RUMEN MICROBIAL CHANGES IN CATTLE FED HIGH GRAIN DIETS WITH OR WITHOUT IONOPHORE ANTIBIOTICS

BY

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GENERAL INTRODUCTION

The constant efforts to produce food from animal sources more efficiently at a lower cost to the consumer has stimulated a continued search for a more suitable combination of nutrients and new additives which will increase the efficiency in growth rate and level of production of animals. Ruminant feeds are subjected to fermentative digestion by rumen microorganisms and hydrolytic digestion by the animal's own enzyme systems. Microbial digestion is beneficial for substances that cannot be digested otherwise or have poor nutritional qualities, but fermentation of dietary protein, starch and sugars and products of fermentation such as sugars and amino acids are accompanied by losses in both energy and amino nitrogen. Therefore, a proper balance of fermentative and hydrolytic digestion is required for optimal efficiency of producing food from fiber with ruminants. A long sought objective of ruminant nutritionists has been to manipulate and improve the efficiency of rumen fermentation. Such beneficial shift in the rumen fermentation should enhance the overall productive efficiency of ruminants. Initial attempts to achieve this goal were restricted to dietary manipulations but during the last decade, a number of chemicals have been discovered that induce fermentation changes and hence improve efficiency of production of ruminants. The alteration in rumen fermentation due to these manipulations are attributed to changes in the microbial ecosystem. Among chemical feed additives that have received wide attention are compounds that belong to a class of antibiotics called polyether ionophores.

The objective of these studies was to compare rumen microbial changes in cattle fed high grain diets with those in cattle fed the same diet supplemented with ionophore antibiotics. The results of these studies are presented in three sections.

RUMEN MICROBIAL CHANGES IN CATTLE FED HIGH GRAIN DIETS SUPPLEMENTED WITH EITHER LASALOCID OR MONENSIN

Summary

Six rumen-fistulated cattle, randomly assigned to three groups (control, lasalocid-fed and monensin-fed) were used to monitor the changes in rumen microbial populations and volatile fatty acids (VFA) concentrations associated with feeding lasalocid or monensin (.66 $mg \cdot kg^{-1} \cdot d^{-1}$). Cattle were adapted to alfalfa hay and grain diet (50:50) before supplementing the diet with lasalocid or monensin and then the diet was changed to 40:60, 30:70 and 20:80 alfalfa hay to grain ratio. Rumen fluid samples for total and selective enumeration of anaerobic bacteria, VFA analysis and for counting protozoa were collected at 50:50 alfalfa hay-grain diet before feeding the antibiotics (pretreatment samples), 50:50, 40:60, 30:70, and 20:80 hay-grain diet with antibiotics (treatment samples) and 20:80 hay-grain diet without antibiotics (posttreatment samples). Lasalcoid or monensin had no effect on rumen pH but decreased total VFA concentration and acetate-propionate ratio. Total anaerobic bacterial counts and the proportions of amylolytic and lactate-fermenting bacteria increased whereas xylanolytic and pectinolytic bacterial counts decreased with increased grain intake. Although there was an initial decline in the total bacterial counts, lasalocid and monensin had no significant effect on total anaerobic, cellulolytic, xylanolytic, pectinolytic and lactate-utilizing bacterial counts. The proportion of amylolytic bacteria was consistently higher in antibiotic-fed cattle than the control cattle. Lasalocid and monensin had no effect on total protozoal number, but holotrichs appeared to be more sensitive to monensin than lasalocid and among the entodiniomorphs, Ophryoscolex was extremely sensitive to monensin.

Introduction

Lasalocid (Bovatec®, Hoffman-La Roche Inc., Nutley, NJ) and Monensin-sodium (Rumensin®, Elanco Products Co., Indianapolis, IN) are polyether ionophore antibiotics widely used as feed additives in cattle. Feed efficiency and increase in daily weight gains have been reported in feedlot cattle and cattle on pasture, fed lasalocid- or monensin-supplemented diets (Brandt, 1982; Goodrich et al., 1984). Improved animal performance is attributed, in part, to altered rumen microbial fermentation (Bartley and Nagaraja, 1982; Bergen and Bates, 1984; Schelling, 1984).

The effects of lasalocid or monensin on rumen microorganisms have been studied in vitro, mainly with isolated or mixed cultures of rumen bacteria (Slyter, 1978; Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). These studies have shown that most starch-hydrolyzing and lactate-utilizing rumen bacterial species were resistant and many of the cellulolytic, xylanolytic and pectinolytic bacteria were sensitive to lasalocid and monensin. However, Bacteroides species, a major cellulolytic, xylanolytic and pectin-utilizing rumen bacteria, though sensitive, rapidly develop resistance to lasalocid or monensin (Chen and Wolin, 1979). It is believed that altered rumen fermentation in lasalocid- or monensin-fed cattle is due to selection of antibiotic-resistant microbial population in the rumen (Chen and Wolin, 1979; Dennis et al., 1981). In cattle, monensin supplementation had no effect on total viable colony counts (Dawson and Boling, 1983), total bacterial and protozoal numbers in the rumen (Dinius et al., 1976). Dawson and Boling (1983) found that the anaerboic bacteria resistant to monensin were higher in cattle receiving the monensin supplemented diet than the control.

Changes in the microbial numbers in the rumen of cattle fed lasalocid- or monensin-supplemented diets have not been thoroughly investigated. The purpose of

this study was to monitor the microbial changes in the rumen of cattle fed lasalocid- or monensin-supplemented diets.

Materials and Methods

Animals and Diet. Six rumen-fistulated mature cattle were randomly assigned to three groups. One group received lasalocid at .66 $mg \cdot kg^{-1} \cdot d^{-1}$, the second group received monensin at .66 $mg \cdot kg^{-1} \cdot d^{-1}$ and the third group that received no antibiotics served as control. The cattle were fed alfalfa hay (IFN 1-00-096) and grain diet composed of 40.6% cracked corn (IFN 4-02-931), 40.6% grain sorghum (IFN 4-05-643), 17.5% soybean meal (IFN 5-04-604), .5% dicalcium phosphate (IFN 6-01-080), .5% trace mineral salt and .2% vitamins A and D supplement containing 1,000,000 I.U. vitamin A and 5,000,000 I.U. vitamin D per 454 g. The cattle were adapted to a 50:50 alfalfa hay-grain diet for four weeks before feeding the antibiotics. Lasalocid (Bovatec premix, 68 g/454 g) or monensin (Rumensin premix, 60 g/454 g) was hand mixed with the grain portion of the diet and fed twice daily at .33 $mg \cdot kg^{-1}$ body weight. The diet was gradually switched from 50:50 to 40:60, 30:70 and 20:80 alfalfa-hay ratio, repectively. The diet change was made every two weeks.

Sampling schedule and processing of samples. Rumen samples were collected twice (pretreatment samples) at two week intervals before feeding the antibiotics. Thereafter, samples were collected every two weeks following each diet change until the animals were on 20:80 alfalfa hay-grain diet, when two more samples were collected at one month interval (treatment samples). The antibiotics were then withdrawn from the feed for one month and two more samples were collected at two week intervals (post-treatment samples). Rumen samples were collected 3 h

post-morning feeding. The rumen contents were hand mixed, as thoroughly as possible, before collecting about 500 ml into a rubber-stoppered flask and transported immediately to the laboratory.

An aliquot of the rumen sample was flushed with oxygen-free CO_2 and blended in a Waring blender for one minute and strained through four layers of cheesecloth. The strained rumen fluid was used to inoculate various culture media, and for volatile fatty acids (VFA) and dry weight determinations. The rest of the unblended rumen sample was strained through one layer of cheesecloth and used to record pH and for counting rumen ciliates.

Culture Media. The following culture media were used to enumerate rumen bacteria: complete carbohydrate agar (CCA), selective carbohydrate agar (SCA) media. The SCA media contained xylan/xylose, pectin, soluble starch or lactate as the single energy sources. A cellulose broth was used to enumerate rumen cellulolytic bacteria. Incubated clarified rumen fluid (ICRF) was used to prepare CCA and SCA and dilution blanks. The ICRF was prepared with rumen fluid obtained from a rumen-fistulated donor cow on a 50:50 alfalfa hay-grain diet. The rumen contents were taken 3 h postfeeding, stained through four layers of cheesecloth and centrifuged at 5500 x g for 15 minutes. The supernatant was incubated according to the procedures of Allison et al. (1979) and Leedle and Hespell (1980). The incubated mixture contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 5.0 ml distilled water, .05 g L-cysteine hydrochloride, 2.5 g agar, and 80 ml centrifuged rumen fluid. The mixture was prepared under oxygen-free CO_2 and the flask was stoppered with a one-way (gravity type) check valve and incubated for 7 d with constant stirring. The pH of the incubated mixture was checked daily and if needed adjusted to 6.8 with saturated sodium carbonate solution. After incubation, the

mixture was centrifuged at 10,000 x g for 15 minutes and the supernatant frozen for media preparation. The sediment containing agar was washed three times by centrifugation. After the final wash, the agar slurry was frozen, lyophylized and ground before using for media preparation.

The cell-free supernatant of the ICRF obtained by centrifugation at 30,000 x g for 30 minutes was used for preparing CCA and SCA media. The CCA medium contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 35 ml distilled water, 1.0 ml B-vitamin mixture (Scheifinger et al., 1975) 1.0 ml of .01% hemin (Leedle and Hespell, 1980) .05 ml (V/V) glycerol, .05 g each of glucose, xylose, cellobiose, maltose and soluble starch, .2 g typticase, 50 ml ICRF, 1.0 ml .01% resazurin, .4 g sodium carbonate and .05 g L-cysteine hydrochloride. The SCA media contained xylan/xylose, pectin, starch or lactate (.3%) as the sole carbohydrate energy source. Because xylan and pectin were not readily soluble, their acqueous suspension was treated with a homogenizer (Polytron, Brinkman Inst., NY) for 90 seconds before including in the medium (Henning, 1979). The media were distributed into roll tubes containing washed incubated agar, closed with butyl rubber stoppers, crimped with aluminum seals and autoclaved. Cellulose broth contained the same ingredients as the CCA minus the carbohydrate energy sources, .2 g cellulose powder (Microgranular CC31 cellulose powder, Whatman Chemical Separation Ltd., England) .01 g cellobiose and 20% (V/V) centrifuged rumen fluid instead of ICRF. The broth was constantly stirred during dispensing to allow even distribution of the cellulose powder. The dilution blank contained minerals I and II, 20% (V/V) ICRF, resazurin, distilled water, sodium carbonate and L-cysteine hydrochloride. The procedures employed for the preparation of pre-reduced media and dilution blank were according to Hungate (1969) as modified by Holdeman et al. (1977).

Inoculation of Media. A ten-fold serial dilutions of the strained rumen fluid were made and .5 ml of the appropriate dilutions were inoculated into various media. Four roll tubes were prepared for each dilution. The tubes were incubated at 39 C for 7 d after which the colonies were counted. Cellulolytic bacteria were estimated by the most probable numer (MPN) technique (Cochran, 1950). One ml of 10^2 to 10^9 dilutions were inoculated into each of three cellulose broth tubes and incubated at 39 C for 21 d (Bryant et al., 1958).

Counting Rumen Ciliates. Rumen fluid samples were strained through one layer of cheesecloth and diluted (1:25) with protozoan dilution fluid (.05 g methyl green in 900 ml phosphate buffer and 100 ml formaldehyde solution). Total number of ciliates and generic composition in 20 microscopic fields were counted in a Sedgewick-Rafter counting chamber (Fisher Scientific, Lexington, NJ). Identification of protozoan genera was according to Hungate (1978).

Volatile Fatty Acids (VFA) Analysis. The VFA was determined according to the procedure of Erwin et al. (1961). Rumen fluid was acidified with 25% metaphosphoric acid and centrifuged at 18,000 x g for 20 minutes. The supernatant was analyzed in a gas chromatograph (Hewlett-packed Model 5890A, Avondale, PA).

Data were analyzed by general linear models procedure of Statistical Analysis Systems with variations partitioned into animal, treatment, sampling time and the interactions of treatment and sampling time. Least square means were tested by least significant difference (Barr et al., 1976).

Results

Rumen pH and VFA Concentrations. Lasalocid or monensin had no significant effect on the rumen pH (table 1). Rumen pH in all three groups declined with increased grain intake but the difference was only significant in the control cattle fed 20:80 hay-grain diet. The monensin-fed cattle had consistently lower rumen pH than those of lasalocid-fed or the control cattle but the difference was not significant. The total VFA concentrations remained unaltered in the control cattle but decreased ($P<.05$) in lasalocid and monensin-fed cattle, during the treatment and posttreatment period (table 1).

The proportion of acetate tended to decrease in all cattle as the level of grain in the diet increased (table 2). This decrease was not significant in the control and lasalocid-fed cattle until the 20:80 and 30:70 hay-grain diet change, respectively, but it was significant in monensin-fed group at the 40:60 hay-grain diet. The proportion of propionate increased slightly at the 40:60 and 30:70 hay-grain diets in the control cattle but was not significant. The increase was significant in lasalocid- and monensin-fed cattle at the 30:70 and 40:60 hay-grain diet, respectively. The proportion of butyrate, isobutyrate, isovalerate and valerate did not significantly change in all cattle during the trial (tables 2 and 3).

Rumen Bacteria. There was a general tendency, in all cattle, for increase in the number of total anaerobic colony counts as the level of grain in the diet increased (table 4). This increase was rather progressive in the control cattle and was significant at the 30:70 hay-grain diet, but appeared stabilized at the 20:80 hay-grain diet. There was an initial decrease, though not statistically significant, in the numbers of total anaerobic counts in lasalocid- and monensin-fed cattle, during the first two weeks of feeding the antibiotics. The counts then gradually

TABLE 1. EFFECT OF LASALOCID OR MONENSIN ON RUMEN pH AND TOTAL VOLATILE FATTY ACIDS (VFA) CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Rumen pH			Total VFA (μmole/ml)		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	6.86[e]	6.66	6.73	89.3	106.4[e]	97.1[e]
Treatment							
2	50:50	6.45[e]	6.30	6.39	80.0	91.1[ef]	76.4[f]
4	40:60	6.57[e]	6.71	6.49	89.8	92.4[ef]	93.9[ef]
6	30:70	6.57[e]	6.55	6.39	73.7	80.6[f]	73.8[f]
8 to 16[c]	20:80	6.41[f]	6.45	6.45	90.4	80.4[f]	80.9[f]
Posttreatment							
2 to 4[d]	20:80	6.58[e]	6.58	6.53	86.3	80.4[f]	81.1[f]
SE		.14			6.1		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding the antibiotics.

[d]Mean of two samples taken at two week intervals after withdrawal of the antibiotics from the feed.

[e,f]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 2. EFFECT OF LASALOCID OR MONENSIN ON RUMEN VOLATILE FATTY ACID CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Acetate (%)			Propionate (%)			Butyrate (%)		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment										
-2 to 0[b]	50:50	63.8^{e}	59.4^{ef}	62.2^{e}	20.6	22.9^{ef}	21.9^{ef}	10.9	11.6	10.7
Treatment										
2	50:50	64.0^{e}	59.2^{ef}	60.2^{e}	18.2	22.4^{ef}	24.6^{ef}	12.5	12.8	10.6
4	40:60	62.8^{e}	62.2^{f}	56.6^{f}	20.0	18.9^{ef}	36.4^{g}	12.3	11.6	14.4
6	30:70	59.4^{ef}	55.7^{e}	52.7^{f}	20.4	26.7^{e}	28.3^{fg}	15.3	11.3	14.1
8 to 16[c]	20:80	57.8^{f}	58.7^{ef}	54.3^{f}	19.4	21.1^{ef}	23.8^{ef}	15.8	13.5	14.6
Posttreatment										
2 to 4[d]	20:80	59.4^{ef}	59.9^{ef}	59.9^{ef}	19.3	18.9^{f}	19.4^{e}	15.1	14.2	12.5
SE		2.2			2.4			2.6		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding the antibiotics.

[d]Mean of two samples taken at two week intervals after the withdrawal of the antibiotics from the feed.

[e,f,g]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 3. EFFECT OF LASALOCID OR MONENSIN ON RUMEN VOLATILE FATTY ACID CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Isobutyrate (%)			Isovalerate (%)			Valerate (%)		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment										
-2 to 0[b]	50:50	1.0	1.2	1.2	2.4	2.4	2.3	1.7	2.5	1 .8
Treatment										
2	50:50	1.3	1.0	0.8	2.5	3.4	2.4	1.7	2.4	1.5
4	40:60	.8	1.7	1.7	2.5	4.2	2.8	1.9	1.9	1.6
6	30:70	1.3	1.3	1.1	2.3	3.2	2.7	1.7	1.7	1.4
8 to 16[c]	20:80	1.5	1.4	1.7	2.7	3.3	4.1	2.2	2.0	1.5
Posttreatment										
2 to 4[d]	20:80	1.5	1.8	1.9	2.9	3.41	4.3	1.9	1.1	2.2
SE		.5			.6			.3		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding the antibiotics.

[d]Mean of two samples taken at two week intervals after the withdrawal of the antibiotics from the feed.

TABLE 4. EFFECT OF LASALOCID OR MONENSIN ON RUMEN TOTAL ANAEROBIC AND MOST PROBABLE NUMBER (MPN) OF CELLULOLYTIC BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Total anaerobic bacterial count ($X10^{10}$/g)			Cellulolytic bacteria (MPN $X10^{8}$/g)		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	6.9[e]	6.7[e]	7.0	6.8	4.9	4.2
Treatment							
2	50:50	7.8[e]	4.6[e]	4.8[e]	1.2	4.5	2.0
4	40:60	10.8[ef]	9.3[ef]	14.5[e]	0.6	0.5	1.7
6	30:70	22.8[fg]	21.9[fg]	15.3[ef]	2.7	4.9	9.1
8 to 16[c]	20:80	31.1[g]	29.4[g]	26.6[f]	1.8	1.1	1.1
Posttreatment							
2 to 4[d]	20:80	30.5[g]	40.7[h]	39.9[g]	5.0	3.2	2.7
SE		3.8			3.8		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding antibiotics.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f,g,h]Means within the same column bearing different superscripts differ significantly ($P<.05$).

increased and became significant by the time the animals were on 20:80 hay-grain diet. After the withdrawal of the antibiotics from the feed, there was still a significant increase in the total anaerobic counts in the lasalocid- and monensin-fed cattle. Antibiotics had no consistent effect on the most probable number (MPN) of cellulolytic bacteria in the rumen (table 4). However, increased grain intake tended to decrease the cellulolytic bacterial counts.

