Streptomyces aureofaciens (Dorman et al., 1976; Boeck et al., 1977; Berg and Hamill, 1978). The cation selectivity for narasin has been determined to be $Na^+ > K^+$, $Rb^+ > Cs^+ > Li^+$ (Wong et al., 1977). Narasin is structurally similar to salinomycin except for the additional methyl group on the 6-member cyclic either at the carboxylic terminal (Fig 4, Occolowitz et al., 1976; Seto et al., 1977). Narasin was active in vitro against gram-positive bacteria, anaerobic bacteria, fungi and some viruses (Berg and Hamill, 1978). Narasin also possesses broad spectrum activity against coccidial infections in chickens (Weppelman et al., 1977) and has been shown to improve feed efficiency in ruminants (Schaeffer et al., 1975).

Dinusson et al. (1979) evaluated narasin as a feed additive in finishing diets in a trial involving 54 Hereford steers. The diet was dry-rolled corn to appetite, limited corn silage, chopped alfalfa hay and a commercial 32% protein liquid

TABLE 8. EFFECT OF SALINOMYCIN ON RUMEN VOLATILE FATTY ACID (VFA) CONCENTRATION IN SHEEP[a,b]

Item	Salinomycin (ppm) 0	5.5	11	22	SE
Rumen pH	5.87	5.95	5.90	5.93	.05
Total VFA, mM	104.4	98.4	91.9	104.6	4.20
Acetate, %[c]	57.8	55.4	50.4	49.7	2.20
Propionate, %[c]	24.1	28.4	35.3	38.0	.94
Butyrate, %[c]	14.3	11.8	10.8	9.0	1.05
Acetate:propionate ratio	2.48	2.04	1.42	1.32	.15

[a]Merchen and Berger, 1985.

[b]Values are means of samples taken 4 h postfeeding on d 4, 7, 10, 13 and 16 of each period.

[c]Linear effect ($P<.05$).

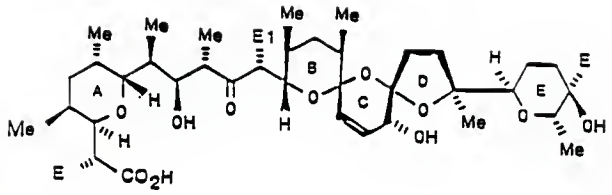

Figure 4. Structure of Narasin

supplement. The antibiotic was added to the supplement to approximate levels of 0, 4 and 12 ppm in the diet. As compared to the control, steers fed 4 ppm narasin gained 9.5% faster required 7.1% less feed dry matter for gain, whereas the steers on 12 ppm narasin gained 12.1% faster and required 11.8% less feed dry matter.

Potter et al. (1979) used 120 cattle to study the effect of narasin upon performance of feedlot cattle fed in group pens or in individual crates for 126 days. Narasin was fed at 0, 2.75, 5.5, 16.5 and 33 ppm. Narasin at all levels had no influence on average daily gain but reduced feed intake and improved feed efficiency in both group fed and individual fed cattle. In a second study, Hereford steers averaging approximately 330 kg were allotted by weight to 10 pens and used to compare the affects of feeding 0, 2, 8, 16 ppm of narasin on performance of feedlot cattle over a 133-d period. Narasin reduced feed intake, improved feed efficiency and increased ruminal molar proportion of propionate (table 9). The data suggested that the optimal dose of narasin was between 8 and 16 ppm.

LYSOCELLIN

Lysocellin, a divalent ionophore (Fig. 5) produced by Streptomyces longwoodensis (Otake et al., 1975), has recently been evaluated as a possible feed additive to improve the growth and feed efficiency of ruminants. Lysocellin has been shown to significantly increase average daily gain and increase feed efficiency in lambs (Wolfrom et al., 1983). Wolfrom et al. (1983) conducted in vitro studies to determine the ability of lysocellin to alter the molar proportions of volatile fatty acids. Lysocellin lowered the molar percentage of acetate and increased the molar percentage of propionate, resulting in a lower acetate:propionate ratio than either the control or the monensin treatments. Calhoun et al. (1983) observed similar responses. They also noted a decrease in the molar percentage of butyrate.

TABLE 9. EFFECT OF NARASIN ON THE PERFORMANCE OF FEEDLOT CATTLE[a]

Item	0	2.0	8.0	16.0
Daily gain, kg	1.27	1.22	1.25	1.17
Dry matter intake, kg/d	11.5	10.3	9.6	9.6
Feed/gain, kg	9.1	8.4	7.8	8.2
Ruminal propionate, %	21.8	23.7	25.4	28.7

[a]Potter et al., 1979.

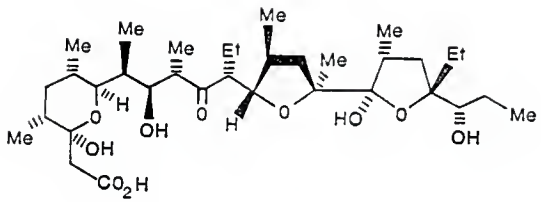

Figure 5. Structure of Lysocellin

LAIDLOMYCIN

Laidlomycin is a monovalent, polyether ionophore antibiotic produced by Streptococcus eurocidicus. The antibiotic is structurally similar to monensin (Kitame et al., 1974; Kitame and Ishida, 1976). The purified form of laidlomycin was inhibitory to various Mycoplasma species, especially against Acholeplasma laidlawii at the concentration of .16 µg/ml, but had no activity against bacteria, fungi or yeast (Kitame et al., 1974). However, the antibiotic was effective in controlling coccidiosis in chickens (Kitame et al., 1974). Acylation of laidlomycin with straight-chain acyl groups from two to 12 carbon atoms tended to improve the potency of the antibiotic (Spires and Algeo, 1983). Laidlomycin butyrate was more potent than monensin in inhibiting lactic acid production and enhancing propionate production in an in vitro fermentation system. In a continuous culture experiment propionic acid production was increased from 22.9 mmol/d in control to 30.5 and 33.7 mmol/d in monensin and laidlomycin treated fermentations, respectively (Spires and Algeo, 1983). In a 56-d trial involving 36 steers average daily gain was 8% better and feed efficiency was improved 20% in steers fed laidlomycin butyrate (33 mg/kg diet) and in monensin-fed steers (33 mg/kg diet) feed efficiency improved by 15% of the control steers (Spires and Algeo, 1983).

ACTAPLANIN

Actaplanin is a glycopeptide antibiotic, similar to avoparcin, produced by a strain of Actinoplanes missouriensis. The antibiotic has been shown to increase propionate production in vitro and in combination with ionophore antibiotics, acta-planin improves efficiency of feed utilization in ruminants (U.S. Patent No. 4,405,609). The interaction of actaplanin and ionophore antibiotics appears to be synergestic (table 10). Gill and Owens (1984) tested the effects of narasin and actaplanin in a trial involving 250 steers fed a high concentrate whole shelled corn

TABLE 10. EFFECTS OF ACTAPLANIN AND NARASIN ON FEEDLOT PERFORMANCE[a]

Item	Control	Narasin + Actaplanin (g/ton) 5 + 8	7.5 + 12	10 + 16
No. of cattle	24	22	31	24
Average daily gain, kg	1.04^{c}	1.10^{bc}	1.14^{b}	1.06^{bc}
Daily feed intake, kg	6.6	6.7	6.8	6.4
Feed/gain	6.42^{b}	6.08^{bc}	5.98^{c}	6.01^{c}

[a]Gill, D.R. and F.N. Owens, 1984.

[b,c]Means in a row with different superscripts differ (P<.05).

grain baled diet for 168 days. Addition of narasin together with actaplanin increased rate of gain slightly and improved feed efficiency (table 10). The lowest levels of both narasin and actaplanin gave benefits equal to that of higher levels. Actaplanin has also been tested as a feed additive to increase level and efficiency of milk production by dairy cows. McGuffy et al. (1983b) fed actaplanin to 64 Holstein heifers to determine lactation performance in an experiment beginning four weeks postpartum. Actaplanin was fed at 0, 400, 800 or 1200 mg daily in a diet commposed of 52.5% corn silage 17.5% alfalfa hay and 30% concentrate. After eight weeks, actaplanin was withdrawn from the diet of half of the heifers in each treatment, while remaining heifers received actaplanin for a complete lactation. Average daily milk production was higher at all levels of actaplanin in both group of heifers. In another study (McGuffy et al., 1983a) involving 399 Holstein cows at seven lacations actaplanin was tested at 0, 400, 800 or 1200 mg per day for 280 days. Milk production and efficiency of production were increased by actaplanin, while percent milk components were decreased by feeding 800 and 1200 mg (table 11).

AVOPARCIN

Avoparcin is a glycopeptide antibiotic (Fig 6) produced by a strain of Streptomyces candidus (Kuntsman et al., 1969). The antibiotic is a water soluble compound and its production, isolation, physiochemical and structural properties have been reported (Kuntsman et al., 1969; Hlavka et al., 1974; McGahren et al., 1980). The antibiotic possesses a strong affinity for the cell walls of gram-positive bacteria and disrupts peptidoglycan synthesis by inhibiting the incorporation of N-acetyl glucosamine and is ineffective against gram-negative bacteria (Redin and Dornbush, 1968). Avoparcin is not active against all gram-positive bacteria. Walton (1978) noted that avoparcin was not inhibitory to some gram-positive enteric

TABLE 11. EFFECT OF ACTAPLANIN ON MILK PRODUCTION AND EFFICIENCY[a]

Item	Actaplanin, mg/d			
	0	400	800	1200
Milk, kg/d	23.3[b]	24.3[c]	24.6[c]	24.4[c]
Fat, %	3.78[b]	3.69[bc]	3.58[cd]	3.51[d]
Protein, %	3.46[b]	3.44[b]	3.38[c]	3.36[c]
Total solids, %	12.62[b]	12.48[bc]	12.41[cd]	12.30[d]
Dry matter intake, kg/d	18.3	18.4	18.3	18.0
Milk/DMI	1.30[c]	1.35[bc]	1.38[b]	1.39[b]

[a]McGuffy et al., 1983b.