Lasalocid and monensin did not appear to have any effect on the rumen xylanolytic bacteria (table 5). The absolute viable colony counts increased significantly at the 30:70 and 20:80 hay-grain diet changes in control and lasalocid-fed groups, respectively, but not in monensin-fed group. However, the mean percent counts in all groups tended to decrease with increased grain intake. The decrease was significant in control and monensin-fed cattle at posttreatment.

Generally, there was no significant effect of lasalocid or monensin on the numbers of pectinolytic rumen bacteria (table 6). Pectinolytic viable colony counts appeared to increase with higher level of grain in the diet and was significant at the 30:70 hay-grain diet in control and lasalocid-fed groups and at 20:80 hay-grain diet monensin-fed cattle. The counts appeared to be slightly lower, though not significant, in monensin-fed groups than the other groups. However, as a percent of total anaerobic bacterial count, pectinolytic bacteria decreased in all cattle, except at 30:70 hay-grain diet where it increased, though not significantly. The mean percent count ($P<.05$) decreased in all groups at the posttreatment period.

The viable colony counts of amylolytic rumen bacteria increased in all cattle as the level of grain in the diet increased (table 7). The counts peaked at the 20:80 hay-grain diet. However, the increase was not statistically significant, except in monensin-fed cattle at 20:80 hay-grain diet ($P<.05$). The proportion of amylolytic bacteria was higher in the lasalocid- and monensin-fed cattle than in

TABLE 5. EFFECT OF LASALOCID OR MONENSIN ON RUMEN XYLANOLYTIC BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Xylanolytic bacterial count ($X10^{10}$/g)			Percent of Anaerobic bacterial count		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	3.6^{ef}	2.7^{e}	3.4	48.9^{e}	39.1	50.2^{e}
Treatment							
2	50:50	2.1^{e}	1.6^{e}	1.8	26.8^{ef}	42.9	37.5^{ef}
4	40:60	2.0^{e}	2.0^{e}	2.6	18.7^{ef}	21.1	17.4^{ef}
6	30:70	8.8^{fg}	7.3^{ef}	4.9	35.3^{ef}	32.3	32.2^{ef}
8 to 16[c]	20:80	8.2^{g}	6.1^{fg}	5.2	26.8^{ef}	21.2	19.4^{ef}
Posttreatment							
2 to 4[d]	20:80	6.1^{efg}	7.6^{f}	6.7	20.1^{f}	18.6	16.4^{f}
SE		1.9			10.2		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f,g,h]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 6. EFFECT OF LASALOCID OR MONENSIN ON RUMEN PECTINOLYTIC BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Pectinolytic bacterial count ($X10^{10}$/g)			Percent of Anaerobic bacterial count		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	2.3^{e}	2.0^{e}	2.0^{e}	31.9^{e}	28.3^{ef}	28.7^{e}
Treatment							
2	50:50	2.0^{e}	1.5^{e}	1.6^{e}	25.1^{ef}	34.2^{ef}	33.7^{e}
4	40:60	2.0^{e}	1.3^{e}	2.1^{e}	18.2^{e}	13.5^{fgh}	14.1^{ef}
6	30:70	8.9^{f}	8.9^{f}	4.5^{ef}	33.3^{e}	43.9^{e}	30.0^{e}
8 to 16[c]	20:80	4.3^{e}	3.6^{e}	6.1^{f}	14.7^{f}	16.4^{fg}	21.4^{ef}
Posttreatment							
2 to 4[d]	20:80	3.7^{e}	4.6^{e}	4.3^{ef}	12.2^{f}	11.4^{gh}	10.8^{f}
SE		1.3			6.5		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f,g,h]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 7. EFFECT OF LASALOCID OR MONENSIN ON RUMEN AMYLOLYTIC BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Amylolytic bacterial count ($X10^{10}$/g)			Percent of Anaerobic bacterial count		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	3.1	3.9	2.7[e]	43.4	54.5[e]	39.9
Treatment							
2	50:50	3.2	3.3	3.2[e]	40.6	86.8[e]	68.1
4	40:60	3.9	4.4	3.9[e]	36.3	47.5[e]	30.4
6	30:70	6.0	8.6	5.6[ef]	27.1	39.9[ef]	37.1
8 to 16[c]	20:80	11.8	10.6	15.8[f]	39.0	35.5[ef]	61.1
Posttreatment							
2 to 4[d]	20:80	7.4	8.7	12.7[ef]	25.0	21.4[g]	31.7
SE		4.0			17.6		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f,g]Means within the same column bearing different superscripts differ significantly ($P<.05$).

the control, during the treatment period. This difference was highest in lasalocid- or monensin-fed cattle at the second week of treatment period.

Lactate-utilizing bacterial numbers in the rumen was not affected by lasalocid or monensin (table 8). The absolute viable counts increased in all cattle but the difference was not significant in the control and lasalocid-fed cattle until at 20:80 hay-grain diet. The proportion of lactate-utilizing bacteria increased in all three groups with increase in the intake of grain in the diet.

Rumen Protozoa. Lasalocid or monensin had no significant effect on the total numbers of rumen protozoa (table 9). The total number increased as the level of grain increased in the diet. The increase was significant in the control and monensin-fed but not in lasalocid-fed cattle. The increase in the total numbers appeared to be due to increase in both holotrichs and entodiniomorphs (table 9). Monensin depressed significantly holotrichs number during the treatment period ($P<.05$).

Among the holotrichs (table 10), Isotricha decreased ($P<.05$) in lasalocid-fed but not in monensin-fed or the control cattle at the 40:60 hay-grain diet. There was a significant increase in the numbers of Dasytricha in the control but not in lasalocid-fed cattle. The numbers of Dasytricha was not affected by feeding monensin during the treatment period, but it significantly increased during the posttreatment period.

Among the entodionomorphs, lasalocid or monensin had no effect on Polyplaston and Epidinium in the rumen (table 11). However, Ophryoscolex increased in lasalocid-fed and control cattle with the increase in the level of grain in the diet (table 12). Ophryoscolex initially disappeared completely from the rumen of monensin-fed cattle during the treatment period until they were on the 20:80 hay-grain diet and the numbers during posttreatment period were similar to

TABLE 8. EFFECT OF LASALOCID OR MONENSIN ON RUMEN LACTATE-UTILIZING BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Lactate-utilizing bacterial count ($X10^{10}$/g)			Percent of Anaerobic bacterial count		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	.3[e]	.3[e]	.4	4.7	4.0	5.9
Treatment							
2	50:50	.7[e]	.5[e]	.9	9.6	9.7	19.0
4	40:60	.7[e]	1.4[ef]	1.0	6.6	15.1	6.9
6	30:70	2.6[ef]	2.3[ef]	1.7	10.2	11.3	11.5
8 to 16[c]	20:80	4.6[f]	4.0[f]	3.6	17.7	18.9	15.3
Posttreatment							
2 to 4[d]	20:80	4.1[f]	4.6[f]	3.6	13.1	11.3	9.1
SE		1.3			7.8		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 9. EFFECT OF LASALOCID OR MONENSIN ON RUMEN PROTOZOA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Total Protozoa ($X10^3$/ml)			Holotrichs ($X10^3$/ml)			Entodiniomorphs ($X10^3$/ml)		
		Control	Lasalocid	Monensin	Control	Lasalocid	Monensin	Control	Lasalocid	Monensin
Pretreatment										
-2 to 0[b]	50:50	264.0^{e}	181.2	278.6^{e}	7.1	6.3	14.6^{e}	257.0^{e}	174.5	268.3^{e}
Treatment										
2	50:50	342.7^{ef}	341.0	228.5^{e}	8.1	8.5	5.0^{e}	334.6^{ef}	332.5	218.5^{e}
4	40:60	228.1^{e}	216.1	286.2^{ef}	9.6	13.4	8.1^{e}	218.6^{e}	202.7	278.0^{ef}
6	30:70	284.1^{ef}	242.6	359.0^{ef}	21.6	13.1	7.4^{e}	257.3^{ef}	229.5	351.6^{ef}
8 to 16[c]	20:80	398.5^{ef}	330.1	570.8^{f}	20.9	11.6	13.1^{e}	377.9^{ef}	318.6	497.9^{f}
Posttreatment										
2 to 4[d]	20:80	505.8^{f}	355.7	729.2^{g}	17.5	15.8	29.5^{f}	488.3^{f}	334.6	698.9^{g}
SE		78.9			7.3			84.9		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b]Mean of two samples taken at two weeks interval before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding the antibiotics.

[d]Mean of two samples taken at two weeks interval after the withdrawal of the antibiotics from the feed.

[e,f,g]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 10. EFFECT OF LASALOCID OR MONENSIN ON RUMEN HOLOTRICHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Isotricha ($X10^3$/g) Control	Lasalocid	Monensin	Dasytricha ($X10^3$/ml) Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	2.0	1.7[e]	2.3	4.6[e]	7.4	12.3[e]
Treatment							
2	50:50	1.1	5.3[f]	.1	7.1[ef]	3.2	2.7[e]
4	40:60	.0	.7[g]	.1	9.6[ef]	12.7	6.0[e]
6	30:70	2.1	1.4[e]	4.9	17.3[f]	11.7	4.6[e]
8 to 16[c]	20:80	3.0	1.5[e]	3.6	16.7[f]	10.4	9.9[e]
Posttreatment							
2 to 4[d]	20:80	2.0	1.8[e]	6.0	15.6[f]	14.3	23.5[f]
SE		1.1			4.2		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e,f,g]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 11. EFFECT OF LASALOCID OR MONENSIN ON RUMEN ENTODINIOMORPHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Polyplastron ($X10^3$/ml) Control	Lasalocid	Monensin	Epidinium ($X10^3$/ml) Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	1.4	2.1	2.3	1.6	1.2	6.3
Treatment							
2	50:50	3.5	2.5	3.2	2.3	1.1	6.3
4	40:60	2.5	2.5	2.0	.0	2.7	7.0
6	30:70	1.4	2.1	1.3	3.0	.0	.0
8 to 16[c]	20:80	2.3	5.0	4.4	2.3	6.1	1.4
Posttreatment							
2 to 4[d]	20:80	2.0	4.2	4.4	2.0	3.7	14.3
SE		1.8			7.9		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding the antibiotics.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

TABLE 12. EFFECT OF LASALOCID OR MONENSIN ON RUMEN ENTODINIOMORPHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Entodinium and others ($X10^3$/ml) Control	Lasalocid	Monensin	Oprhyoscolex ($X10^3$/ml) Control	Lasalocid	Monensin
Pretreatment							
-2 to 0[b]	50:50	247.8^{f}	170.5	255.0^{f}	3.9^{fg}	0.8^{f}	4.0
Treatment							
2	50:50	322.6^{fg}	326.5	219.3^{f}	3.2^{g}	5.0^{f}	.0
4	40:60	204.4^{f}	197.0	276.2^{fg}	9.2^{fg}	5.7^{f}	.0
6	30:70	248.0^{fg}	220.4	120.1^{f}	5.7^{fgh}	15.6^{g}	.1
8 to 16[c]	20:80	363.9^{fg}	310.5	488.2^{g}	11.5^{h}	5.9^{f}	3.4
Posttreatment							
2 to 4[d]	20:80	475.9^{g}	322.7	700.7^{h}	8.3^{fgh}	8.0^{f}	5.0
SE		86.3			2.1		

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixtures.

[b]Mean of two samples taken at two week intervals before feeding the antibiotics.

[c]Mean of samples taken at 8, 12 and 16 weeks of feeding antibiotics.

[d]Mean of two samples taken at two week intervals after withdrawal of antibiotics from feed.

[e]Diplodinium, Eudiplodinium, Metadinium and Ostracodinium.

[fgh]Means within the same column bearing different superscripts differ significantly ($P<.05$).

that of the pretreatment period. Other species of the entodimomorphs, including Entodinium showed similar increasing trend as the total protozoal numbers.

Discussion

Feeding lasalocid or monensin has been shown to cause no change in rumen pH and total VFA concentration (Dinius et al., 1976; Prange et al., 1978; Gutierrez et al., 1982; Thonney et al., 1982; Dawson and Boling, 1983). However, in cattle with soluble carbohydrate stress, both antibiotics have been shown to increase rumen pH (Nagaraja et al., 1981). Although we found no change in rumen pH, total VFA concentration decreased in lasalocid or monensin-fed cattle. Increased VFA concentration has been reported in cattle fed low levels of monensin with concentrate diets (Thornton and Owens, 1981). Dawson and Boling (1983) found that the mean total VFA concentration in monensin-fed animals was greater than those of the control cattle during the entire treatment period, but were not significantly greater during the posttreatment period and had returned to values comparable to those in control animals within ten days after the withdrawal of the antibiotic from the diet. The characteristic decrease in acetate:propionate ratio observed is consistent with the previous reports (Dinius et al., 1976; Prange et al., 1978; Gutierrez et al., 1982; Dawson and Boling, 1983).

Increased propionate production in antibiotic-fed cattle is attributed to selection of bacteria that produce propionate as major product (Chen and Wolin, 1979; Dennis et al., 1981; Nagaraja et al., 1982). However, Dawson and Boling (1983) have observed that although monensin increased resistant bacteria in the rumen, the change was not correlated with the characteristic decrease in acetate:propionate ratio. Significant increase in resistent bacteria was not found immediately after monensin was included in the diet and remained high for more than 18 days after it had been withdrawn from the diet. In contrast, changes in

acetate:propionate ratio were observed before the increase in the resistant bacteria, suggesting that rumen fermentation changes were not necessarily associated with increased proportion of monensin-resistent bacteria. It has been suggested that the effect of ionophore antibiotics on rumen fermentation may be due to altered metabolic pathways of rumen bacteria (Van Nevel and Demeyer, 1977; Bergen and Bates, 1984). The initial decrease (though not significant) in the total anaerobic viable colony counts observed in the lasalocid- and monensin-fed cattle was probably due to the inhibitory effects of the antibiotics on the growth of the sensitive rumen bacterial species before the development or adaptation of the resistent species (Chen and Wolin, 1979; Henderson et al., 1981). The resistent rumen bacteria would then rapidly proliferate to replace the sensitive bacterial species.

The absence of inhibitory effects of lasalocid or monensin on cellulolytic rumen bacterial population observed in this study agrees with the report of Dinius et al. (1976) who found that monensin had little or no effects on crude fiber digestion in cattle. It has been suggested that the intake of readily fermentable substrates causes a reduction in the numbers of cellulolytic bacteria by lowering the pH of the rumen content (Krogh, 1961; Slyter et al., 1970) and/or by increasing other bacterial types in the rumen which may in turn deplete the required nutrients for cellulolytic bacteria (Bryant, 1973). Because the rumen pH did not change much in this study, the inconsistent effect observed due to increase in grain intake may probably be explained by the competition from other fast growing rumen bacteria (Bryant, 1973).

Pectin and xylan are complex groups of polysaccharides and are utilized by a wide variety of rumen bacteria, including Bacteroides succinogenes, Bacteroides rumincola, Butyrivibrio fibrisolvens, Streptococcus bovis and Lachnospira multiparus (Baldwin and Allison, 1983). Bacteroides species are known to be resistant or

rapidly develop resistance to lasalocid or monensin, while B. fibrisolvens S. bovis and L. multiparus are sensitive to these antibiotics (Slyter, 1978; Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). It is possible that when the growth of the sensitive bacterial species are inhibited, the resistant species or those that readily develop resistance to the antibiotics would proliferate to replace the sensitive ones (Bryant, 1973). This could explain why lasalocid or monensin in the diet had no effect on the viable colony counts of xylanolytic or pectinolytic bacteria from the rumen of antibiotic-fed cattle. B. ruminicola a major xylanolytic and pectinolytic bacteria occurs in high numbers in the rumen (Bryant and Small, 1953; Bryant et al., 1958). Grubb and Dehority (1975) found no significant differences in the mean percent counts of xylan and pectin-utilizing rumen bacteria in sheep fed 100% roughage and those fed 60:40 concentrate-roughage diets. They, however, observed that daily variations in colony counts were highest for those two carbohydrate-utilizing bacteria.

Increased proportion of amylolytic bacteria observed during the treatment period in this study could be explained by the fact that most of the major amylolytic bacteria were among the rumen bacterial species resistant to lasalocid or monensin (Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). Resistance of lactate-utilizing bacteria to lasalocid or monensin (Dennis et al., 1981) would explain why there was no significant differences in the viable and proportion of lactate-utilizing bacteria between the treated and control cattle.

Selective carbohydrate media with specific energy sources have been used successfully to delinate carbohydrate-utilizing subgroups in the bacterial population from the rumen of sheep (Dehority and Grubb, 1976) and cattle (Leedle and Hespell, 1980) and from the cecum and colon of pigs (Allison et al., 1979). From the results of this study, it would seem difficult to use the selective carbohydrate media to determine the effect of dietary ionophore antibiotics on the culturable

subgroups of rumen bacterial population. In vitro studies have shown an array of rumen bacterial species which utilize a wide range of energy sources, such as xylan, pectin, lactate, etc., to be sensitive or resistant to lasalocid or monensin (Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). Inhibition of sensitive bacterial species results in the growth of the resistant species (Bryant, 1973). This explains why in most cases in this study, there were no significant differences in the viable colony counts of rumen bacteria between antibiotic-treated and the control cattle.