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

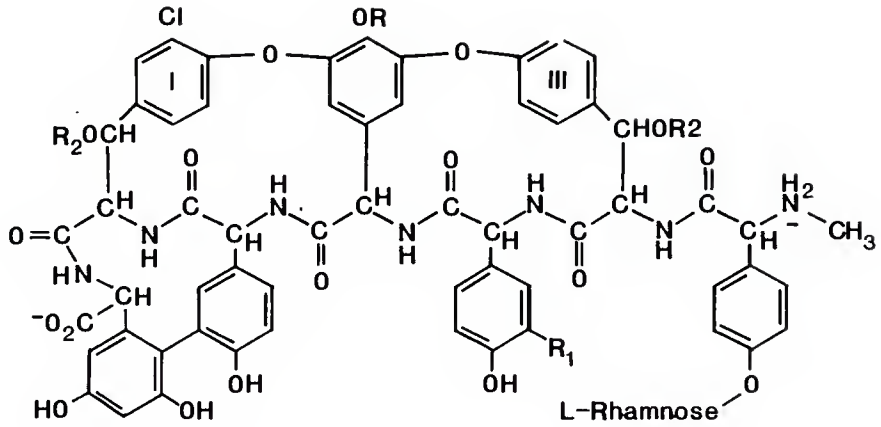

Figure 6. Structure of Avoparcin

streptococci and pharyngeal staphylococci in chickens and Dutta and Devriese (1981) have described a number of lactobacilli against which avoparcin had no activity. Stewart et al. (1983) studied the effect of avoparcin on pure and mixed cultures of rumen bacteria. Of the gram-negative bacteria tested, all except B. succinogenes were able to grow in the presence of 200 μg avoparcin ml^{-1}. Among the gram-positive bacteria, L. acidophilus and L. casei were the most highly resistant. Those bacteria which often stain gram-negative but possess cell walls with gram-positive structure such as B. fibrisolvens, L. multiparus and the ruminococci were all among the most sensitive organisms. However, avoparcin had no effect on the in vitro digestion of dried grass and straw.

Avoparcin has been shown to be a growth promotant in broiler chickens (Leeson et al., 1980), turkeys (Leeson and Summers, 1981) and in pigs (Schneider et al., 1979). Avoparcin has been used extensively in the feed of broiler chickens and pigs under commercial conditions in the United Kingdom (trade name 'Avotan') and Europe since 1976 to improve rate of live-weight gain and efficiency of feed conversion, and since 1981 in turkey feeds for the same purposes.

Since the last 5 to 6 years avoparcin has been tested as a possible feed additive for ruminants. Ingle et al. (1978) demonstrated from in vitro studies that avoparcin decreased the acetate:propionate ratio without altering the production of total VFA. Addition of avoparcin at 100-1000 ppm to an in vitro rumen fluid incubation system depressed acetate:propionate ratio by 20-200%. In another study rumen samples were collected from three groups of six steers each fed a 60% roughage diet containing 0, 33 and 99 ppm avoparcin. The acetate:propionate ratio and percent change from control for the samples taken from 0, 33 and 99 ppm avoparcin fed steers were 3.74, 0%; 2.80, 23%; and 2.08, 44% at 28 days and 3.39, 0%; 2.54, 21%; and 2.08, 39% at 56 days, respectively. Over the 56-day period avoparcin supplementation decreased feed intake and improved feed conversion

(Ingle et al., 1978). Further studies conducted in the United States and Europe have confirmed the effect of avoparcin on rumen fermentation (Johnson et al., 1979; Dyer et al., 1980; Chalupa et al., 1981; Macgregor and Armstrong, 1982; and Froetschel et al., 1983) and on the performance of beef cattle (Embry et al., 1979; Johnson et al., 1979; Sherrod et al., 1979; Dyer et al., 1980; Owens and Gill, 1981; Mudd and Smith, 1982; Cuthbert et al., 1984).

Froetschel et al. (1983) studied the effect of avoparcin on ruminal propionate production and amino acid degradation by using four rumen-fistulated wethers. Wethers were fed each of four diets during 28-day peirods in a 4 x 4 Latin square design. Diets were high fiber, high-fiber plus 50 ppm avoparcin, low fiber and low-fiber plus 50 ppm avoparcin. Propionate production was determined by isotope dilution technique (single injection). Avoparcin decreased total VFA concentration, increased ruminal pH and molar proportion of propionate on both high fiber and low-fiber diets (table 12). Daily propionate production and pool turnover rate increased in sheep fed avoparcin. Avoparcin decreased ruminal ammonia (50 vs 45 mg/100 ml) and increased α-amino nitrogen (8.6 vs 14.3 mM) in low fiber fed sheep. They concluded that avoparcin modifies rumen fermentation by increasing propionate production and inhibiting protein or amino acid degradation.