The effect of increased energy intake on rumen protozoal numbers have been studied in sheep and cattle (Warner, 1962; Nakamara and Kanegasaki, 1969; Abe et al., 1973; Grubb and Dehority, 1975). The reports generally indicated that rumen protozoal concentrations increased as the level of available energy intake increased. The increase in total protozoa was essentially due to increase in the entodiniomorphs (Abe et al., 1973). Our findings are in general agreement with their report. The effect of monensin on the rumen protozoal population appears to depend on the diet. Richardson et al. (1978) reported that under different feedlot conditions and in sheep, monensin may decrease rumen protozoal numbers by 4 to 63%, whereas, Dinius et al. (1976) found no significant changes in the total number or any discernible shift in any specific type of rumen protozoa in cattle fed grass hay diet supplemented with the antibiotic. In this study, we observed that, although lasalocid and monensin had little or no effect on the total numbers of rumen protozoa, holotrichs appeared to be more sensitive to monensin than lasalocid. Among the entodiniomorphs, Ophryoscolex was extremely sensitive to monensin.

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RUMEN MICROBIAL CHANGES IN CATTLE FED DIETS WITH OR WITHOUT SALINOMYCIN

Summary

Four rumen-fistulated cattle, randomly assigned to two groups (control and salinomycin-fed) were used to monitor the changes in rumen microbial populations and volatile fatty acids (VFA) concentrations associated with feeding salinomycin (.22 $mg \cdot kg^{-1} \cdot d^{-1}$). Steers were adapted to alfalfa hay and grain diet (80:20) before supplementing the diet with salinomycin and then the diet was changed to 50:50 and 20:80 alfalfa hay to grain at two week intervals. Rumen fluid samples for total and selective enumeration of anaerobic bacteria, VFA analysis and for counting protozoa were collected at 80:20 alfalfa hay-grain diet before salinomycin feeding, and at 80:20, 50:50 and 20:80 hay-grain diet with salinomycin. At each sampling period, rumen samples were collected at 3 h postfeeding on three consecutive days. Salinomycin feeding had no effect on rumen pH and total VFA concentration. Acetate proportion was lower and propionate proportion was higher in salinomycin-fed steers than the control steers. The acetate-propionate ratio was significantly lower in salinomycin-fed steers than that of the control. Molar proportion of butyrate increased in both control and salinomycin-fed steers. Total anaerobic bacterial counts were lower in salinomycin-fed steers than the control steers after 8 wks of salinomycin feeding. Salinomycin-resistant bacteria increased from 7.6 to 15.6% in salinomycin-fed steers but remained unchanged in control steers. Salinomycin had no effect on cellulolytic bacteria but the proportions of amylolytic bacteria were higher in salinomycin-fed steers than control steers. Total protozoal numbers decreased initially in salinomycin-fed cattle. Initial reduction was due to reduced numbers of entodiniomorphs, particularly that of Entodinium species. Holotrichs were unaffected by salinomycin feeding.

Introduction

Salinomycin is a polyether antibiotic produced by Streptomyces albus (Kinashi et al., 1973). It is a relatively new monocarboxylic acid ionophore with a cation selectivity in the order of $K^+ > Na^+ > Cs^+ >> Ca^{++}$ (Westley, 1982). Like other ionophores, salinomycin is highly effective against gram-postive bacteria, including Mycobacterium, but it is ineffective against gram-negative bacteria and fungi (Miyazaki et al. 1974; Liu, 1982). Although not officially approved as a feed additive for cattle, salinomycin has been shown to enhance feed efficiency and daily weight gains in steers, heifers and sheep (Nakashina et al. 1982; McClure et al., 1980; Webb et al., 1980; Ferrell et al., 1983; Zinn and Axe, 1983; Merchen and Berger, 1985). Its potency is about three times higher than that of lasalocid or monensin (Heinemann, 1984; Merchen and Berger, 1985). Other effects of salinomycin as growth promoter for weaning and finishing pigs (Blair and Shires, 1981; Leeson et al., 1981), control of coccidiosis in poultry and steers (Danforth et al., 1977; Merchen and Berger, 1985) and improved performance of broilers (Danforth et al. 1977) have been reported. Currently, it is marketed as a coccidiostat under the trade name BioCox® (1985 Feed Additive Compendium).

Much information has accumulated on the effects of lasalocid and monensin on cattle performance (Brandt, 1982; Stuart, 1982; Goodrich et al., 1984), and on alteration in ruminal fermentation patterns (Bartley and Nagaraja, 1982; Bergen and Bates, 1984; Schelling, 1984). Fermentation changes are attributed to a shift in microbial population in the rumen (Chen and Wolin, 1979; Bartley and Nagaraja, 1982). Salinomycin has also been found to alter rumen fermentation patterns by increasing the molar proportion of propionate while decreasing acetate (Fontenot et al. 1980; Zinn and Axe, 1983; Merchen and Berger, 1985). The effect of salinomycin on rumen microbial population has not been reported. The purpose of

this study therefore, was to monitor the changes in the rumen microbial population in cattle fed diet supplemented with salinomycin.

Materials and Methods

Animals and Diet. Four rumen-fistulated Holstein steers (mean body weight 382 kg) were randomly assigned to two groups. One group (two steers) received salinomycin at .22 $mg \cdot kg^{-1} d^{-1}$, whereas the other group (two steers) received no antibiotic and served as control. The steers were fed a diet of alfalfa hay (IFN 1-00-0961) and a grain mixture, composed of 49.2% cracked corn (IFN 4-02-931), sorghum grain (IFN 4-05-643), 1.0% dicalcium phosphate (IFN 6-04-080), .25% trace mineral salts and .1% vitamins A and D mixture. The animals were adapted to an 80:20 alfalfa hay-grain diet for two weeks before feeding antibiotic and the diet was gradually switched to 50:50, 20:80 hay to grain ratio every two weeks. The salinomycin premix (A.H. Robins Co., Richmond, VA) was thoroughly hand-mixed with the grain component of the diet and fed twice daily.

Sampling schedule, collection and processing samples. Rumen samples were collected before feeding the antibiotic (pretreatment samples) and two weeks after the antibiotic feeding, when the cattle were on 80:20 hay to grain diet and thereafter at two weeks interval following each diet change (treatment samples). At each sampling period, samples were collected from each animal at 3 h post-morning feeding on three consecutive days. The rumen contents were as thoroughly hand mixed as possible and about 500 ml was collected into a rubber-stoppered flask and transported immediately to the laboratory.

An aliquot of the rumen sample was flushed with oxygen-free CO_2 and blended in a Waring blender for 1 min. and strained through four layers of

cheesecloth. The strained rumen fluid was used to inoculate various culture media and for volatile fatty acids (VFA) and dry weight determination. The rest of the unblended rumen sample was strained through one layer of cheesecloth and used to record pH and for counting rumen ciliates.

Culture Media. Culture media used to enumerate rumen bacteria included complete carbohydrate agar medium (CCA) for total viable anaerobic counts, selective carbohydrate agar medium (SCA) with soluble starch as the sole energy source, for the enumeration of amylolytic rumen bacteria and cellulose broth for the determination of cellulolytic bacterial counts. To determine the proportions of rumen bacterial population that were resistant to salinomycin, rumen samples were inoculated into CCA, SCA and cellulose broth media containing 3.5 $\mu g \cdot ml^{-1}$ of salinomycin.

Incubated rumen fluid (ICRF) was used to prepare CCA and SCA media. The ICRF was prepared from rumen fluid obtained from a rumen-fistulated donor cow on 50:50 alfalfa hay-grain diet. The rumen contents were taken 3 to 4 h post-feeding, strained through four layers of cheesecloth and centrifuged at 5500 x g for 15 minutes. The supernatant was incubated according to the procedures of Allison et al. (1979) and Leedle and Hespell (1980). The incubated mixture contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 5.0 ml distilled water, .05 g L-cysteine hydrochloride, 2.5 g agar, 80 ml centrifuged rumen fluid. The mixture was prepared under oxygen-free CO_2 and the flask was stoppered with one-way (gravity type) check valve and incubated for 7 d with constant stirring. The pH of the incubated mixture was checked daily and adjusted if needed to 6.8 with saturated sodium carbonate solution. After incubation, the mixture was centrifuged at 10,000 x g for 15 minutes and the supernatant was frozen for media preparation. The sediment

containing agar was washed in distilled water three times by centifugation. After the final wash, the agar slurry was frozen, lyophylized and ground before using for media preparation.

Cell-free supernatant of the ICRF obtained by centrifugation at 30,000 x g for 30 minutes was used for preparing CCA and SCA media. The CCA contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 35 ml distilled water, 1.0 ml B-vitamin mixture (Scheifinger et al. 1975), 1.0 ml .01% Hemin (Leedle and Hespell, 1980), .05 ml (V/V) glycerol, .05 g each of glucose, xylose, cellobiose, maltose and soluble starch, .2 g trypticase, 50 ml ICRF, 1.0 ml .01% resazurin, .4 g sodium carbonate and .05 L-cysteine hydrochloride. The SCA medium contained soluble starch (.3%) as the only carbohydrate energy source. The media were distributed into roll tubes, stoppered, crimped with aluminum seals and autoclaved.

Cellulose broth contained per 100 ml: 7.5 ml each of minerals I and II, .01 g cellobiose, 1.0 ml B-vitamin mixture, 1.0 ml .01% Hemin, 20 ml non-incubated centrifuged (cell-free) rumen fluid, 65 ml distilled water, .05 g L-cysteine hydrochloride, 4 g sodium carbonate and 1.0 ml .01% resazurin. Ten ml portion of it was distributed into tubes containing 75 mm x 8 mm strips of Whatman No. 1 filter paper, as the only energy source for cellulolytic bacteria.

The dilution blank contained minerals I and II, 20% (V/V) ICRF, resazurin, distilled water, sodium carbonate and L-cysteine hydrochloride. The procedure for the preparation and distribution of the pre-reduced media and dilution blank was according to Hungate (1969) as modified by Holdeman et al. (1977).

Inoculation of Media. A ten-fold serial dilution of the strained rumen fluid was made and .5 ml of the appropriate dilutions was inoculated into the culture

media. Four roll tubes were prepared for each dilution. The tubes were incubated at 39 C for 7 d before counting colonies.

To determine the cellulolytic bacterial counts, 1 ml of dilutions 10^2 to 10^9 was inoculated into each of three tubes of cellulose broth and incubated at 39 C for 21 d. Cellulose digestion was determined by the disintegration of the filter paper strips and the most probable number (MPN) of cellulolytic rumen bacteria was estimated (Cochran, 1950).

Counting rumen ciliates. Rumen fluid strained through one layer of cheesecloth was diluted (1:25) with protozoan dilution fluid (.05 g methyl green in 900 ml phosphate buffer plus 100 ml fermaldehyde solution). The ciliates were counted in a Sedgwick-Rafter counting chamber (Fisher Scientific, Lexington, NJ). Identification of the protozoa genera was according to Hungate (1978).

VFA Analysis. VFA was determined according to the procedure of Erwin et al. (1961). Rumen fluid was acidified with 25% metaphosphoric acid and centrifuged at 18,000 x g for 20 min. The supernanant was then analysed in a gas chromatograph (Hewlett-packed Model 5890A, Avondale, PA).

Statistical Analysis. Data were analyzed by General Linear Models procedure of Statistical Analysis system with variations partitioned into animals, treatment, sampling time and interactions of treatment and sampling time (Barr et al., 1976). Treatment effect at each sampling time was tested by t-test (Steel and Torrie, 1960).

Results

Effect of salinomycin on rumen pH and VFA concentrations. Salinomycin in the diet had no significant effect on ruminal pH (table 1). However, the salinomycin-fed cattle tended to have a slightly higher rumen pH than the control cattle, during the first four weeks of antibiotic feeding. The pH decreased in both groups as the level of grain increased in the diet. Salinomycin also had no effect on the total VFA concentration in the rumen (table 1). Molar proportion of acetate decreased in both control and antibiotic-fed cattle. The decrease was significant at 20:80 hay to grain diet in control cattle and in antibiotic-fed cattle the difference was significant at 50:50 or higher hay to grain diet. Propionate proportion remained unchanged in control cattle but increased ($P<.05$) in salinomycin-fed cattle. In samples collected at 50:50 and 20:80 hay to grain diet, salinomycin-fed cattle had significantly higher propionate than control (table 2). Accordingly acetate:propionate ratio did not change significantly in control cattle but decreased ($P<.05$) in salinomycin-fed cattle. The molar proportion of butyrate increased with increased grain intake in both groups while isobutyrate decreased (table 3). Isovalerate also showed some increasing trend in salinomycin-fed but not in the control cattle. There was no change in molar proportion of valerate.

Effect of salinomycin on rumen bacteria. The effect of salinomycin on total viable and resistant anaerobic counts are in table 4. An increasing trend, though no statistically significant was observed for total anaerobic viable counts in control cattle as the level of grain increased in the diet. In salinomycin-fed cattle, the total anaerobic counts, during treatment period were lower than that of the pretreatment period, but the difference was not significant ($P>.05$). Total anaerobic bacterial counts in salinomycin-fed cattle were lower ($P<.05$) than that of control cattle in sample collected after 8 wk antibiotic feeding. The

TABLE 1. EFFECT OF SALINOMYCIN ON RUMEN pH AND TOTAL VOLATILE FATTY ACIDS (VFA) CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Rumen pH		Total VFA (µmole/ml)	
		Control	Salinomycin	Control	Salinomycin
Pretreatment					
0	80:20	6.44^{bc}	6.38^{b}	93.0^{b}	87.8^{bc}
Treatment					
2	80:20	6.69^{b}	6.79^{c}	85.4^{c}	86.0^{b}
4	50:50	6.12^{cd}	6.39^{b}	89.9^{bc}	91.8^{bcd}
6	20:80	5.99^{d}	6.01^{d}	93.6^{b}	94.3^{cd}
8	20:80	6.08^{d}	5.94^{d}	95.3^{b}	95.8^{d}
SE		.10		2.1	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and vitamins A & D mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 2. EFFECT OF SALINOMYCIN ON RUMEN VOLATILE FATTY ACIDS CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Acetate (%)		Propionate (%)		Acetate:Propionate ratio		Butyrate (%)	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment									
0	80:20	62.3^{bc}	62.3^{b}	18.4	18.5^{b}	3.4	3.4^{b}	11.7^{b}	12.0^{bc}
Treatment									
2	80:20	62.8^{b}	61.7^{b}	18.8	19.0^{bc}	3.3	3.3^{b}	11.5^{b}	11.4^{b}
4	50:50	60.8^{cd}	59.1^{c}	18.1	20.6^{ef}	3.4	2.9^{cdf}	13.9^{bcd}	13.2^{bc}
6	20:80	59.1^{d}	56.4^{de}	18.3	23.6^{df}	3.2	2.4^{ef}	14.7^{d}	12.4^{bc}
8	20:80	61.0^{bc}	56.8^{de}	18.5	19.6^{bc}	3.3	3.0^{df}	14.3^{cd}	14.3^{c}
SE		.6		.6		.1		.8	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b,c,d,e]Means within the same column bearing superscripts differ significantly ($P<.05$).

[f]Significantly different from the control ($P<.05$).

TABLE 3. EFFECT OF SALINOMYCIN ON RUMEN VOLATILE FATTY ACIDS CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Isobutyrate (%)		Isovalerate (%)		Valerate (%)	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment							
0	80:20	1.7[b]	1.7[b]	2.8	3.4[b]	3.1	2.8
Treatment							
2	80:20	1.6[bc]	1.9[bc]	2.9	3.9[bc]	2.5	2.2
4	50:50	1.5[c]	1.5[c]	2.8	3.4[b]	2.8	2.7
6	20:80	1.6[bc]	1.5[c]	3.3	3.3[b]	2.9	2.8
8	20:80	1.3[d]	1.5[ce]	2.6	5.3[ce]	2.4	1.6
SE		.1		.4		.5	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM and vitamins A & D mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

[e]Significantly different from control ($P<.05$).

TABLE 4. EFFECT OF SALINOMYCIN ON RUMEN ANAEROBIC AND CELLULOLYTIC BACTERIAL COUNTS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Anaerobic bacterial count ($X10^{10}$/g)		Resistant Anaerobic count ($X10^{10}$/g)		Percent Anaerobic bacterial count		Cellulolytic bacterial count ($X10^{8}$/g)	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment									
0	80:20	20.9	22.4	1.2	1.0	7.7	7.6	4.0^{b}	3.8^{b}
Treatment									
2	80:20	25.3	19.3	.9	2.2	3.7	11.4	2.6^{bc}	$.4^{c}$
4	50:50	24.0	20.0	.5	1.7	3.4	9.6	4.6^{b}	4.7^{b}
6	20:80	21.5	18.6	.5	2.6	2.6	15.5	$.8^{c}$	$.8^{c}$
8	20:80	30.0	18.3^{d}	1.1	2.5	3.5	15.6	3.1^{b}	3.8^{b}
SE		2.8		.7		4.4		3.7	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b,c]Means within the same column bearing superscripts differ significantly ($P<.05$).

[d]Significantly different from the control ($P<.05$).

salinomycin-resistant anaerobic bacterial counts increased in the antibiotic-fed, but remained unchanged in the control cattle. The proportion of salinomycin-resistant bacteria increased from 7.6% before to 15.6% after 8 wk of feeding salinomycin.

Salinomycin in the diet had no consistent effect on rumen cellulolytic bacteria. Although there was a trend towards lower counts with increased grain intake (table 4), the difference was not significant ($P>.05$). In the salinomycin-fed cattle, the count was lowest ($P<.05$) at the first two weeks of antibiotic feeding. Although salinomycin in the diet had no effect on cellulolytic bacteria in the rumen, the growth of these organisms were completely inhibited when rumen fluid from both groups were inoculated into cellulose broth containing .9, .18 or .35 $\mu g \cdot ml^{-1}$ of salinomycin.

Rumen amyloltyic bacterial counts increased both in the control and salinomycin-fed cattle, as the level of grain increased in the diet (table 5). However, the increase was not significant until after 8 wk of antibiotic feeding, when the cattle were on 20:80 hay to grain diet. Amylolytic bacterial counts in salinomycin-fed cattle was not significantly different from that of the control cattle. However, the proportion of amylolytic bacteria were consistently higher in the salinomycin-fed cattle than the control, throughout the treatment period (table 5). The resistant amylolytic bacterial colony counts were higher in salinomycin-fed cattle than that of the control throughout the treatment period. The proportion of resistant amyloltyic bacteria remained unchanged in the control cattle but increased in salinomycin-fed cattle (table 5).

Effect of salinomycin on rumen protozoa. Total protozoal numbers increased in the control with increased grain intake (table 6). In salinomycin-fed cattle total protozoal numbers decreased during week 2 and 4 of antibiotic feeding and was

TABLE 5. EFFECT OF SALINOMYCIN ON RUMEN AMYLOLYTIC BACTERIA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Amylolytic bacterial count ($X10^{10}$/g)		Percent of total bacterial count		Resistant Amylolytic count ($X10^{10}$/g)		Percent of amylolytic bacterial count	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment									
0	80:20	2.8^{b}	3.1^{b}	17.4^{bc}	22.8^{b}	.9	.8	32.2	26.3^{bc}
Treatment									
2	80:20	3.0^{bc}	3.6^{b}	12.3^{c}	21.2^{b}	.4	1.5	12.9	43.5^{cd}
4	50:50	4.4^{b}	4.2^{bc}	22.2^{b}	21.9^{b}	.5	2.6	11.7	61.9^{de}
6	20:80	4.7^{b}	7.9^{c}	22.5^{b}	42.9^{b}	1.8	3.7	18.6	46.8^{cd}
8	20:80	12.4^{c}	11.9^{d}	44.9^{b}	74.1^{ce}	1.3	3.5	10.7	29.1^{bc}
SE		1.2		8.8		1.0		21.0	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b,c,d]Means within the same column bearing superscripts differ significantly ($P<.05$).

[e]Significantly different from the control ($P<.05$).

TABLE 6. EFFECT OF SALINOMYCIN ON RUMEN PROTOZOA

Sampling time (weeks)	Diet[a] (Hay:Grain)	Total Protozoal number ($X10^4$/ml)		Holotrichs ($X10^4$/ml)		Entodiniomorphs ($X10^4$/ml)	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment							
0	80:20	33.4^{b}	32.4^{b}	2.6	2.5	30.8^{b}	29.3^{b}
Treatment							
2	80:20	29.6^{b}	18.6^{b}	2.1	2.0	27.5^{b}	16.6^{be}
4	50:50	60.7^{cd}	15.0^{c}	2.0	1.0	58.7^{c}	14.0^{e}
6	20:80	56.7^{bcd}	70.9^{c}	1.9	.9	54.8^{c}	70.4^{c}
8	20:80	48.5^{bcd}	65.3^{c}	1.0	2.7	47.5^{c}	62.6^{c}
SE		7.8		.9		7.3	

[a]Alfalfa hay and grain diet composed of corn, milo, SBM and vitamins A & D mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

[e]Significantly different from the control ($P<.05$).

significantly lower than the control at week 4. The reduction in the total numbers of rumen protozoa was due to the reduction of entodiniomorphs. Holotrich numbers were not significantly affected but entodiniomorphs declined during the final 4 weeks of salinomycin feeding. Salinomycin in the diet had no effects on the total and individual numbers of the Holotrichs (table 7). Also Polyplastron, Epidinium or Ophryoscolex (table 8) were not affected. The effects on Entodinium and other species of entodiniomorphs followed the same pattern as that of the total numbers of rumen protozoa. Apparently, Entodinium species became adapted to the antibiotics.

Discussion

Salinomycin in the diet has been shown to cause no change in rumen pH and total VFA concentrations (McCure et al., 1980; Webb et al., 1980; Nakashima et al., 1982; Merchen and Berger, 1985). Although we found no change in total VFA concentration and no significant effect on rumen pH, the rumen pH was slightly but consistently higher in the salinomycin-fed than the control cattle except at the 20:80 hay to grain diet. Like the other ionophore monensin, salinomycin at the dietary level may produce no significant change in ruminal pH as long as the cattle are not stressed with soluble carbohydrate (Dinius et al., 1976; Nagaraja et al., 1982).

Many workers have found a significant decrease in acetate:propionate ratio when salinomycin was fed in the diet at a concentration as low as a 5 g/ton or 5.5 ppm of feed (Fontenot et al., 1980; Webb et al., 1980; Zinn and Axe, 1983; Merchen and Berger, 1985). Others (Fontenot et al., 1980; Webb et al., 1980) have reported a decline in the molar proportion of butyrate and isovalerate with a concurrent linear increase in valerate as the level of salinomycin intake increased. The results of this study showed significant decrease in acetate:propionate ratio

TABLE 7. EFFECT OF SALINOMYCIN ON RUMEN HOLOTRICHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Isotricha ($X10^4$/ml)		Dasytricha ($X10^4$/ml)		Charonina ($X10^4$/ml)	
		Control	Salinomycin	Control	Salinomycin	Control	Salinomycin
Pretreatment							
0	80:20	1.4	.5	1.6	2.1^{bc}	2.3	1.1
Treatment							
2	80:20	.7	.3	1.6	1.7^{bc}	.0	.0
4	50:50	.7	1.8	1.3	1.1^{b}	.0	.1
6	20:80	.5	.3	1.6	$.8^{c}$	.0	.0
8	20:80	.4	.8	.7	1.9^{bc}	.0	.0
SE		.5		3.0			

[a]Alfalfa hay and grain diet composed of corn, milo, SBM and vitamins A & D mixture.

[b,c]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 8. EFFECT OF SALINOMYCIN ON RUMEN ENTODINIOMORPHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Polyplastron (X10⁴/ml) Control	Polyplastron (X10⁴/ml) Salinomycin	Epidinium (X10⁴/ml) Control	Epidinium (X10⁴/ml) Salinomycin	Ophryoscolex (X10⁴/ml) Control	Ophryoscolex (X10⁴/ml) Salinomycin	Entodinium and Others[b] (X10⁴/ml) Control	Entodinium and Others[b] (X10⁴/ml) Salinomycin
Pretreatment									
0	80:20	.3	.3	.8	.9[cd]	1.0[c]	.6	29.0[c]	27.9[c]
Treatment									
2	80:20	.4	.2	.2	1.3[cf]	.4[c]	.3	26.9[c]	16.5[c]
4	50:50	.4	.2	.5	.6[cd]	1.7[d]	.1	57.0[e]	14.3[cf]
6	20:80	.6	.5	.5	.3[d]	3.6[d]	1.1	52.3[de]	69.4[d]
8	20:80	.5	.7	.5	--	2.1[cd]	--	45.3[cde]	60.7[d]
SE		.2	.2	.5		7.0			

[a]Alfalfa hay and grain diet composed of corn, milo, SBM, salts and Vitamins A & D mixture.

[b]Others include Diplodium, Eudiplodinium, Metadinium and Ostracodinium.

[c,d,e]Means within the same column bearing superscripts differ significantly ($P<.05$).

[f]Significantly different from the control ($P<.05$).

and little or no change in isobutyrate and valerate in antibiotic-fed cattle. Molar proportions of butyrate and isovalerate increased in both the control and antibiotic-fed groups, with increased grain intake. A level of 5.5 ppm or 5 g/ton of salinomycin in the feed has been shown to enhance animals performance similar to 22 ppm or 30 g/ton of monensin in the feed (Heinemann, 1984; Merchen and Berger, 1985). Improved animal performance is attributed to alterations in rumen fermentation produced by the antibiotics (Bergen and Bates, 1984; Schelling, 1984; and Merchen and Berger, 1985).

Monensin has been shown to have no significant effect on the total numbers of rumen bacteria (Dinius et al., 1976; Dawson and Boling, 1983). However, greater numbers of monensin-resistant bacterial colony counts were observed from the rumen of monensin-fed cattle than the control (Dawson and Boling, 1983). Similarly in this study, salinomycin-resistant bacterial colony counts were consistently higher in salinomycin-fed cattle then the control, although the increase was not statistically significant. Merchen and Berger (1985) found no decrease in crude fiber and organic matter digestibility in the diet, when salinomycin was fed. Webb et al. (1980) had reported earlier that crude fiber digestibility decreased linearly in response to salinomycin in steers fed 80% concentrate diets containing 0, .4 or .8 $mg \cdot kg^{-1}$ of salinomycin. In our study, while salinomycin in the diet appeared to have no effects on the numbers of cellulolytic bacteria from the rumen of treated cattle, these organisms were completely inhibited when the rumen contents of salinomycin-fed cattle were inoculated into cellulose broth containing as low as .9 $\mu g \cdot ml^{-1}$ of the antibiotics. Dennis et al. (1981a) have estimated that the concentration of lasalocid or monensin in the rumen fluid of cattle fed these antibiotics at 30 g/ton of feed was much higher than the minimum inhibitory concentration (MIC) for the rumen bacteria. Similarly, one would expect that the minimum concentration of salinomycin in the rumen fluid when fed at .22

$mg \cdot kg^{-1} d^{-1}$, would be much higher than the MIC for the sensitive species of cellulolytic bacteria and probably inhibit their cellulolytic activities. However, Bacteroides succinogenes, a major cellulolytic bacteria in the rumen, has been found to be resistant or readily develop resistance to lasalocid or monensin (Chen and Wolin, 1979; Henderson et al., 1981). It seemed possible that the absence of inhibitory effect of salinomycin in vivo might be due to an enhanced role of crude fiber digestion by Bacteroides succinogenes. Also, there may be other complex interactions in the rumen that may prevent the full inhibitory effects of salinomycin on cellulolytic bacteria. Henderson et al. (1981) have reported the inhibitory effect of monensin on the digestion of powdered filter paper and grass incubated with rumen contents while it had no effects on the digestion of cotton fiber and straw.

The higher proportion of resistant amylolytic bacteria in salinomycin-fed cattle as compared to control is suggestive of selection of succinate and propionate producing bacteria. Major amylolytic bacteria, except Streptococcus bovis and Lactobacilli, being gram-negative, are resistant to lasalocid or monensin (Chen and Wolin, 1979; Dennis et al., 1981a; Henderson et al., 1981). The antibacterial spectrum of salinomycin is similar to that of lasalocid or monensin (Taylor and Nagaraja, unpublished observations). It has been suggested that altered rumen fermentation patterns in lasalocid- or monensin-fed cattle is because of the inhibition of rumen bacteria that produce hydrogen, formate, acetate, butyrate, and lactate, and selection of succinate- and propionate-producing and lactate-utilizing bacteria that are resistant to these antibiotics (Chen and Wolin, 1979; Dennis et al., 1981a). The results of this study suggests that similar selection takes place in the rumen of salinomycin-fed animals, as evidenced by increase in the proportion of resistant bacteria in salinomycin-fed cattle as compared to the control.

The effects of lasalocid or monensin on the total numbers of rumen protozoa have been found to depend on the diet. In cattle fed grass hay supplemented with monensin, there was no change in the total number or any discernible shift in any specific type of rumen protozoa (Dinius et al., 1976). But in grain-fed cattle and sheep, monensin depressed the total numbers of rumen protozoa by 4 to 63% (Richardson et al., 1978). In a previous study (Dennis et al., 1981b) monensin, but not lasalocid, had a marked depressing effect on Ophryoscolex. From this study, salinomycin appeared to have only an initial inhibitory effect on Entodinium, which apparently became adapted to the antibiotic within four weeks.

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RUMEN MICROBIAL CHANGES IN CATTLE FED EITHER CORN OR MILO GRAIN

Summary

Four rumen-fistulated Holstein steers randomly assigned to two groups (corn-fed and milo-fed) were used to monitor the effects of type (corn or milo) and level (0 to 100%) of grain in the diet on rumen microbial population and volatile fatty acids (VFA) concentrations. Steers were initially adapted to 100% alfalfa hay diet and then switched every two weeks to 80:20, 50:50, 20:80 and 0:100 hay to grain diet. Rumen fluid samples for total and selection enumeration of anaerobic bacteria, VFA analysis and for counting protozoa were collected on three consecutive days from steers at 100:0, 80:20, 50:50, 20:80 and 0:100 hay to grain diet. Rumen pH decreased with increased grain intake but total VFA concentration remained unchanged. Acetate:propionate ratio was significantly lower in steers on 100% grain diet. Molor proportions of butyrate and isovalerate increased while that of isobutyrate decreased and valerate remained unchanged with increased grain intake. Total anaerobic bacterial colony counts increased with increased grain intake in both groups. Amylolytic, cellobiose-, maltose-, and lactate-utilizing bacterial counts were higher and xylose-utilizing bacterial counts were lower in corn-fed steers than milo-fed steers. Total protozoal counts increased with increased grain intake. Steers fed 100% corn-diet had higher total protozoa and entoiniomorphs particularly that of Entodinium species than steers fed 100% milo diet.

Introduction

Among the dietary factors influencing changes in the numbers and types of rumen microbial populations, the major one is the amount of grain. Changes in rumen microbial population due to high grain feeding include increased total bacterial numbers, due mainly to increase in amylolytic, lactate-fermenting and other aciduric bacteria, accompanied by a decrease in the numbers of cellulolytic

bacteria (Bryant and Burkey, 1953; Slyter et al., 1970; Grubb and Dehority, 1975; Leedle and Hespell, 1980). Also, increase in total rumen protozoal numbers, due to increase in entodiniomorphs has been observed (Abe et al., 1973; Hobson and Wallace, 1982).

It is well recognized that the consumption and digestibility of high-grain diets fed to cattle depends on the type of grain fed (Wise et al., 1968). Corn and milo grains represent about 80% of all-concentrate diets fed to ruminants in the United States (Waldo, 1973). Generally, milo grain is considered to be inferior to corn because of lower digestibility. Dry matter, nitrogen and starch digestibility of milo grain in the rumen have been reported to be lower than that of corn (Oltjen et al., 1967; Hale, 1973; McNeill, 1973; Waldo, 1973). There is very little information as to whether rumen microbial population differs between cattle fed diets that differs in digestibility and in feedlot performance. Slyter et al. (1970) observed that steers fed milo grain contained less ruminal DNA, lactobacilli and viable counts than cattle fed corn. The objective of this study was to monitor the changes in the rumen microbial population in cattle fed either corn or milo grain diet.

Materials and Methods

Animals and Diet. Four rumen-fistulated Holstein steers (mean body weight 511 kg) were randomly assigned to two groups. One group (two steers) was fed corn while the other group (two steers) was fed milo. The composition of the grain diet is shown in table 1. The steers were adapted to 100% alfalfa hay (IFN 1-00-096) for four weeks before feeding the grain diets. The diets were gradually switched every two weeks to 80:20, 50:50, 20:80 hay to grain ratio and finally to 100% grain diets. The steers were fed twice daily.

TABLE 1. COMPOSITION OF THE GRAIN DIET

Ingredients	Corn Diet (% DM)	Milo Diet (% DM)
Corn	93.99	—
Milo	—	97.1
Soybean Meal	3.25	—
Dicalcium phosphate	.27	.56
Limestone	1.14	.95
Salts	.30	.30
Trace mineral salts	.025	.025
Fat	1.0	1.0
Vitamin A, D & E mixture	.03	.03

Sampling Schedule, Collection and Processing of Samples. Rumen samples were collected from each animal before initiating grain feeding (100% hay diet) and thereafter at two week intervals following each diet change. At each sampling time, rumen samples were collected on three consecutive days. Rumen contents were hand mixed as thoroughly as possible at 3 h post-morning feeding and about 500 ml was collected into a rubber stoppered flask and transported immediately to the laboratory. An aliquot of each sample was flushed with oxygen-free CO_2 and blended in a Waring blender for one minute and strained through four layers of cheesecloth. The strained rumen fluid was used to inoculate various media and for volatile fatty acids (VFA) and dry weight determinations. The rest of the unblended rumen fluid was strained through one layer of cheesecloth and used to record the pH and counting rumen ciliates.

Culture Media. The following culture media were used to enumerate rumen bacteria: complete carbohydrate agar (CCA) and selective carbohydrate agar (SCA) media containing xylose, cellobiose, maltose, soluble starch or lactate as the single energy source.

Incubated clarified rumen fluid (ICRF) was used to prepare all culture media and dilution blanks. The ICRF was preparred from rumen fluid obtained from a rumen-fistulated donor cow on 50:50 alfalfa hay to grain diet. The rumen content was taken 3-4 h post-feeding, strained through four layers of cheesecloth and centrifuged at 5500 x g for 15 min. The supernatant was incubated according to the procedures of Allison et al. (1979) and Leedle and Hespell (1980). The incubated mixture contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 5.0 ml distilled water, .05 g L-cysteine hydrochloride, 2.5 g agar, 80 ml centrifuged rumen fluid. The mixture was prepared under oxygen-free CO_2 and the flask was stoppered with a one-way (gravity type)

check valve and incubated for 7 d with constant stirring. The pH of the incubated mixture was checked daily and if need be adjusted to 6.8 with saturated sodium carbonate solution. After incubation, the mixture was centrifuged at 10,000 x g for 15 minutes and the supernatant frozen for media preparation. The sediment containing agar was washed three times by centrifugation. After the final wash, the agar shurry was frozen, lyophylized and ground before using for media preparation.

The cell-free supernatant of the ICRF obtained by centrifuging at 30,000 x g for 30 minutes was used for preparing CCA and SCA media. The CCA medium contained the following ingredients per 100 ml: 7.5 ml each of minerals I and II (Bryant and Burkey, 1953), 35 ml distilled water, 1.0 ml B-vitamin mixture (Scheifinger et al., 1975), 1.0 ml .01% Hemin (Leedle and Hespell, 1980), .05 ml (V/V) glycerol, .05 g each of glycose, xylose, cellobiose, maltose and soluble starch, .2 g trypticase, 50 ml ICRF, 1.0 ml .01% resuzurin, .4 g sodium carbonate and .05 g L-cysteine hydrochloride. The SCA media contained .3% of xylose, cellobiose, maltose, soluble starch or lactate as the single carbohydrate energy source. The media were distributed into roll tubes containing the incubated and washed agar, closed with butyl rubber stoppers, crimped with aluminium seals and autoclaved.