The effect of avoparcin on feedlot performance is equal to or superior to that of monensin (Johnson et al., 1979; Dyer et al., 1980; Owens and Gill, 1981). Johnson et al. (1979) used 150 yearling steers to evaluate the effect of 0, 16.5, 33 and 60 ppm avoparcin and 33 ppm monensin in the feed on growth rate, feed efficiency, and the concentration of volatile fatty acids in the rumen. Avoparcin at all levels improved feed efficiency and produced daily gains that were greater than untreated control or monensin treated steers (table 13). Ruminal propionate increased due to avoparcin ingestion. Dyer et al. (1980) used 150 crossbred yearling heifers fed a 77% barley diet for 140 days to evaluate the effect of 0, 33, 49.5

TABLE 12. EFFECT OF AVOPARCIN ON RUMEN VOLATILE FATTY ACID (VFA) CONCENTRATION IN SHEEP[a]

Item	High-fiber diet[b]		Low-fiber diet[b]	
	Control	Avoparcin (50 ppm)	Control	Avoparcin (50 ppm)
Total VFA, mM	124.0	110.9	112.0	88.9
Acetate, %	62.8[c]	61.8[c]	56.9[d]	57.5[d]
Propionate, %	19.0[c]	23.2[d]	19.5[c]	23.9[d]
Isobutyrate, %	1.4	1.5	1.6	1.9
Butyrate, %	13.4[c]	10.7[d]	16.1[c]	12.6[d]
Isovalerate, %	2.1	1.6	3.5	2.5
Valerate, %	1.3	1.3	2.3	1.6
Acetate:Propionate ratio	3.4[c]	2.7[d]	3.1[c]	2.5[d]

[a]Values are means of 72 observations collected from 4 sheep. Froetschel et al., 1983.

[b]Composition of the diets (%): High-fiber diet - alfalfa pellets 71, corn 24, molasses 4.5, trace mineral salt 0.5, low-fiber diet - alfalfa pellets 25, corn 50, soybean meal 20, molasses 4.5, trace mineral salt 0.5 (crude protein 19.0 and 22.1 respectively).

[c,d]Means within a row with unlike superscripts differ significantly ($P<.05$).

TABLE 13. EFFECT OF AVOPARCIN AND MONENSIN ON STEER PERFORMANCE[a]

Item	Control	Avoparcin			Monensin
		16.5 ppm	33.0 ppm	66.0 ppm	33.0 ppm
No. of steers	29	30	30	30	30
Initial weight, kg	272.40	273.3	273.8	274.8	275.8
Final weight (112 d), kg	404.1	407.2	415.5	418.9	399.0
Daily gain, kg	1.18^{bc}	1.19^{cd}	1.27^{cd}	1.28^{d}	1.10^{b}
Daily feed intake, kg[e]	10.55^{b}	10.37^{bc}	10.62^{b}	10.54^{b}	9.87^{c}
Feed/gain	8.97^{b}	8.71^{bc}	8.38^{cd}	8.19^{d}	8.97^{b}

[a]Adapted from Johnson et al., 1979.

[b,c,d]Means in a row with unlike superscripts differ (P<.05).

[e]Composition of the diet (%): alfalfa pellets 50.0, steam rolled barley 44.4, salt .50, trace minerals .05, vitamin A premix .05, fine ground corn 4.87 and antibiotic premix 0.02.

and 66 ppm avoparcin and 33 ppm monensin on growth rate feed efficiency and ruminal VFA concentation. Average daily feed intake for cattle fed 49.5 ppm avoparcin or 33 ppm monensin was lower than that for cattle fed the control diet but not different from average intake for heifers fed cattle 33 or 66 ppm avoparcin. Since there were no treatment differences in average daily gain, the lower feed intake by heifers given 49.5 ppm avoparcin indicated that the level of avoparcin was near to optimal dose for cattle. Feed efficiency at each level of avoparcin was higher than that of the control but no difference in feed efficiency between control and monensin-fed heifers was observed. Although there was no difference in rumen VFA concentration between treatments, there was a trend for lower acetate and higher propionate with increased level of avoparcin. Owens and Gill (1981) have reported that avoparcin fed at 60 g per ton of feed decreased feed intake by 11.5% and rate of gain by 6.8% for a feed efficiency improvement of 5.1%. Similar values for monensin at 30 g per ton of feed were a 4.6% decrease in feed intake, a 1.5% increase in rate of gain and a 6.0% improvement in feed efficiency. Fecal pH tended to be lower and fecal starch higher with avoparcin feeding (Owens and Gill, 1981).