The dilution blank contained mineral I and II, 20% (V/V) ICRF, resazurin, distilled water, sodium carbonate and L-cysteine hydrochloride. The procedure for preparing the pre-reduced media and the dilution blank were according to Hungate (1969) as modified by Holdeman et al. (1977).

Inoculation of Media. A ten-fold dilution of the strained rumen fluid were made and .5 ml of the appropriate dilutions were inoculated into each culture

medium. Four roll tubes were prepared for each dilution. The tubes were incubated at 39 C for 7 d and the colonies counted.

Counting Rumen Ciliates. The rumen fluid strained through one layer of cheesecloth was diluted (1:25) with a protozoan dilution fluid (.05 g methyl green in 900 ml phosphate buffer and 100 ml formaldehyde solution). The ciliates were counted in a Sedgwick-Rafter counting chamber (Fisher Scientific, Lexington, NJ). Identification of the genera was according to Hungate (1978).

Volatile Fatty Acids (VFA) Analysis. The VFA concentration was determined according to the procedure of Erwin et al. (1961). Rumen fluid was acidified with 25% metaphosphoric acid and centrifuged at 18,000 x g for 20 minutes. The supernatant was then analyzed in a gas chromatograph (Hewlett-Packard Model 5890 A, Avondale, PA).

Results

Rumen pH and Volatile Fatty Acids Concentrations. Rumen pH decreased in both corn- and milo-fed cattle, as the level of grain increased in the diet (table 2). The increase was significant in the milo-fed cattle at the 20:80 hay to grain diet and in corn-fed at 100% grain diet. However, the total VFA concentrations remained unchanged ($P>.05$) with increased level of grain feeding. Also, the type of grain had no effect on total VFA concentration. The molar proportion of acetate decreased while that of propionate increased with increased grain intake (table 3). These changes were significant only in cattle on 100% grain diets. The molar proportion of butyrate also increased ($P<.05$) with increased grain intake (table 3). The molar proportion of isobutyrate decreased and isovalerate increased ($P<.05$)

TABLE 2. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN pH AND VOLATILE FATTY ACIDS (VFA) CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Rumen pH		Total VFA (μmole/ml)	
		corn	milo	corn	milo
0	100:0	6.56^{b}	6.77^{b}	122.8	118.6
2	80:20	6.68^{b}	6.79^{b}	116.9	115.8
4	50:50	6.50^{bc}	6.60^{b}	126.8	121.9
6	20:80	6.30^{bc}	6.10^{b}	110.3	137.9
8	0:100	6.02^{c}	5.73^{c}	123.7	128.1
SE		.13		9.4	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM. salts and vitamins A, D & E mixture.

[b,c]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 3. EFFECT OF TYPE AND LEVEL OF GRAIN ON VOLATILE FATTY ACIDS CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Acetate (%)		Propionate (%)		Butyrate (%)	
		corn	milo	corn	milo	corn	milo
0	100:0	69.0[b]	68.9[b]	18.8[b]	18.1[b]	7.4[b]	6.2[b]
2	80:20	68.4[b]	70.8[b]	18.5[b]	18.2[b]	9.2[b]	8.2[b]
4	50:50	67.1[b]	66.7[b]	17.3[b]	18.5[b]	11.3[bc]	11.8[bc]
6	20:80	68.9[b]	64.6[b]	17.3[b]	18.1[b]	11.0[bc]	14.5[c]
8	0:100	56.7[c]	55.4[c]	23.4[c]	22.4[c]	13.6[c]	14.3[c]
SE		1.9		1.1		1.3	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c]Means within the same column bearing different superscripts differ significantly ($P<.05$).

but valerate proportion did not change with increased level of grain in the diet (table 4). Isovalerate proportion tended to be higher in milo-fed than corn-fed cattle. The difference was significant in samples collected at 20:80 and 0:100 hay to grain diet.

Rumen Bacterial Counts. The anaerobic viable counts increased ($P<.05$) in both groups with the introduction of grain in the diet (table 5). The increase in counts observed with increased grain intake was not significant ($P>.05$). Amylolytic bacterial counts increased ($P<.05$) with the introduction of grain in the diet in both groups (table 6). Although the counts were highest at the 100% grain diet, the increase was not significant. However, the proportion of amylolytic bacteria tended to increase as the level of grain in the diet increased. Both numbers and proportion of amylolytic bacteria were higher in all samples in the corn-fed than milo-fed groups, but the differences were not significant. Likewise, maltose-utilizing bacterial counts increased ($P<.05$) with the introduction of grain in the diet and was highest at 100% grain diet in the corn-fed cattle but showed no significant change in the milo-fed cattle. The proportion of maltose-utilizing bacteria appeared to increase in the corn-fed while it tended to decrease in milo-fed cattle. Xylose-utilizing bacterial counts increased ($P<.05$) in both groups as grain was introduced into the diet (table 7) and generally the counts were higher in the milo-fed groups than corn-fed group, especially at 100% grain diet ($P<.05$). However, there was a general decreasing trend in the proportion of xylose-utilizing bacteria in the corn-fed cattle but the proportion decreased ($P<.05$) in the milo-fed cattle with the introduction of grain in the diet and then tended to increase as the level of grain increased. Cellobiose-utilizing bacterial colony counts increased as the level of grain was raised in the diet and the increase was significant at the 100% grain diet (table 7). However, the proportion

TABLE 4. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN VOLATILE FATTY ACIDS CONCENTRATION

Sampling time (weeks)	Diet[a] (Hay:Grain)	Isobutyrate (%) corn	Isobutyrate (%) milo	Isovalerate (%) corn	Isovalerate (%) milo	Valerate (%) corn	Valerate (%) milo
0	100:0	1.3^{bc}	1.3^{b}	1.8^{b}	2.3^{b}	1.9	1.3
2	80:20	1.2^{cd}	1.2^{b}	1.8^{b}	2.3^{b}	2.0	1.2
4	50:50	1.2^{cd}	1.3^{b}	1.9^{b}	2.6^{b}	1.9	.9
6	20:80	1.0^{d}	1.0^{b}	1.9^{be}	2.7^{bf}	1.2	.8
8	100:0	1.0^{d}	$.8^{c}$	3.9^{ce}	4.6^{cf}	1.3	2.5
SE		.1		.2		.7	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c]Means within the same column bearing different superscripts differ significantly ($P<.05$).

[e,f]Significantly different ($P<.05$).

TABLE 5. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN TOTAL ANAEROBIC BACTERIAL COUNT

Sampling time (weeks)	Diet[a] (Hay:Grain)	Anaerobic bacterial count ($X10^{10}$/g) corn	milo
0	100:0	7.6[b]	6.0[b]
2	80:20	15.5[c]	21.2[c]
4	50:50	16.1[c]	16.7[d]
6	20:80	18.6[c]	18.6[d]
8	100:0	25.5[c]	23.3[c]
SE		1.6	

[a] Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c,d] Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 6. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN AMYLOLTYIC AND MALTOSE-UTILIZING BACTERIAL COUNTS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Amylolytic bacterial count ($X10^{10}$/g) corn	milo	Percent of anaerobic bacterial count corn	milo	Maltose-utilizing bacterial count ($X10^{10}$/g) corn	milo	Percent of anaerobic bacterial count corn	milo
0	100:0	1.9^{b}	0.9^{b}	25.1	28.0	2.0^{b}	2.5^{b}	26.8^{bc}	39.9^{b}
2	80:20	6.2^{c}	5.3^{c}	41.5	26.4	5.2^{cd}	5.1^{c}	36.3^{c}	23.5^{c}
4	50:50	5.9^{c}	4.3^{c}	40.0	40.0	4.1^{bc}	3.3^{bcf}	30.2^{bc}	20.5^{c}
6	20:80	5.3^{c}	4.8^{c}	29.4	29.4	3.5^{bc}	3.6^{bc}	18.6^{b}	19.4^{c}
8	0:100	8.4^{c}	7.2^{c}	34.3	34.3	7.0^{d}	4.6^{bc}	27.4^{bc}	20.1^{c}
SE		1.0		5.8		.8		4.4	

[a]Alfalfa hay and grain diet composed of corn or milo grain, SBM, salts and Vitamins A, D & E mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 7. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN XYLOSE- AND CELLOBIOSE-UTILIZING BACTERIAL COUNTS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Xylose-utilizing bacterial count ($X10^{10}$/g) corn	milo	Percent of anaerobic bacterial count corn	milo	Cellobiose-utilizing bacterial count ($X10^{10}$/g) corn	milo	Percent of anaerobic bacterial count corn	milo
0	100:0	1.9	1.9	25.6^{bc}	31.7^{b}	1.9^{b}	1.2^{b}	26.2	21.0^{b}
2	80:20	3.0	4.0	21.3^{cd}	19.2^{c}	3.4^{b}	3.0^{bcf}	23.5	14.3^{c}
4	50:50	2.9	3.6	19.1^{cd}	22.2^{bc}	3.5^{bc}	2.9^{b}	22.8	20.0^{b}
6	20:80	2.8	3.8	15.8^{d}	21.0^{bc}	3.5^{c}	6.4^{c}	19.0	15.6^{bc}
8	0:100	4.6	6.8	18.8^{cde}	30.0^{bf}	5.6^{c}		21.7	28.2^{d}
SE		.3		2.9		.7		4.0	

[a]Alfalfa hay and grain diet composed of corn or milo grain, SBM, salts and Vitamins A, D & E mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

[e,f]Significantly different ($P<.05$).

remained unchanged except for the sample collected at 0:100 hay to grain diet from the milo-fed cattle. The lactate-utilizing bacterial counts and the proportion increased in both groups as the level of grain was raised in the diet and the increase was significant in the diet containing 50% or higher grain (table 8).

Rumen Protozoa. Total protozoal numbers increased in both groups as the level of grain intake in the diet increased (table 9). The increase was significant on the 50:50 hay to grain diet for the corn-fed group but not in the milo-fed group until the 100% grain diet. The numbers were generally higher in the corn-fed group than the milo-fed throughout the experiment and the difference was significant at the 100% grain diets. The holotrichs decreased while the entodimiomorphs increased with increase in grain intake but did not differ between the two groups. The entodiniomorphs showed the same trend as that of the total protozoal numbers.

Among the holotrichs, the numbers of Isotricha remained unchanged but that of Dasytricha tended to decrease as the level of grain intake increased. The decrease in Dasytricha number was significant in the corn-fed group (table 10). Among the Entodiniomorphs, Entodinium showed a similar trend and differences between the two groups as the total protozoal numbers (table 11). Metadinium decreased in both groups with increasing grain intake. The decrease was significant ($P<.05$) in the milo-fed group. Polyplastron and Ophryoscolex increased ($P<.05$) with increase in grain intake until the 20:80 hay to grain diet (table 12). Diplodinium, Epidinium, Metadinium and Ophryoscolex completely disappeared in the cattle fed 100% grain diet (tables 11 and 12).

Discussion

The consumption and digestibility of all-concentrate diet fed to cattle depends on the type of grain fed (Wise et al., 1968). The digestibility of milo grain

TABLE 8. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN LACTATE-UTILIZING BACTERIAL COUNT

Sampling time (weeks)	Diet[a] (Hay:Grain)	Lactate-utilizing bacterial count ($X10^{10}$/g) corn	milo	Percent of anaerobic count corn	milo
0	100:0	0.5^{b}	0.4^{b}	6.4^{b}	7.9^{bcd}
2	80:20	1.0^{b}	1.0^{bc}	6.8^{b}	6.0^{c}
4	50:50	3.5^{cd}	2.6^{c}	23.0^{c}	16.9^{e}
6	20:80	3.0^{c}	2.7^{c}	16.6^{c}	15.4^{de}
8	0:100	5.0^{d}	6.0^{d}	19.1^{c}	25.9^{f}
SE		.6		2.5	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c,d,e]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 9. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN PROTOZOAL NUMBERS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Total Protozoal count ($X10^4$/ml) corn	milo	Holotrichs ($X10^4$/ml) corn	milo	Entodiniomorphs ($X10^4$/ml) corn	milo
0	100:0	7.0^{b}	5.6^{b}	1.8^{b}	1.9^{b}	5.2^{bc}	3.7^{b}
2	80:20	32.7^{b}	23.9^{b}	1.2^{bc}	0.7^{bc}	31.5^{cd}	23.2^{b}
4	50:50	45.9^{c}	31.3^{c}	1.7^{b}	1.5^{bc}	44.2^{dc}	29.8^{bc}
6	20:80	51.4^{c}	41.5^{cd}	1.1^{bc}	1.8^{bc}	50.3^{d}	39.7^{bc}
8	0:100	155.1^{de}	69.5^{df}	0.1^{c}	0.2^{c}	155.0^{efg}	69.3^{cg}
SE		11.2		.4		11.4	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

[e,f,g]Means within the same row under the same parameter bearing different superscripts differ significantly ($P<.05$).

TABLE 10. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN HOLOTRICHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	*Isotricha* (X10⁴/ml) corn	*Isotricha* (X10⁴/ml) milo	*Dasytricha* (X10⁴/ml) corn	*Dasytricha* (X10⁴/ml) milo
0	100:0	.5	.5	1.3[b]	1.4
2	80:20	.5	.2	.7[bc]	.5
4	50:50	.6	.6	1.1[bc]	.9
6	20:80	.3	.6	.8[bc]	1.3
8	0:100	.0	.1	.1[c]	.1
SE		.1		.3	

[a] Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c] Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 11. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN ENTODINIOMORPHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Entodinium (X10⁴/ml) corn	Entodinium (X10⁴/ml) milo	Diplodinium X10⁴/ml) corn	Diplodinium X10⁴/ml) milo	Metadinium (X10⁴/ml) corn	Metadinium (X10⁴/ml) milo
0	100:0	4.0^{b}	3.1^{b}	.1	.0	$.9^{b}$	.3
2	80:20	29.4^{bc}	21.9^{b}	.4	.4	$.6^{bc}$	.6
4	50:50	40.7^{bc}	29.0^{b}	.2	.4	$.4^{c}$	.4
6	20:80	47.3^{c}	37.1^{bc}	.1	.0	$.2^{c}$	.1
8	100:0	155.0^{d}	69.3^{c}	.0	.0	.0	.0
SE		11.4		.1		.1	

[a]Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c,d]Means within the same column bearing different superscripts differ significantly ($P<.05$).

TABLE 12. EFFECT OF TYPE AND LEVEL OF GRAIN ON RUMEN ENTODINIOMORPHS

Sampling time (weeks)	Diet[a] (Hay:Grain)	Polyplastron (X10⁴/ml) corn	Polyplastron (X10⁴/ml) milo	Epidinium (X10⁴/ml) corn	Epidinium (X10⁴/ml) milo	Ophryoscolex (X10⁴/ml) corn	Ophryoscolex (X10⁴/ml) milo
0	100:0	.2[b]	.2[b]	.1	.0	.3[b]	.3[b]
2	80:20	.3[b]	.3[b]	.6	.1	.4[b]	.3[b]
4	50:50	.6[c]	.3[b]	.1	.1	.3[b]	.1[b]
6	20:80	.6[c]	.7[c]	.6	.0	2.0[c]	2.0[c]
8	100:0	.0	.2[b]	.0	.0	.0	.0
SE		.1		.7		.4	

[a] Alfalfa hay and a grain diet composed of corn or milo grain, SBM, salts and vitamins A, D & E mixture.

[b,c] Means within the same column bearing different superscripts differ significantly ($P<.05$).

fed to steers has been observed by many workers to be lower than that of corn grain (Oltjen et al., 1967; McNeill et al., 1971; Rumsey et al., 1970). The 1984 NRC Nutrition Requirements for Beef Cattle publication implied that milo grain is almost 12% inferior to corn grain as fed to beef cattle. Riley (1984) in a recent review of the performance of beef cattle on a number of grains, has suggested that milo grain, on the average, is 93 to 96% the value of corn (on weight basis).

Considering the differences in the digestibility of corn and milo grains, one would expect differences in the rumen microbial population and fermentation products in cattle fed corn or milo grain. Slyter et al. (1970) reported that steers fed milo grain contained less ruminal DNA, Lactobacilli and viable bacteria than steers fed corn, barley or wheat and suggested that this might be the reason why steers fed milo grain had the poorest feed utilization in their trial. This study indicated no differences in the total VFA concentrations from the two groups, the acetate-propionate ratio decreased for both groups at all-grain diets but there appeared to be no significant differences between the two groups. There was a higher anaerobic viable bacterial counts in the corn grain-fed than milo grain-fed cattle at all-grain diets. Also there were higher counts of amylolytic-, cellobiose-, maltose-, and lactate-utilizing bacteria in corn fed than milo fed cattle during the period of grain feeding. The higher bacterial counts may probably be due to the higher digestibility of corn grain starch than milo grain starch (McNeill et al., 1971). Milo grain starch is more resistant to rumen bacterial attack than corn grain starch (Davis and Harbers, 1974). In fact, Waldo (1973) has suggested that milo grain starch is probably the most resistant to digestion of all common feed starch. Diets high in starch result in high numbers of amylolytic bacteria that compete favorably for soluble carbohydrates and the products of starch and hemicellulose hydrolysis. Thus propionate production is increased when cereals are fed not only because of the high starch fermentation, but also the fermentation

products formed from other carbohydrate are altered to favor propionate (Baldwin and Allison, 1983). This observation may explain why there were no significant differences in the rumen fermentation products in cattle fed those two grains.

The increase in the numbers of total protozoa, particularly that of entodiniomorphs, in the rumen with increase in grain intake has been reported by other workers (Abe et al., 1973; Slyter et al., 1970). The corn grain diet consistently supported a higher total protozoal numbers, particularly at 100% grain diet than the milo grain diet. These differences may probably be due to the lower rumen pH of the milo fed than the corn grain fed cattle, at 100% grain diets. Protozoal numbers decrease drastically in the rumen at a pH below 6 (Hobson and Wallace, 1982; Britton et al., 1984). The more readily available corn grain starch may have been engorged by the rumen protoza in corn grain-fed cattle, thus protecting starch from bacterial fermentation and preventing pH to fall below 6 (Russel and Hespell, 1981; Veira and Ivan, 1983). This would therefore keep the protozoal numbers higher.