THIOPEPTIN

Thiopeptin, a sulfer containing peptide antibiotic (Fig 7) produced by Streptomyces tateyamensis (Miyairi et al., 1970), exhibits strong antimicrobial activity against gram-positive microorganisms (Miyairi et al., 1972). The antibacterial activity is because of inhibition of protein synthesis by blocking elongation of peptide chain on the 505 ribosomal unit (Liou et al., 1976). Thiopeptin supplemented feeds have been shown to improve weight gain, feed efficiency in chickens and swine and the egg performance of layers (Mine et al., 1972). Muir and Barreto (1979) evaluated the ability of various antibiotics including

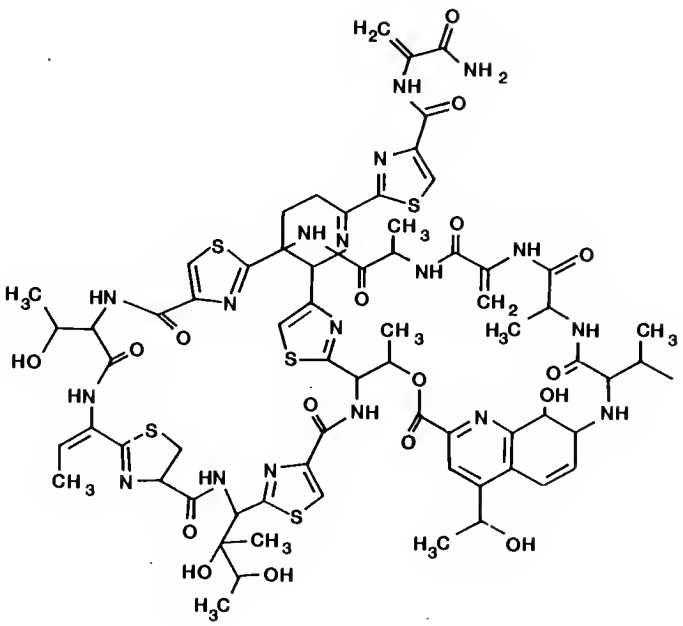

Figure 7. Structure of Thiopeptin

thiopeptin to inhibit S. bovis. Of the antibiotics evaluated thiopeptin was among the most effective inhibitor of S. bovis Thiopeptin's effect on S. bovis prompted the researchers to investigate its potential at controlling lactic acidosis. Muir et al. (1980b) determined the efficacy of thiopeptin in preventing lactic acidosis in lambs challenged by intraruminal administration of ground wheat (40 g/kg body weight). Thiopeptin given as a single dose prevented lactic acidosis by reducing rumen lactate 80 to 90%. In addition thiopeptin permitted normal rumen fermentation to continue as indicated by a significant increase in volatile fatty acids. The minimum effective dose of thiopeptin to control acute lactic acidosis was .18 mg/kg body weight. Similar protection from induced lactic acidosis with thiopeptin supplementation was also observed by Kezar and Church (1979). Because acidosis is frequently encountered when high concentrate diets are fed to feedlot cattle, supplementation of thiopeptin may improve performance and feed efficiency by preventing clinical and subclinical acidosis. Gill et al. (1979) fed thiopeptin (11 ppm) with a high-concentrate rolled corn diet in a factorial experiment with two roughage levels (15 and 5% cottonseed hulls) and two diet adaptation schemes (diet changes from 40 to 20% roughage and to the final roughage level at 6- or 12-d intervals) to 111 growing steers. Thiopeptin supplementation increased rate of gain by 5.3% and feed efficiency by 7.8% over the 129-day trial (table 14). Incidence and severity of liver abscesses tended to be higher in thiopeptin supplemented steers. Muir et al. (1980a) evaluated thiopeptin at 0, 2.75, 5.5, 8.25, 11, and 22 ppm in the feed in 8-week growth trials involving 252 lambs. An abrupt shift to micronized milo at the start of the trials was used to provide a lactic acidosis challenge. Five of 78 control lambs died within 48 h after the challenge. In lambs fed diets containing thiopeptin at 11 ppm or more, there was no evidence of lactic acidosis. Lambs given thiopeptin at 11 ppm or more ate 11% more and gained 20% more than control during the 8-week trial. Incidence of death was lower among

TABLE 14. EFFECT OF THIOPEPTIN ON CATTLE PERFORMANCE[a]

Item	Thiopeptin level, g/ton		SE
	0	11	
No. of steers	55	56	
Initial weight, kg	301	300	
Daily gain (129 d), kg	1.38	1.45	.025
Daily feed intake, kg[d]	8.93	8.73	.118
Feed/gain	6.48[b]	6.00[c]	.073
Liver abscesses	29.5	35.7	8.3

[a]Adapted from Gill et al., 1979.

[b,c]Means in a row with different superscripts differ significantly.

[d]Composition of the diet (%): Rolled corn 73.8, cottonseed hulls 15.0, alfalfa meal 4.0, cane molasses 4.0, cottonseed meal 4.5, limestone .75, urea, .5, dicalcium phosphate .15, salt .3, vitamin A .015 and thiopeptin premix (11.8% crude protein).