Among the holotrichs and entodiniomorphs, Dasytricha and Entodinium and Polyplastron species are less sensitive to low rumen pH, but Isotricha and other species of entodiniomorphs are more sensitive to low rumen pH and that may be the reason why they completely disappeared from the rumen of cattle fed 100% grain diets, when the rumen pH was lowest (Slyter et al., 1970).

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EFFECTS OF IONOPHORE ANTIBIOTICS, TYPE AND LEVEL OF GRAIN DIETS ON RUMEN MICROBIAL POPULATION AND FERMENTATION.

A LITERATURE REVIEW.

IONOPHORE ANTIBIOTICS

Feed cost can represent up to 80% of the cost of producing cattle. Because of high cost of production and a low dollar return, there have been wide efforts since the last 25 years to find suitable combinations of nutrients which will increase the efficiency and rate of growth and level of production of animals. These efforts have led to the present use of antibiotics, hormones and other chemicals in animal production. Thus, while these materials are not nutrients and cannot be considered as dietary essentials, it is important to understand their effects on animals and the meat, milk and eggs they produce. It is easy to see why feed additives, which promote improvements in feed efficiency and rate of weight gain are readily adopted by cattlemen.

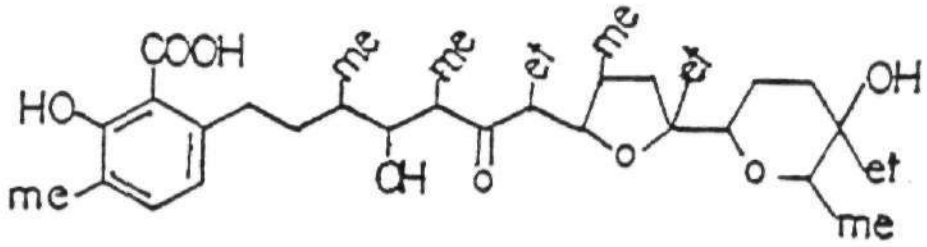
Lasalocid- (Bovatec®, Hoffmann-LaRoche Inc., Nutley, NJ) and Monensin-sodium (Rumensin®, Elanco Products Co., Indianapolis, IN) are two such polyether antibiotics that have been approved and popularly used as feed additives in cattle feeding. Salinomycin is another ionophore that improves beef cattle performance (McClure et al., 1980) and produces alterations in rumen fermentation similar to monensin (Fonentot et al., 1980). Salinomycin has only been studied to a limited extent and has not been approved as a feed additive in cattle feeding. Narasin is another ionophore that appears to mimic monensin (Potter et al., 1979). Both antibiotics appear to be more potent than monensin. Avoparcin is a glycopeptide antibiotic that produces performance responses in beef cattle (Ingle et al., 1978), similar to that of ionophore antibiotics.

Lasalocid, monensin and salinomycin belong to the valinomycin class of antibiotics produced by strains of Streptomyces. Along with about 76 others, they are classified as ionophores because of their ability to

facilitate the transport of cation across lipid barriers including artificial and biological membranes (Pressman, 1973). The ionophores catalyze transport by (a) enveloping an ion at a membrane interphase with a consequent dehydration of the ion (b) diffusing across the membrane as a cation complex (c) releasing the ion which undergoes concomitant rehydration at the opposite interphase and (d) diffusing back uncomplexed to the original interphase to complete the catalytic circle. Thus, ionophores function as mobile carriers for cations in membranes (Pressman, 1973). The mechanism of ionophore interaction with biological membranes has been extensively reviewed by Reed (1982). This literature review only attempts to cover the work done on lasalocid, monensin and salinomycin as representatives of the ionophore antibiotics.

Structure and Properties of Lasalocid, Monensin and Salinomysin. Lasalocid, monensin and salinomycin are structurally very similar (Figs. 1,2&3). The sodium salt of lasalocid has a molecular weight of 613 with an emperical formula of $C_{34}H_{53}O_{11}.Na$ (Westley 1982), monensin-sodium has a molecular weight of 692 and an emperical formula of $C_{36}H_{61}O_{11}.Na$ (Westley, 1982), while salinomycin has a molecular weight of 772 and an emperical formula of $C_{42}H_{69}O_{11}.Na$ (Miyazaki et al., 1974). They are very slightly soluble or insoluble in water but are quite soluble in most organic solvents. These antibiotics have been found to be generally active against gram-positive bacteria including Mycobacterium, but are inactive on gram-negative bacteria and fungi (Westley, 1982; Miyazaki et al., 1974).

Approved Usage in Livestock. Lasalocid and monensin were initially marketed as anticoccidial drugs in poultry. Lasalocid is currently marketed



LASALOCID

Fig. 1.

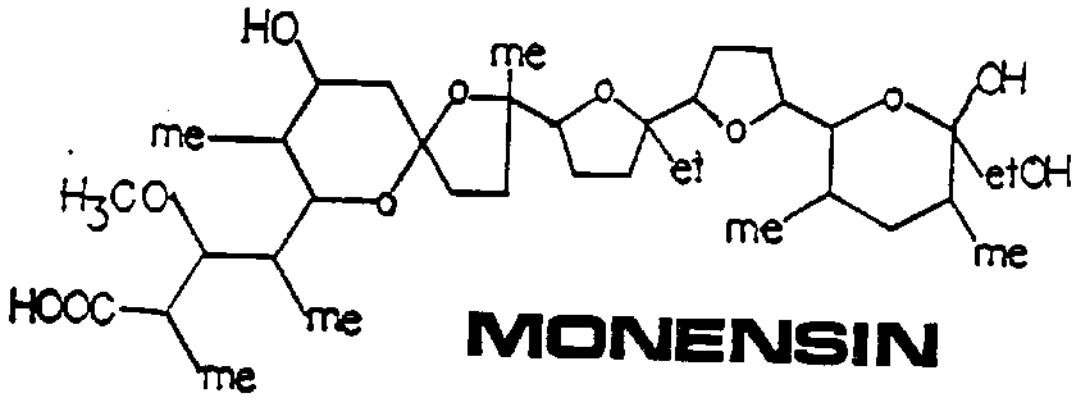


Fig. 2.

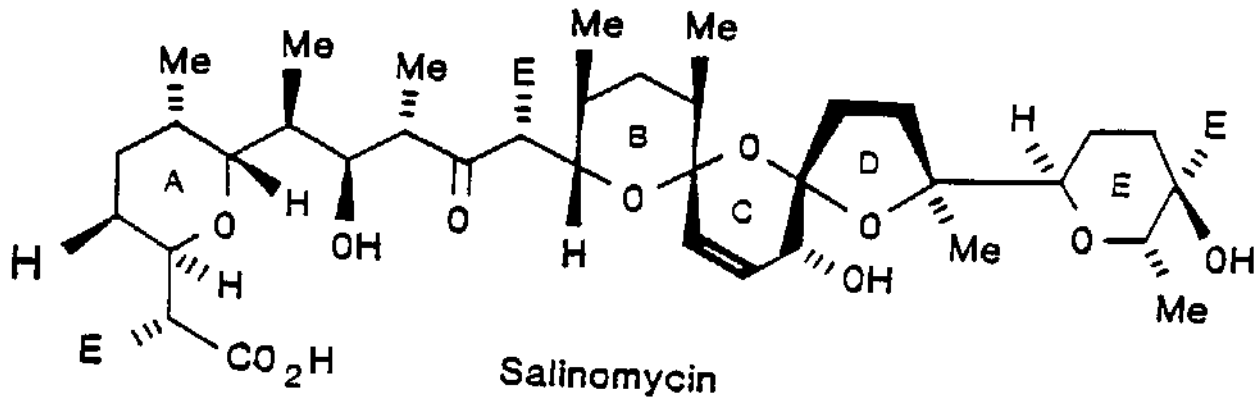


Fig. 3.

as Bovatec® 68 medicated premix - a light brown free-flowing powder containing 68 g (15%) lasalocid sodium per 454 g in a ground rice hull carrier. Rumensin® premix is currently marketed by Eli Lilly Co. and has a concentration of 60 g/454 g of carrier and has a blackish to brown, tan-speckled meal appearance. Both Bovatec® and Rumensin® are marketed in 23 kg (50 lb) multiwall bags. Both are included in cattle diets at the rate of 30 to 33 g/ton of feed. Salinomycin is being marketed as a coccidiostat by the trade name of BioCox® (1985 Feed Additive Compendium) and the premix being tested (A. H. Robins Co., Richmond, VA) contains 30 g/454 of salinomycin activity. It has a brown to tan-speckled meal appearance and at the moment it is only used in cattle feed for beef cattle production on an investigational purposes, since it has not been officially approved as feed additive.

Presently, Bovatec has been cleared for use in feedlot cattle diet at levels of 25 to 30 g/ton of feed. The claim is for the improvement of both feed efficiency and rate of weight gain. Roche has also obtained a subsequent clearances for cattle on pasture and liquid feed. A clearance for feed efficiency imporovement has a range of 10 to 30 g/ton of feed (1985 Feed Additive Compendium). Rumensin has several clearances, since it has been on the market for a considerable period. For confined beef cattle for slaughter, monensin has a general claim for feed efficiency improvement at 5 to 30 g/ton of complete feed. For slaughter stocker and feeder cattle weighing more than 400 lb on pasture, monensin has a claim for increased rate of weight gain at 5 to 30 g/ton of feed. In this regime, cattle should receive no more than 100 mg/head/day for the first five days. Following this adjustment period, the cattle should receive 200 mg/head/day of monensin. Monensin can also be added to liquid feed with

the following conditions: (1) for only cattle being fed in confinement for slaughter (2) expiration date of eight weeks from the date of manufacture (3) minimum of 20% molasses in combination with specific ingredients (4) pH of 4.3 to 6 and (5) agitate well before use. Monensin has also got a combination clearance with tylosin for feedlot cattle. Monensin is cleared at 10 to 30 g/ton for improvement of feed efficiency with tylosin at 10 g/ton for reduction of incidence of liver abscess. Salinomycin has been found to improve feed efficiency and rate of weight gain at the rate of 5 to 10 g/ton of feed (Zinn and Axe, 1983; Ferrel et al., 1983; Merchen and Berger, 1985).

Effect of Lasalocid, Monensin or Salinomycin on Cattle Performance. Lasalocid, monensin and salinomycin improved the feed efficiency and daily weight gain in cattle (Wedegaertner et al., 1983; Goodrich et al., 1984; Zinn and axe, 1983; Heinemann, 1984; Merchen and Berger, 1985). Grutter et al. (1975) found that 10 g/ton of rumensin in the feed increased daily gain by 5% and feed efficiency by 4%, while 30 g/ton of feed increased daily weight gain by 1% and feed efficiency by 9% in growing cattle. During high concentrate feeding phase which followed, 10 g/ton improved daily gain by 4% and feed efficiency by 6% while 30 g/ton of feed improved daily gain by 1% and feed efficiency by 11%. Cattle fed rumensin consumed 6.4% less feed and had gains equal to or better than those on the control diet (Goodrich et al., 1984).

Increasing Rumensin® dosage caused decreased feed intake, but average gain tended to increase slightly at 11 ppm and was approximately equal to that of the control steers at 33 ppm (Goodrich et al., 1984). The optimal level for feed efficiency response was reported to be at 33 ppm, which is equal to 300 mg/head/day, assuming 20 lb consumption or 30 g/ton of feed.

The increase in performance with Rumensin® in the ration is additive to the metabolic effects of implants such as DES, Ralgro and Synovex (Goodrich et al., 1984; Heinemann, 1984). The response would be as expected, since the mode of action of these compounds are different.

In contrast to cattle fed high concentrate diet, there is an increased average daily weight gain for roughage-fed cattle (Goodrich et al., 1984). The difference observed in gain between pasture and feedlot studies has been explained by Potter et al. (1976). On high roughage diets, feed intake is limited by the capacity of the reticulo-rumen. The amount of roughage consumed depends on the initial bulk of the feed and the rate and extent to which it is digested. Forage with comparatively high nutritive value will be digested rapidly in the rumen, thus more can be consumed and the rate of weight gain should increase. Once the energy density of a diet increases beyond a point, chemostatic factors limit its intake. According to Potter et al. (1976), increased energy availabilty due to the rumensin in roughage diets will tend to increase gains with consumption being the same, while in high concentrate diets, the increase in energy density will decrease consumption. The amount of energy retained will however remain constant, since intake is controlled by available energy, while less feed will be required. A major problem with feeding Rumensin® to pasture cattle is the delivery system. If the cattle are going to be regularly fed with a

daily supplement, there may be no problem in incorporating Rumensin into the supplement. Feeding a daily supplement is often not practicable. Rumensin incorporated into a molasses block, have shown mixed results (DeMuth et al., 1976; Neumann, 1976).

Isichei et al. (1977) observed that monensin improved feed efficiency without affecting feed intake. The adjusted DM digestibility was increased from 73.3% (control) to 75.9% in monensin-fed cattle. The percentage digestible energy (DE) was also increased from 71.8% (control) to 74.5% in monensin-fed animals (Wedegaertner and Johnson 1983). The increase in DM and DE were largely accompanied by an increase in NDF, which increased from 50.5% (control) to 57.7% in monensin-fed steers. Rust et al. (1979) determined that the digestibility of starch (not adjusted to level of intake differences) was increased from 96.5% to 97.5%, when monensin was added to a corn-based diet fed to 300 kg steers. This must be due to less intake (12.3% of DM) in the monesin-fed cattle than the control in this experiment. The ratio of ME to DE was higher when the steers were fed monensin than when they were not. There was also a trend towards an increase in the ration as intake increased. A higher proportion of dietary GE was also stored by cattle while consuming monensin (Wedegaertner and Johnson 1983). Rumensin has been found to have no effect on the carcass composition of cattle (Gruetter et al., 1976). However, Goodrich et al. (1984) reported that the effect of monensin was highly variable. Monensin lowered dressing percentage and fat depth more when measured values were high than when they were low. Marbling score was depressed most when measured values were low. The improvement in feed efficiecy realized by feeding monensin is not due to a change in the energy content of the carcass but the addition allows ruminants more energy from their feed

daily supplement, there may be no problem in incorporating Rumensin into the supplement. Feeding a daily supplement is often not practicable. Rumensin incorporated into a molasses block, have shown mixed results (DeMuth et al., 1976; Neumann, 1976).

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(Gruetter et al., 1976).

Salinomycin has also been found to increase average daily weight gain and improve feed efficiency in cattle (Webb et al., 1980; McClure et al., 1980; Owens et al., 1982; Zinn and Axe, 1983; Merchen and Berger, 1985). Crude protein digestibility increased while crude fiber digestibility decreased linearly as salinomycin intake increased, but did not affect nitrogen retention (Webb et al., 1980; Zinn and Axe, 1983). Merchen et al. (1985) however found no effect on crude fiber, ADF and organic matter digestibiity when salinomycin was fed. Ruminal pH was not consistently affected but the ammonia level was decreased (Webb et al., 1980; Nakashina et al., 1982; Fontenot et al., 1980; McClure et al., 1980). Improved feed efficiency was best noticed at a concentration of 15-33 g/ton of feed (Webb et al., 1980; McClure et al., 1980; Owens et al., 1982). Hoowever, better performance in daily weight gain was observed at a lower concentration of 5-10 g/ton of feed (Owens et al., 1982).

Effect of Lasalocid, Monensin or Salinomycin on Rumen Microbial Flora and Fermentation. The effects of lasalocid or monensin on rumen microorganisms have been studied in vitro mainly with isolated or mixed cultures of most important species of rumen bacteria (Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). Monensin had no significant effect on the total bacteria concentration in the rumen (Dinius et al., 1976; Dawson and Boling, 1983), but there was a shift to the gram-negative bacteria in the rumen of monensin-fed cattle (Haney and Hoehn, 1968; Richardson et al., 1974; Westley et al., 1983). Lasalocid, monensin or salinomycin are generally effective against gram-positive bacteria (Westley et al., 1973; Miyazaki et al., 1974; Westley, 1977), the only exception was

the resistance of some strains of Streptococcus bovis to monensin (Bartley et al., 1983). Among the gram-negative bacteria, Butyrivibrio fibrisolvens, Ruminococcus albus and R. flavefaciens were sensitive to lasalocid or monensin, which was probably due to their gram-positive-like cell wall (Patterson et al., 1975; Cheng and Costerton, 1977; Chen and Wolin, 1979).

The effect of lasalocid or monensin on rumen bacteria appeared to be bacteriostatic rather than bacteriocidal. Bartley and Nagaraja (1982) observed that after 24 hours of incubation, viable cells were found in the media containing double the minimum inhibitory concentration of lasalocid or monensin, as evidenced by their growth when inoculated into media containing no antibiotics. Dawson and Boling (1983) have suggested that these monensin resistant bacteria may be present in greater number in the rumen of the animals fed monensin-supplemented diet

Recent in vitro studies of representatives of rumen isolates have shown that hydrogen-, formate-, acetate-, butyrate- and lactate-producing bacteria tend to be inhibited by lasalocid or monensin, whereas succinate- and propionate-producing bacteria and lactate-utilizing organisms are resistant or rapidly develop resistance to these antibiotics (Chen and Wolin, 1979; Dennis et al., 1981; Henderson et al., 1981). As a result of their studies, Chen and Wolin (1979) have suggested a model in which lasalocid or monensin induce changes in rumen fermentation. The Ruminococcus and Butyrivibrio are inhibited by very low concentrations of these antibiotics. These species are very important producers of acetate, butyrate and substrates for methanogens, H_2 and CO_2. Selenomonas are very insensitive and Bateroides, although sensitive, rapidly develop resistance to the antibiotics. Both organisms are important in the production of propionate. The methanogens are inhibited by the antibiotics but only at a

much higher concentrations than are necessary for the inhibition of Ruminoccocus or Butyrivibrio. Wolin and Miller (1983), believed that the antibiotics affect rumen fermentation by selecting against organisms that participate in the production of relatively more acetate, butyrate and precursors of methane and for those that produce propionate. Methane decrseased not by a direct effect of the antibiotics on the methanogens, but because the organisms that are major producers of the precursors of methane are inhibited by the antibiotics.

Bulla and Byrant (1980) showed that Bacteroides ruminicola and Butyrivibrio. fibrisolvens were the major ruminal bacteria in steers fed high-roughage diet. When lasalocid or monensin was added, B. fibrisolvens decreased while B. ruminicola increased and B. succinogenes became the more predominant cellulolytic bacterial species. This results were consistent with the predictions of the in vivo effects, based on the pre-culture studies of activity of these antibotics (Chen and Wolin, 1979).

Dawson and Boling (1983) however, have observed that although monensin increased the resistant bacterial population in the rumen, the change was not correlated with the characteristic decrease in acetate:propionate ratio. Significant increase in resistant bacterial population was not found immediately after monensin was included in the diet and remained high for more than 18 days after it had been withdrawn from the diet. In contrast, changes in acetate:propionate ratio were observed before the increase in the bacteria resistance, suggesting that rumen metabolic changes were not necessarily associated with increased proportions of monensin resistant bacteria. Van Nevel and Demeyer (1977) found that one of the important properties of monensin was to inhibit microbial growth yield. They also

observed that the growth inhibition was not accompanied by a decrease in fermentation activity. The part of energy (ATP) generated during fermentation and normally used for maintenance and growth of microbial cells must be used for other purposes when monensin is added to the substrate. Van Nevel and Demeyer (1977) were therefore tempted to suggest that monensin uncouples growth from fermentation. The mechanism of this action could be related to altered cell membrane activity, since monensin is known to be an ionophore (Pressman and deGuzman, 1975). The effect of ionophores on rumen fermentation may therefore be to alter metabolic pathways of rumen bacteria (Bergen and Bates, 1984).

Van Nevel and Demeyer (1977) did not find any direct toxic effect of monensin on the methanogen flora, since no effect on methane formation was observed when gaseous carbon dioxide and hydrogen were supplied as substrates. However, with formate as a substrate, inhibition of methanogenesis was observed. This indicates that monensin superficially inhibits formate decomposition, whether added as a substrate or formed in the pyruvate lyase reaction during carbohydrate fermentation. An increase in propionate production accompanying inhibition of methanogenesis reflects a shift from the flow of electrons from formate and methane to succinate or propionate as alternate electron sink products in the mixed microbial population.

Thus, One of the most consistent changes occuring when lasalocid, monensin or salinomycin is added to a ration is to shift the molar concentrations of the VFA components, with no change in the total amount of VFA produced (Goodrich et al., 1984; Schelling, 1984; Fonentot et al., 1980; Zinn and Axe, 1983; Merchen and Berger, 1985).The molar proportion of propionate is usually consistently increased while that of acetate is

decreased,thus reducing the acetate:propionate ratio. Dawson and Boling (1983) found that the decreased acetate:propionate ratios were only observed during the period that the antibiotics were included in the ration and suggested that this was directly associated with monensin supplementation. It has been observed that monensin has an effect on the pathway in which propionate is produced. Propionate can be produced in two ways, either by the succinate or acrylate pathways, with the succinate more important. When monensin is added to a diet, propionate production via the acrylate pathway tended to become more predominant (Beede and Farlin, 1975; Raun et al., 1976).

Lasalocid or monensin had no effect on the total numbers of protozoa in the rumen of animals fed the supplemented diets (Dinius et al., 1976; Potter et al., 1976a). Bartley and Nagaraja (1982) monitored the rumen protozoal population in six fistulated cattle on 80% alfalfa hay and 20% grain diet for a period of 20 weeks and found that lasalocid had no effect on the total number. However, there was an initial decrease in the total number in monensin-fed animals, but during the subsequent weeks it returned to normal. Among the entodiniomorphs, Epidinium disappeared completely in both lasalocid- or monensin-fed animals, even one month after the antibiotics were withdrawn from the feed. One week after lasalocid feeding, Ophryoscolex disappeared, but subsequently the number reached normal level, but in monensin-fed cattle, they were not detected as long as the antibiotic was admnistered to the cattle. The effect on Ophryoscolex appears to be an important difference between lasalocid and monensin. With both drugs, Diplodinium decreased at the beginning and later returned to normal. Richardson et al. (1976) reported that Diplodinium was more resistant to monensin than any other species. Richardson et al. (1978)

observed that under different feedlot conditions, monensin may depress rumen protozoa by 4 to 63% in steers and sheep on pasture. Sakauchi and Hoshino (1981) also found that dietary monensin tended to decrease rumen protozoa content without markedly altering protozoal composition.

Another very interesting effect of monensin is the considerable decrease in the protein degradation in incubation with casein (Van Nevel and Demeyer 1977). This effect is in line with the lowered rumen ammonia level found in vivo (Dinnus et al., 1976). Accumulation of alpha-amino nitrogen as an intermediate indicates a faster rate of proteolysis than the rate of amino acid metabolism (Blackburn, 1965). The overall effect on casein fermentation suggests that deaminase activity is more inhibited than proteolysis, although it is possible that monensin has an inhibitory effect on peptide transport into the microbial cells, since bacteria can only use ammonia or peptides as nitrogen source (Bryant, 1970). All available information strongly suggests that besides the influence of monensin on fermentation, the beneficial effect of this drug may be the inhibition of feed protein degradation in the rumen, thus altering the final site of protein digestion in the animal (Schelling, 1984). This is however coupled with decreased efficiency of rumen microbial protein synthesis, which could be a disadvantage to the animal, depending on the nature of the diet and the amount of protein escaping rumen degradaion.

Effect of Lasalocid, Monensin and Salinomycin on Animal Health. Aside from improving feed efficiency, the antibacterial and coccidiostat properties of these antibiotics have the added advantage of improving the health of the animals and therefore better performance. Levels of 5 g/ton of feed are sufficient to suppress the number of oocyst in cattle and sheep. Eimeria

bovis has been controlled with lasalocid or monensin in calves (Fritzgerald and Mansfield, 1973) and in sheep, E. ovina, E. shata, E. ninakohlyakinorae, E. parva and E. pallida have been effectively controlled by feeding monensin at or below the levels required for maximum response in feed efficiency (Horton, 1982). Salinomycin has been found to be effective against coccidiosis in sheep and steers (Merchen and Berger, 1985).

Although lasalocid and monensin depressed feed intake, they were found to have no adverse effects on the health of calves received at the feedlot (Prigge et al., 1975). Calves also appeared to recover rapidly from stress of relocation and processing when fed salinomycin (Zinn and Axe, 1983). A slight increase in the number of condemned liver (3%) due to abscesses has been reported in monensin-fed cattle (Prigge, 1976) and salinomycin-fed cattle (Owens et al., 1982; Heinemann, 1984). Although this increase was not much, its potential economic input could be great in the light of the widespread use of the antibiotic. However, the clearance of monensin at the rate of 10 to 30 g/ton, to be used with tylosin at the rate of 10 g/ton of feed has decreased the incidence of liver abscesses.

Lactic acidiosis, which is a serious problem of feedlot cattle has been controlled by feeding lasalocid or monensin (Dennis et al., 1981; Nagaraja et al., 1981). Feedlot bloat, another serious problem of the feedlot enterprise has been controlled by feeding lasalocid or monensin. Bartley et al. (1983) found that 300 mg/kg of lasalocid was sufficient in reducing bloat, but 600 mg/kg was more effective. Both lasalocid and monensin reduced the severity of bloat but lasalocid appeared to be better. It was pointed out that the difference in the lasalocid effectiveness may, in part, be attributed to its ability to inhibit the growth of all important strains of S. bovis.

Lasalocid and monensin have been shown to reduce the in vitro and in vivo rumen production of 3-methylindole (3MI) (Carlson et al., 1983; Carlson and Breeze, 1984; Honeyfield et al., 1985), and therefore the incidence of acute bovine pulmonary edema and emphysema (ABPE) (Hammond et al., 1980; Hammond et al., 1982; Carlson et al., 1983; Nocerini et al., 1985). There are strong evidences to show that 3MI may be the cause of ABPE (Yokoyama et al., 1975; Carlson et al., 1975). The feeding of lasalocid or monensin to cattle at the recommended levels is quite safe (Potter et al., 1984). Rumensin fed to cattle at levels up to 100 g/ton of feed for a period of 160 days showed no changes indicative of toxicity from symptoms, hematology, serum chemistry, urinalysis or organ weight parameters. No gross or microscopic tissue changes relating to treatment were found (Van Duyn, 1975). However, the 100 g/ton reduced gain. When lasalocid was fed at the rate of 150 g/ton of feed to cattle for 182 days, only slight diarrhea, which lasted for 3 days, was observed. No other clinical signs were noticed from symptoms, biochemical and cellular profiles (Messersmith and Hanson, 1982). Monensin has been found to be toxic to horses and lasalocid and monensin toxic to pigs (Messersmith and Hanson, 1982). There is yet no report in the literature on the toxic effect of salinomycin in cattle or any other species of animal.

INFLUENCE OF TYPE AND LEVEL OF GRAIN DIETS ON RUMEN MICROBIAL POPULATION

Much information has been accummulated on the effects of diet on ruminal microorganisms. Early observations indicated that a lot of fluctuations in numbers and types exist when different diets were fed (Bryant and Burkey, 1953, Maki and Foster, 1957, Warner, 1962, Thorley et al., 1968, Latham et al, 1971, Abe et al., 1973). Gall et al., (1949) found no differences in number of bacteria present in the rumen of sheep and cattle fed practical farm rations, except an increase in the number of fast-growing organisms, with an increasing amount of grain in the ration. Rapid changes were also observed in the ruminal flora when large amount of grain or glucose was placed in the rumen of sheep that had been maintained on a ration of alfalfa hay (Hungate et al., 1952). The changes tended towards a rapid increase in Streptococcus bovis and a decrease in the other kinds of rumen bacteria.

Bryant and Burkey (1953), found little difference in bacteria numbers from the rumen of cattle on diets other than concentrates. The number of culturable bacteria was higher when concentrate diet was fed and became progressively higher with the length of time the animals were on the diet. They found that a straw diet gave colony counts of about the same number as diets on hay and concentrate or hay alone and concluded that the level of feeding the hay-concentrate diet had little effect on the microbial numbers grown. However, when alfalfa hay or alfalfa hay-concentrate diets were fed there were relatively more groups of bacteria present and in higher numbers, with no particular dominant groups. Wheat straw presented a far less complex and fewer groups. All the groups found in straw-fed animals were also found in the animals fed alfalfa hay or alfalfa hay-concentrate

diet. The cocentrate diet also supported a less complex flora than the alfala hay diet, but it contained more groups of bacteria than the straw diet with a tendency towards fewer predominant groups.

Effect of Physical Chacteristic of Diet on Rumen Microbial Population.

The physical characteristic of the diet fed may also affect the microbial population in the rumen. Thorley et al., (1968) found a significantly higher viable counts in animals fed ground grass as compared to those fed long grass. They indicated, however, that the significantly higher count might be due to interactions betwen physical characteristic of the diet fed and the type of culture medium used. They therefore cautioned that these definite differences did not necessarily mean that the actual numbers of bacteria were higher when ground grass was fed. Slyter et al., (1966) showed with in vitro cultures of rumen ecosystem that, with the pH maintained at 5.5, there was a greater increase in total viable cells but a small decrease in DNA as compared to systems with the pH at 6 or 6.7. One possible explanation of the work of Slyter et al., (1966) is that with the drop in pH, a much larger proportion of the organism selected by the system became viable in the culture medium. It is possible that such factors may explain the greater increase in the colony counts when ground grass was fed.

Maki and Foster (1957) have reported that the viable bacterial counts in the rumen contents of cows fed a high roughage diet represents only about 3 to 12% of the number determined by direct counts, whereas 57 to 73% of the bacteria from cows that were fed a diet without roughage (grain and alfalfa haymeal), could be cultured. A similar proportion was also obtained by Bryant and Burkey (1953) from all concentrate-fed animals. However, they

also observed that the percentatge value of viable counts was similar to that of direct count, about 8.4%, when animals were fed all roughage or a mixture of roughage and concentrate diets. A possible explanation proposed for this discrepancy between direct and viable counts is that direct counts measure dead cells and those that are adhering to cell-walls which cannot be grown on artificial medium (Hungate, 1966). The difference between roughage- and concentrate-fed animals must then be attributed to a lower proportion of these two types of cells in the concentrate-fed animals, a shift in the population to more organisms that can be grown or possibly sequestration of cells in the fibrous materials in hay-fed animals that are not readily disrupted by mixing.

Effect of Type of Grain Diet. The type of grain in the concentrate diet seems to have some effect on the numbers and types of rumen bacteria. Slyter et al. (1970) found that the numbers of viable bacteria were higher in steers fed corn but lower in steers fed wheat when DES was part of the treatment. The number of viable bacteria was also less in steers fed milo than those fed other grains. This trend in steers fed milo was also reflected by the DNA content but not by the total bacterial numbers. There were some differences in the distribution of morphological groups of bacteria, when different grains were fed (Slyter et al., 1970). The steers on milo contained greater cocci concentration but due to too much varibility this was not significant. In cattle fed wheat, 13% of the gram-negative rods were large curved rods. The rods were similar to the shape of Selenomonas ruminantium. In steers fed corn, barley and milo, those large curved rods comprised 40, 25 and 28% of the gram-negative groups, respectively.

Effect of Diet on Bacterial Physiological Characteristics of Bacteria. The

physiology of bacteria isolated from the rumen of cattle fed all-concentrate diet ad libitum, differed from that of cattle fed maintenance levels of roughage or roughage and concentrate diets (Bryant and Burkey, 1953). The numbers of amylolytic bacteria cultured varied with the type of diet fed, being low on the straw diet, intermediate on the alfalfa and alfalfa-concentrate diets and highest on all concentrate diet. The percentage of the strains capable of fermenting glucose and cellobiose were high in all diets. However, the number of cellobiose fermenters cultured was depressed on all-concentrate diet. Cultures producing acid from xylose were fewer in number than those producing acid from glucose or cellobiose, but higher in the straw diet which contained more xylan than the other diets.

Dehority and Grubb (1976), examined the percentage of rumen bacteria that utilized starch, xylan, and pectin in two sheep, one on 60:40 concentrate to roughage diet and the other on 100% orchardgrass hay. The total count and the percentage starch digesting bacteria were significantly higher in the sheep fed the 60:40 concentrate to roughage diet. The mean percentage of xylan- and pectin-utilizing bacteria were not different, however, daily variations tended to be greater for these two carbohydrates. In the concentrate fed sheep, the percentage of starch-utilizing bacteria was significantly higher than those for xylan- and pectin-utilizing bacteria. There was no difference in these three carbohydrate group in sheep fed 100% orchardgrass hay.

Leedle and Hespell (1980), observed the same trend of much higher total direct counts and culturable colony counts in the rumen of cattle on high grain diet as compared to the counts from cattle on high roughage diet. They explained that the resident rumen bacterial population in the animal

on high roughage diet reflected the high proportion on cellulosic materials and lower proportion of readily digestible feedstuff, since the cellulolytic and xylanolytic bacteria were the predominant groups. However, the colonies on soluble starch medium were numerically grater than either of these groups within a given diet and reflect the general capacity of many rumen bacteria to ferment or hydrolyze starch or to utilize its breakdown product, maltose. with the high grain diet the rumen bacterial population was predominantly soluble carbohydrate utilizers.

The proportion of bacteria in steers fed all-carbohydrate diet ad libitum that was amylolytic and/or produce acid from glucose was greater than those that were cellulolytic (Slyter et al., 1970). Perhaps as the quantity of starch and sugar increases in the rumen, bacteria that are capable of converting starch and sugars to their final fermentation end products are increased, at the expence of bacteria producing metabolic intermediates. Presumably, increased intake of rapidly fermentable substrate could bring about a reduction in cellulolytic bacteria by lowering the pH of the ruminal content (Krogh, 1961) and/or by increasing other bacteria in the rumen which could in turn deplete required nutrients for the cellulolytic bacteria (Bryant, 1973). The concentration of cellulolytic bacteria, as a function of pH shows that the most consistent effect upon these bacteria appears to be at a pH below 5.2. At less than pH 5.2, cellulolytic bacteria were less numerous and lactobacilli and aciduric bacteria were more numerous at that pH (Slyter et al., 1970).

Effect of Concentrate Diet on Rumen Protozoal Population. The type and level of concentrate in the diet also affect the rumen protozoan population. When high levels of concentrates were fed to cows, there was an increase in the protozoan number, which tended towards an increase in the

Entodiniomorph fraction in proportion to the concentrate level in the diet, with no tendency in the number of Holotrichs (Abe et al. (1973).These workers found no significant change in pH, VFA, or ammonia concentrations and they concluded that the presence of protozoa in the rumen did not appear to be related to the VFA concentration or the bacterial population patterns. However, the protozoal concentration increased in all sheep when available energy intake was raised (Warner, 1962, Nakamura an Kanegasaki, 1969, Abe et al., 1973, Grubb an Dehority, 1975). This increase became significant seven days after changing to high concentrate diet (Abe et al., 1973, Grubb and Dehority, 1975), with a three-fold increase in Entodiniomorphs and a slight increase in Holotrichs (Abe et al., 1973).

The protozoan number became less in cattle fed all-concentrate diet ad libitum as compared to when they were fed restricted diets (Slyter et al., 1970). They could only detect Entodiniomorphs protozoa in steers fed wheat or wheat and corn. Protozoan numbers were lower though not significant in steers fed wheat, when the rumen pH was lowest. Holotrichs were detected in only one steer fed wheat and the number was 1.1 and 3.8% Isotricha intestinalis and Dasytricha ruminantium, respectively, of the total protozoal counted. These findings were consistent with the results obtained by Chou and Walker (1964), Christiansen et al., (1964), Purser and Moir (1959) and Slyter et al., (1968). Eadie et al., (1967) had suggested that the inclusion of dried grass or hay in the diet may enhance the growth of rumen protozoa.

All Entodiniomorph protozoa, except Entodinium utilize starch, whereas Holotrichs only assimilate soluble sugars (Hungate, 1966). Entodiniomorphs are therefore usually aboundant in the rumen of animals given a high concentrate diet and Holotrichs appear in greater numbers in animals given

hay or forage rich in soluble sugars (Warner, 1962).