lambs given thiopeptin at 2.75 to 8.25 ppm but these animals showed no improvement in performance. In another study, forty angus steers were tested for the effect of thiopeptin on performance following a lactic acidosis challenge (Muir et al., 1981). Steers that were abruptly shifted to micronized milo developed ruminal lactic acidosis. Thiopeptin at 11 ppm in the feed prevented lactic acidosis and improved weight gain, feed intake and efficiency of feed utilization at 2 weeks. At 4 weeks steers fed thiopeptin were still performing better than controls, but the differences were not statistically significant. The efficacy of thiopeptin in preventing lactic acidosis was compared with that of lasalocid or monensin by Nagaraja et al. (1982). Lasalocid, monensin or thiopeptin was administered intraruminally each at .33, .65 or 1.3 mg/kg body weight. Four rumen-fistulated cattle were used for each dosage level and the design was a 4 x 4 Latin square, with each animal receiving lasalocid, monensin, thiopeptin or no antibiotic. Acidosis was reduced by intraruminal administration of glucose at 12.5 g/kg body weight. At all three dosage levels cattle given lasalocid had higher rumen pH and lower lactate concentration than did control cattle or cattle given monensin or thiopeptin. Cattle given monensin had a slightly higher rumen pH and a lower lactate concentration than the controls only at the .65 and 1.3 mg/kg body weight dosages, whereas thiopeptin was effective only at 1.3 mg dosage. Colony counts of Streptococcus bovis and Lactobacillus were significantly reduced in rumen fluid of cattle given 1.3 mg antibiotic/kg body weight. Counts of lactate-utilizing bacteria increased in both control cattle and cattle given antibiotics.

TYLOSIN

Tylosin is a macrolide antibiotic (Fig 8) produced from the fermentation of a strain of Streptomyces fradiae (Berkman et al., 1961). Macrolides contain a large lactone ring (12-16 atoms) having few double bonds and no nitrogen atoms; in

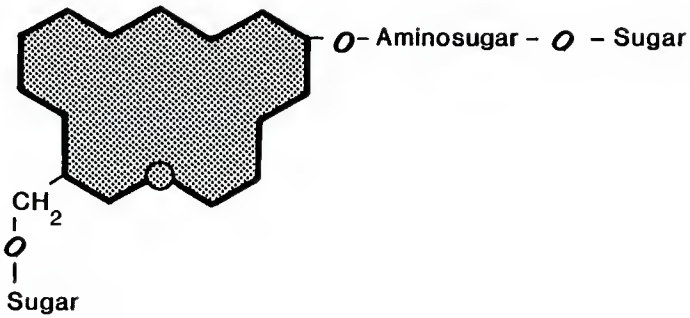

Figure 8. Structure of Tylosin

addition, the ring is substituted with one or more sugar residues, some of which may be amino sugars. The antibiotic has in vitro activity against many gram-positive bacteria, some gram-negative bacteria (not coliforms), spirochetes and most species of mycoplasma (McGuire et al., 1961). The mechanism of antibacterial action of tylosin, like other macrolide antibiotics is believed to be due to inhibition of bacterial protein synthesis possibly by binding with ribosomes (Vazquez, 1967). The usefulness of tylosin as a therapeutic agent against many diseases of animals has been established by experimental studies and clinical evaluations (Sampson et al., 1974a,b,c, 1975; Matsuoka et al., 1980, 1983).

Tylosin is commercially available under the trade name Tylan^{R} (Elanco Products Co., Indianapolis, IN) and is approved in chickens and swine to increase rate of weight gain and improved feed efficiency. Tylosin is also recommended for use in the control of chronic respiratory disease caused by Mycoplasma gallisapticum and in the prevention of swine dysentary (vibrionic). In beef cattle tylosin is fed at 8-10 g/ton of feed for the reduction of incidence of liver abscesses caused by Fusobacterium necrophorum and Corynebacterium pyogenes. Tylosin is also indicated for use in the treatment of bovine respiratory complex associated with pasteurella multocida and corynebacterium pyogenes, foot rot and diphtheria caused by Fusobacterium necrophorum and metritis caused by Corynebacterium pyogenes in beef cattle. (Feed Additive Compendium, 1985).

Akkad and Hobson (1966) tested the antibacterial activity of tylosin towards certain species of rumen bacteria. Gram-positive bacteria like S. bovis, L. bifidus and L. fermenti were sensitive and gram-negative bacteria like M. elsdenii, Veillonella gazogenes, B. amylophilus and S. ruminatium were resistant. R. albus and B. fibrisolvens that have gram-positive cell wall structure were also sensitive. Wang et al. (1969) tested 15 species of rumen bacteria against tylosin in concentrations ranging from 0.1 to 200 µg/ml in an anaerobic tube dilution system.

R. flavefaciens, E. limosum and a Spirillum species were most sensitive to tylosin (MIC 1 μg/ml) and B. ruminicola, B. succinogenes and R. albus were moderately sensitive (MIC 10 μg/ml). B. fibrisolvens, E. ruminantium, L. multiparus, M. elsdenii, S. bovis, Succinimonas amylolytica and Succinivibrio dextrinosolvens were sensitive only at high concentration (MIC $\geq$ 100 μg/ml), whereas B. amylophilus and S. ruminantium were completely resistant to tylosin. Although the major lactate-producing bacteria S. bovis and Lactobacillus species were resistant to tylosin, Beede and Farlin (1977) reported that in an in vitro fermentation system tylosin reduced lactate production.