Effect of Diet Change on Rumen Microbial Fluctuations. When animals are changed from one diet to the other, a period of micrbial adaptation occurs, which can be defined as the interval required for the rumen microbial population to stabilize. Several investigators have estimated the lengh of this adaptation period by measuring the digestibility data as a relative indictors of rumen microbial activity (Hall and Woolfolk, 1952, Lyod et al., 1956, Nickolson et al., 1956). In general, results of these studies indicated that the length of time required for adaptation depends upon how radical a change is made in the diet.

Warner (1962), reported changes in the rumen microbial concentrations of a single sheep when changed from roughage to a concetrate diet at a fixed level of intake. He observed that 10 days was required for microbial changes to occur and concentrations were stable after that time. These findings differ from the results of Grubb and Dehority (1975), in which the bacterial concentrations appear be increasing still after 14 to 21 days of diet change, but the protozoan population had stabilized after 5 days.

Animal Variations in Rumen Microbial Population Changes. A fairly wide variations in microbial populations between animals fed the same diet, suggest that animal plus some other factors, apart from the diet fed, may have some important effect on the number and occurence of different groups of bacteria in the rumen when grain level in the diet changes, (e.g. wheat, corn or milo). These animal factors are not known. Warner (1962) has suggested that there appeared to be a tendency for the ruminant fed a particular diet to re-establish some organisms in concentrations characteristic for that particular animal after its ingesta were exchanged with another animal. Perhaps other factors to consider include the type of

diet the animal was fed before an indentical diet is fed and the environment under which the animal was raised. The age of the animal and the breed seem to be of importance too.

These factors that cause variability in the ruminal microbial concentrations between two animals may be responsible for the different responses to changes in rumen microbial populations when animals are placed on the same diet. For example, an increase in bacterial colony counts in response to increased intake of available energy was reported by Bryant and Burkey (1953) and Maki and Foster (1971). In contrast, Latham et al., (1971) did not find any increase in bacterial concentrations in the rumen when available energy increased. Kern et al., (1973) found no increase in the bacteria numbers in steers' ruminal ingesta when oats was added to forage diets. Grubb and Dehority,(1975) found different responses from animal to animal, in viable bacterial numbers when sheep were abruptly changed from all-roughage to high-concentrate diets. A need exists to more fully establish the degree to which the animal exert control over its ruminal bacterial population as supposed to the effect that feed exerts.

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APPENDIX

EFFECT OF INCUBATION OF RUMEN FLUID ON ITS CHEMICAL COMPOSITION AND ON ENUMERATION OF RUMEN BACTERIA

Summary

Rumen fluid from a rumen-fistulated cow on 50:50 alfalfa hay to grain diet was used to determine the effect of incubation with or without agar on the chemical composition of rumen fluid and to determine whether incubation and the type of agar used in the media preparation had any effect on the enumeration of rumen bacteria. Incubation of rumen fluid had no effect on total nitrogen content, L(+) and D(-) lactate and total VFA concentrations. The total carbohydrate content and the proportion of acetate was reduced. Other components of VFA were not affected. However, incubation of rumen fluid with agar reduced the total nitrogen content, total VFA concentration and the proportion acetate, but markedly increased the total carbohydrate content and slightly increased the L(+) and D(-) lactate concentrations. Nonincubated rumen fluid medium supported a higher total anaerobic bacterial counts than incubated rumen fluid medium. Also, incubated agar medium supported a higher bacterial count than purified agar medium. It is suggested that incubation of rumen fluid without agar is more selective than rumen fluid incubated with agar.

Introduction

The inclusion of rumen fluid in the media for cultivation of rumen bacteria provides conditions approximating the ruminal environment. Rumen fluid contains both known and unidentified factors required for the growth of a wide variety of rumen bacteria. However, the addition of rumen fluid to a medium without added carbohydrate energy source supported the growth of rumen bacteria, suggesting that sufficient substrates might be present in the medium to mark the selective utilization of any specific carbohydrate energy source added to the medium (Grubb and Dehority, 1976). Preincubation of rumen fluid reduced the concentration of endogenous energy sources to a level suitable for use in a selective medium

(Dehority and Grubb, 1976). To increase the selectivity of such a medium agar is also incubated with the rumen fluid (Allison et al., 1979; Leedle and Dehority, 1980). However, the process of recovering from rumen fluid, washing and lyophilizing the agar before use is tedious and time consuming. Incubated rumen fluid has been used to enumerate subgroups in bacterial populations in the rumen of cattle (Leedle and Hespell, 1980) and in the colon and cecum of pigs (Allison et al., 1979). The chemical composition of incubated rumen fluid has not been reported. The objectives of this study therefore, were to determine the differences in chemical composition between incubated and nonincubated rumen fluid, to determine the effect of addition of agar on the chemical composition of incubated rumen fluid and the determine whether a commercial purified agar could be used instead of incubated agar in the preparation incubated rumen fluid medium for the enumeration of rumen bacteria.

Materials and Methods

Preparation of incubated centrifuged rumen fluid (ICRF) and washed agar. Rumen samples were collected on three different occasions from a rumen-fistulated donor cow on a diet of 50:50 alfalfa hay (IFN 1-00-096) and a grain diet composed of 49.2% cracked corn (IFN 4-02-931), 49.2% sorghum grain (IFN 4-05-643), 1.0% dicalcium phosphate (IFN 6-04-080), .25% trace mineral salts and .1% vitamin A and D mixture. Samples were collected 3 h postfeeding and strained through four layers of cheesecloth. The strained rumen fluid was centrifuged at 5500 x g for 15 min. The centrifuged rumen fluid was divided into two parts and incubated with or without agar. Before incubation, an aliquot of each rumen fluid (with or without agar) was removed and frozen immediately for later analyses and media

preparation. Rumen fluid incubation was according to the procedures of Allison et al. (1979) and Leedle and Hespell (1980).

The incubated mixture contained the following ingredients per 100 ml: 7.5 ml of each mineral I and II (Bryant and Burkey, 1953), 5.0 ml distilled water, .05 g L-cysteine hydrochloride, 2.5 g agar, and 80 ml centrifuged rumen fluid. Minerals I and II, distilled water and a large stirring rod were placed in a flask. Oxygen-free CO_2 was then bubbled through the mixture for 15 min. L-cysteine hydrochloride was added and CO_2 bubbled through for another 5 min. The centrifuged rumen fluid was then added to the flask under CO_2 to yield an 80% (V/V) rumen fluid mixture. The undissolved agar was added to one portion while the mixture was constantly stirred. The pH of the mixture was adjusted to 6.8 with saturated sodium carbonate solution. The flask was closed with a one-way check valve (gravity type) and incubated at 39 C for 7 d, with constant stirring. The pH of incubated mixture was checked daily and adjusted to 6.8 if necessary. After 7 d incubation the mixture was centrifuged at 10,000 x g for 15 min. and the supernatant frozen immediately for media preparation. The sediment containing agar was washed three times by centrifugation. After the final wash, the agar slurry was frozen, lyophilized and ground before using for media preparation. In order to minimize batch differences, the three batches of each incubated and nonincubated rumen fluid with or without agar were thawed and combined together in equal proportion for use in media preparation (Leedle and Hespell, 1980) and chemical analysis.

Media preparation. The cell-free supernatants of the pooled incubated (ICRF) and nonincubated (CRF) rumen fluid with or without agar, obtained by centrifugation at 30,000 x g for 30 min. were used for the preparation of complete carbohydrate-agar medium (CCA) and medium without carbohydrate energy sources.

The CCA contained the following ingredients per 100 ml: 7.5 ml of each minerals I and II (Bryant and Burkey, 1953), 35 ml distilled water, 1.0 ml B-vitamin mixture (Scheifinger et al., 1973), 1.0 ml .01% Hemin (Leedle and Hespell, 1980), .05 ml (V/V) dycerol, .05 g each of glucose, xylose, cellobiose, maltose and soluble starch, .2 g trypticase, 50 ml of either ICRF or CRF, 1.0 ml .01% resazurin, .4 g sodium carbonate and .05 g L-cysteine hydrochloride. The medium without carbohydrate energy sources contained all other ingredients except the carbohydrates, glycerol and trypticase. The media were distributed into roll tubes containing either incubated and washed agar or a commercially purified agar (Difco 0560-01, Difco Laboratories, Detroit, MI), closed with butyl rubber stoppers, crimped with aluminum seals and autoclaved. The dilution blank contained minerals I and II, 20% (V/V) ICRF, distilled water, L-cysteine hydrochloride, resazurin and sodium carbonate.

Enumeration of rumen bacteria. Rumen samples for inoculation were collected from a rumen-fistulated donor cow on the 50:50 alfalfa hay to grain diet. Samples were collected 3 h postfeeding on three consecutive days. The sample was flushed with oxygen-free CO_2, blended for 1 min. before straining through four layers of cheesecloth, and .5 ml of the appropriate dilutions of the strained rumen fluid were inoculated into roll tubes. Four roll tubes were prepared for each dilution and incubated at 39 C for 7 d before counting the colonies.

Chemical analyses of rumen fluid. Analysis of the cell-free supernanants of the pooled ICRF or CRF with or without agar, obtained by centrifugation at 30,000 x g for 30 min. were used to analyze for: total carbohydrate (Dubois et al., 1956), Kjeldahl nitrogen (AOAC, 1970), L(+) and D(-)lactate concentrations (Dennis

et al., 1981) and total and individual volatile fatty acids by gas chromatography (Erwin et al., 1961).

Results and Discussion

Incubation of rumen fluid did not affect the total nitrogen content, L(+) and D(-) lactate concentrations and total volatile fatty acid (VFA) concentration. The total carbohydrate content of the incubated rumen fluid was lower than that of nonincubated rumen fluid, while the proportion of acetate tended to increase. Other components of VFA were similar in incubated and nonincubated rumen fluid (table 1). However, when agar was incubated along with the rumen fluid (table 2), the total nitrogen content, total VFA concentration and the proportion of acetate decreased while total carbohydrate content and L(+) and D(-) lactate concentrations increased as compared to the incubated rumen fluid without the addition of agar. Other components of VFA were similar in incubated rumen fluid with or without agar. Dehority and Grubb (1976) first suggested preincubation of rumen fluid for selective or differential enumeration of rumen bacteria. They demonstrated that 40% rumen fluid without carbohydrate energy source supported a considerable colony growth but preincubation for 7 to 10 d reduced the concentration of the endogenous energy sources to a level suitable for a selective medium without removing the required nutritional factors. From this study, incubation of rumen fluid without agar reduced the carbohydrate content, while other chemical ingredients examined were not appreciably altered. However, incubation of rumen fluid with agar markedly increased the carbohydrate content while reducing the nitrogen and total VFA content. It would seem that the source of the increase in carbohydrate content was due to a contribution of carbohydrate released from the agar matrix during fermentation. Leedle and Hespell (1980) observed that preincubation of rumen fluid medium without pH adjustment to 6.8

TABLE 1. EFFECT OF INCUBATION ON CHEMICAL COMPOSITION OF RUMEN FLUID

Ingredients	Centrifuged Rumen Fluid	
	Nonincubated	Incubated
Total nitrogen, mg/dl	12.9	13.1
Total carbohydrate, μg/ml	93.1	82.7
L(+) Lactate, mg/dl	.3	.4
D(-) Lactate, mg/dl	.3	.3
Total volatile fatty acids, μmole/ml	94.4	93.6
Acetate, %	66.0	73.0
Propionate, %	16.3	16.2
Isobutyrate, %	1.2	1.3
Butyrate, %	12.5	13.1
Isovalerate, %	1.8	2.0
Valerate, %	1.8	2.1

TABLE 2. EFFECT OF ADDITION OF AGAR ON CHEMICAL COMPOSITION OF INCUBATED RUMEN FLUID

Ingredients	Incubated rumen fluid	
	Without agar	With agar
Total nitrogen, mg/dl	13.1	11.9
Total carbohydrate, µg/ml	82.7	145.5
L(+) Lactate, mg/dl	.4	.7
D(-) Lactate, mg/dl	.3	.5
Total volatile fatty acids, µmole/ml	93.6	87.9
Acetate, %	73.0	65.4
Propionate, %	16.2	16.4
Isobutyrate, %	1.3	1.3
Butyrate, %	13.1	13.2
Isovalerate, %	2.0	1.7
Valerate, %	2.1	1.9

did not lead to a reduction in the level of endogenous carbohydrate level rather the total carbohydrate level increased presumably due to the release of the carbohydrate components from the agar matrix. Even with the adjustment of pH some carbohydrate appeared to be released from the agar matrix which when fermented also increased the L(+) and D(-) lactate concentrations.

Nonincubated rumen fluid medium supported a higher rumen anaerobic bacterial growth than incubated rumen fluid medium, whether or not carbohydrate energy sources were added (table 3). This was in agreement with the report of Dehority and Grubb (1976) who found that, although colony counts in incubated rumen fluid medium with added carbohydrate energy source were significantly lower than the regular RGCSA medium, the incubation procedure had considerable potential for the development of a selective medium.

The type of agar used for the preparation of medium seemed to affect the total anaerobic counts (table 4). The medium prepared with incubated agar supported a higher bacterial colony counts than the are prepared with a purified agar, when carbohydrate energy source was added. However, without carbohydrate energy source the counts appeared to be similar in both types of medium. It is difficult to explain whey the incubated agar medium supported a higher bacterial counts than the purified agar medium. However, it was observed during medium preparation that the highly hygroscopic nature of purified agar caused a temporary oxidation of the medium when distributed into roll tubes. It was not certain whether this could have an effect on the bacterial growth.

Dehority and Grubb (1976) estimated that only 9.6% of the total culturable bacteria would grow as pinpoint colonies on the basal medium without any carbohydrate energy source. Leedle and Hespell (1980) found that the number of pinpoint colonies were a little less than that of complete carbohydrate medium. However, Allison et al. (1979) found less than 1% colony counts on the basal

TABLE 3. EFFECT OF INCUBATION OF RUMEN FLUID ON RUMEN ANAEROBIC BACTERIAL COUNTS

Rumen fluid used for preparation of medium	Anaerobic bacterial counts		
	With carbohydrate ($X10^9$/g)	Without carbohydrate ($X10^9$/g)	%
Nonincubated rumen fluid	128.0	1.2	1.0
Incubated rumen fluid	118.0	.8	.7

TABLE 4. EFFECT OF AGAR TYPE USED FOR INCUBATED RUMEN FLUID MEDIUM PREPARATION ON RUMEN ANAEROBIC BACTERIAL COUNTS

Agar Type	Anaerobic bacterial counts		
	With carbohydrate ($X10^9$/g)	Without carbohydrate ($X10^9$/g)	%
Incubated agar	131.0	.8	.6
Purified agar	105.0	.7	.7

medium without added substrates as compared to colony counts on medium 98.5. In this study, bacterial colony growth was also pinpointed and ranged from .6 to 1% of the colony counts on a complete carbohydrate medium. The differences in these counts in the medium without carbohydrate energy sources were most likely due to the differences in medium preparation.

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RUMEN MICROBIAL CHANGES IN CATTLE FED HIGH GRAIN DIETS WITH OR WITHOUT IONOPHORE ANTIBIOTICS

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Abstract

Rumen-fistulated cattle were used in three experiments to monitor rumen microbial and volatile fatty acids (VFA) changes in cattle fed high grain diets with or without ionophore antibiotics.

Six rumen-fistulated cattle assigned to three groups (control, lasalocid-fed and monensin-fed) were used in the first experiments. Lasalocid or monensin had no effect on rumen pH but decreased total VFA concentration and acetate-propionate ratio. Total anaerobic bacterial counts and the proportion of amylolytic and lactate-fermenting bacteria increased whereas xylanolytic and pectinolytic bacterial counts decreased with increased grain intake. Although there was an initial decline in the total bacterial counts, lasalocid and monensin had no significant effect on total anaerobic, cellulolytic, xylanolytic, pectinolytic and lactate-fermenting bacterial counts. The proportion of amylolytic bacteria was consistently higher in antibiotic-fed cattle than the control cattle. Lasalocid and monensin had no effect on total protozoal number, but holotrichs appeared to be more sensitive to monensin than lasalocid and among the entodiniomorphs, Ophryoscolex was extremely sensitive to monensin.

Four rumen-fistulated steers, assigned to two groups (control and salinomycin-fed) were used for the second experiment. Salinomycin feeding had no effect on rumen pH and total VFA concentration. Acetate proportion was lowered and propionate proportion was higher in salinomycin-fed steers than the control steers. The acetate-propionate ratio was significantly lower in salinomycin-fed than that of the control. Molar proportion of butyrate increased in both control and salinomycin-fed steers. Total anaerobic bacterial counts were lower in the salinomycin-fed steers than the control steers after 8 weeks of salinomycin feeding. Salinomycin-resistant bacteria increased from 7.6 to 15.6% in salinomycin-fed steers but remained unchanged in the control steers. Salinomycin had no effects on

cellulolytic bacteria but the proportions of amylolytic bacteria were higher in salinomycin-fed than the control steers. Total protozoal numbers decreased initially in salinomycin-fed cattle. Initial reduction was due to reduced number of entodiniomorphs, particularly that of Entodinium species. Holotrichs were unaffected by salinomycin feeding.

Corn or milo grain was fed to four rumen-fistulated Holstein steers assigned to two groups in the third experiment. Total VFA concentration remained unchanged. Acetate: proprionate ratio was significantly lower in steers on 100% grain diet. Molar proportion of butyrate and isovalerate increased while that of isobutyrate declined and valerate remained unchanged with increase in grain intake. Total anaerobic bacterial colony counts increased with increased grain intake in both groups. Amylolytic, cellobiose, maltose, utilizing and lactate-utilizing bacterial counts were higher and xylose-utilizing bacterial counts were lower in corn-fed steers than milo-fed steers. Total protozoal counts increased with increased grain intake steers fed 100% corn diet had higher total protozoal and entodiniomorphs, particularly that of Entodinium species than steers fed 100% milo diet.