The extensive use of tylosin in feedlot cattle is for the reduction of the incidence of liver abscesses. It is routinely used in combination with monensin. Liver abscesses in cattle are part of a disease complex where the abscessation is secondary to primary foci of infection in the rumen epithelium (Jensen et al., 1954). Ruminal lesions induced by increased acidosis that follows rapid change in the diet from high roughage to highly fermentable carbohydrate or prolonged feeding of high-grain diet (Brent, 1976) permit rumen bacteria to penetrate into the hepatic portal venous system, which are then carried to the liver. Numerous studies on the bacterial flora of bovine liver abscesses (Scanlan and Hathcock, 1983) have suggested, F. necrophorum (a normal inhabitant of the rumen) alone or in combination with Corynebacterium pyogenes as the main etiologic agents. Liver absecess in feedlot cattle is a serious economic problem in the cattle industry. The incidence of abscessed livers in grain-fed cattle varies from as low as 4 to 5% to as high as 90%. It is not uncommon to find 25 to 30% abscessed livers in feedlot cattle at slaughter (Farlin, 1980). In addition to the loss of liver at slaughter, reduced weight-gain and feed efficiency in cattle with abscessed livers compared to cattle with healthy livers account for the economic loss (Goodrich et al., 1976).

Brown et al. (1973) conducted four 4 feedlot experiments with 774 cattle on high concentrate diets to evaluate two forms of tylosin (tylosin phosphate and tylosin urea adduct) fed at 0, 50, 75 and 100 $mg \cdot hd^{-1} \cdot d^{-1}$. Continuous administration of tylosin of both forms at three levels reduced the incidence of liver abscesses (24.1% control vs 4.2% treated), increased average daily gain (1.01 kg control vs 1.07 kg treated) and improved feed efficiency (7.65 control vs 7.41 treated). In another study involving 1829 cattle, Brown et al. (1975) compared the effect of 75 mg tylosin with that of 70 mg chlorotetracycline on the incidence of liver abscesses. Continuous administration of these antibiotics reduced the incidence of liver abscesses and improved average daily gain and feed conversion (table 15).

VIRGINIAMYCIN

Virginiamycin, a mixutre of two distinct antibiotic entities (Fig 9) that are synergistic in their antimicrobial effect directed primarily against gram-positive microorganisms, is produced by Streptomyces virginiae (Cocito, 1979). Cocito (1979) characterized virginiamycin as non-toxic, poorly absorbed from the intestinal tract, and highly biodegradable in animal waste. Virginiamycin improves feed utilization and growth rate for non-ruminating animals. Growth studies in poultry (March et al., 1978), veal calves (Parigi-Bini and Chiericato, 1979) and swine (Cromwell et al., 1981; Pelura et al., 1980) indicate virginiamycin is a positive factor in the production of non-ruminating livestock.

Virginiamycin is primarily active against gram positive bacteria (Cocito, 1979). Virginiamycin has been shown to control necrotic enteritis infection in chickens, the causitive agent of which is the gram-positive Clostridium perfringens. Virginiamycin has been reported to control swine dysentery and inhibit the growth

TABLE 15. EFFECT OF TYLOSIN AND CHLORTETRACYCLINE ON THE INCIDENCE OF LIVER ABSCESSES IN FEEDLOT CATTLE[a]

	Control	Tylosin 75 mg/d	Chlortetrocycline 70 mg/d
Incidence of liver abscesses, %	56.1	18.6	44.2
Improvement over control, %		66.9	21.3
Average daily gain, kg	1.10	1.17	1.14
Improvement over control, %		+5.8	+3.3
Feed/gain	8.21	8.14	7.87

[a]Brown et al., 1975.

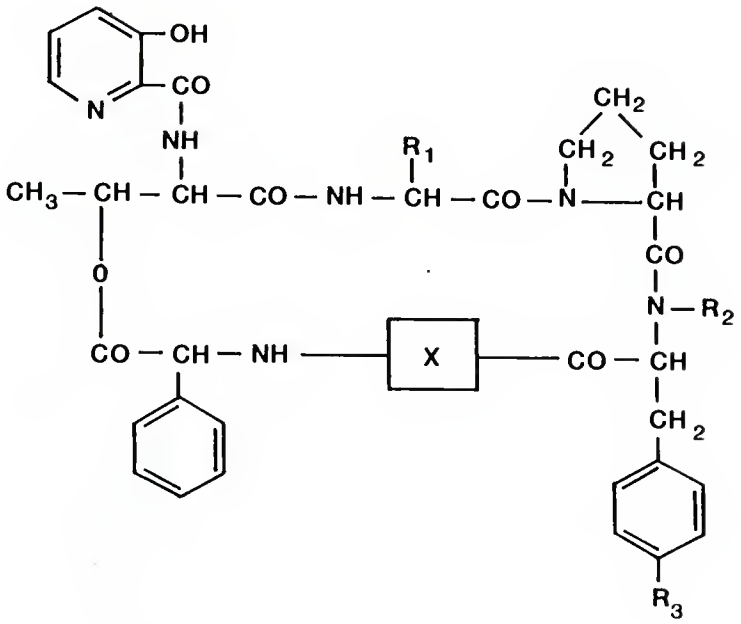

Figure 9. Structure of Virginiamycin

of Treponema hyodysenteriae, the primary cause of swine dysentery (Williams and Babcock, 1976; Williams and Shively, 1978).

In vitro studies (R.E. Hedde, Personal Communication) found that virginiamycin shifts volatile fatty acid production proportions toward propionate with a corresponding reduction in butyrate. They concluded that virginiamycin has selective effects on rumen ecology resulting in fermentation changes similar to ionophores in volatile fatty acid effect. Virginiamycin was specifically inhibitory to D(-) and L(+) lactic acid producing bacteria (Streptococcus bovis and Lactobacillus ruminis) but not to lactate-utilizing bacteria (M. elsdenii) selective inhibition of lactic acid production by virginiamycin was noted during in vitro glucose fermentation in a nonbuffered inoculum derived from roughage-fed cattle. Virginiamycin at 30 ppm in the diet protected against increased production of rumen lactic acid during the change from roughage to concentrate diet in cattle. Rumen L(+) lactate concentration increased in the control treatment but not in the virginiamycin treatment following 35 days of continuous concentrate feeding. Also, virginiamycin appeared to increase feed intake during concentrate feeding (R.D. Hedde, Personal Communication).

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EFFECTS OF ANTIMICROBIAL FEED ADDITIVES ON RUMEN BACTERIA AND IN VITRO LACTIC ACID AND VOLATILE FATTY ACID PRODUCTION

by

Mitchell Brian Taylor

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ABSTRACT

Sensitivity and resistance of rumen bacterial species to avoparcin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin and two new ionphore antibiotics, RO22-6924/004 and RO21-6447/009, were determined. Generally, antimicrobial compounds were inhibitory to gram-positive bacteria and those bacteria that have gram-positive-like cell wall structure. Minimum inhibitory concentration (MIC) ranged from 0.09 to 24.0 μg/ml. Gram-negative bacteria were resistant at the highest concentration tested (48.0 μg/ml). Based on the fermentation products produced, rumen bacteria that produce lactic acid, butyric acid, formic acid or hydrogen were sensitive and bacteria that produce succinic acid or ferment lactic acid were resistant to the antimicrobial compounds. Selenomonas ruminantium was the only major lactic acid-producing bacteria sensitive to all the antimicrobial compounds tested. Avoparcin and tylosin appeared to be less inhibitory (MIC > 6.0 μg/ml) than the other compounds to the two major lactic acid-producing bacteria, Streptococcus bovis and Lactobacillus sp. Ionophore compounds seemed to be more inhibitory (MIC 0.09 - 1.50 μg/ml) than the non-ionophore compounds (MIC 0.75 - 12.0 μg/ml) to the major butyric acid-producing bacteria. Treponema bryantii, an anaerobic rumen spirochete was less sensitive to virginiamycin than to the other antimicrobial compounds. It appears that minimum inhibitory concentration is not a good indicator of the potency of the antimicrobial compounds in altering rumen fermentation characteristics.

Batch culture fermentations were used to determine the effect of avoparcin, lasalocid, monensin, narasin, salinomycin, thiopeptin, tylosin, virginiamycin and two new ionophore compounds (RO22-6924/004 and RO21-6447/009) on lactic acid and volatile fatty acid production. Preliminary experiments were conducted with salinomycin to determine the effects of incubation time and rumen fluid inoculum source on lactic acid and VFA

production. Maximum inhibition of lactic acid by salinomycin was at 6 h incubation, but 12 h incubation showed a more graded response to antibiotic concentration. Lactic acid inhibition by salinomycin was unaffected by rumen fluid inoculum source. All antimicrobial compounds were effective in inhibiting lactic acid production. Among the ionophores, narasin and salinomycin were more inhibitory than others. Monensin and tylosin in combination was more effective than monensin alone. Maximum alternations in VFA production by salinomycin were obtained in fermentations incubated for 12 h with rumen fluid inoculum from low-grain fed cattle. In general, total VFA concentration was unaffected by antimicrobial compounds except that of RO22-6924/004, tylosin and virginiamycin which caused a reduction at high concentrations. The acetate proportion was not affected by avoparcin, RO22-6924/004, RO21-6447/009, lasalocid, monensin, narasin and salinomycin. However, tylosin, monensin and tylosin in combination, thiopeptin and virginiamycin at high concentrations (> 6.0 µg/ml) increased the acetate proportion. All compounds increased the molar proportion of propionate. Tylosin and virginiamycin at high concentrations (> 6.0 µg/ml) decreased the proportion of propionate. Monensin and tylosin combination had no effect on propionate portion. Narasin and salinomycin were the most effective among the compounds tested, in enhancing propionate production. Ionophore antibiotics were more inhibitory to butyrate production than the nonionophore compounds. Molar proportions of isobutyrate, isovalerate and valerate were generally not affected by the addition of antimicrobial compounds.