REPORT ON

XVII CONFERENCE ON RUMEN FUNCTION

held at
Americana Congress Hotel, Chicago Illinois
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Americana Congress Hotel
520 South Michigan Boulevard
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For the purpose of discussion, the program was divided into three panels. The identity of the panels and the chairman of each follows:

General Chairman --- C. R. Richards --- ARS, USDA

(a)	Nutrition	J. T. Huber
(b)	Microbiology	M. P. Bryant
(c)	Physiology and Physiopathology	A. D. McGilliard

NUTRITION

In Vitro and In Vivo Effects of a New Polyether Antibiotic (Lysocellin) to Enhance the Feedlot Performance of Ruminants - G. W. Wolfrom, D. R. Bright, P. J. Schalburg and A. Clingerman, International Minerals & Chemical Corporation, Terre Haute, IN.

Lysocellin, a divalent ionophoric polyether antibiotic produced from fermentation by the mold Streptomyces cacaoi var. asoensis, has recently undergone investigations related to its potential ability to improve the growth and feed efficiency performance of ruminants. Two series of in vitro rumen screening studies were conducted to evaluate the ability of lysocellin in various forms to alter the molar proportions of volatile fatty acids (VFAs). The basic procedure used in both series, adapted from various literature procedures, involved the harvesting of ruminal digesta from one of two fistulated steers, filtration through cheesecloth, dilution with McDougall buffer, incubation for 18-24 hours, centrifugation and determination of VFAs by gas-liquid chromatography. In Series I an additional factor was introduced: washing the solid digesta residue several times and combining the washings with the original filtrate (Senshu et al., 1980). Polyether treatments for Series I and II, respectively, were: control, 5 ppm Na-monensin, and 1, 5, and 10 ppm Na-lysocellin; and control and 5 ppm of either Na-monensin, Zn-lysocellin, lysocellin free acid, or Mn-lysocellin. The results were as follows:

	SERIES I					SERIES II (5 ppm)				
			ppm lysocellin					-lysocellin		
Item	control	5 ppm Na-monensin	1	5	10	control	monensin	Zn-	Acid-	Mn-
A/P	1.83	1.16	1.14	1.02	0.97	2.12	1.37	1.27	1.28	1.26
M/A	51.00	44.20	42.20	41.70	41.10	55.10	47.60	46.90	46.10	46.00
M/P										
unwashed	27.10	33.70	32.20	36.00	38.30	26.10	34.90	36.90	36.20	36.50
washed	29.20	43.70	43.60	47.30	47.50	-	-	-	-	-

All forms of lysocellin lowered the molar percentage of acetate (M%A), increased the molar percentage of propionate (M%P), and lowered the acetate: propionate ratios (A/P) compared to the control and monensin treatments. Additionally, one-hundred Columbia wether lambs were utilized in an eight-week study to evaluate the effects of dietary addition of various salts of lysocellin at a level of 30 g/T. The study was conducted as a 4x6 unreplicated factorial design. Only the results pertaining to the effects of lysocellin are presented below.

		-Lysocellin			
Item	Control	Na-	Zn-	Acid-	Mn-
ADG (kg)	.21	.24*	.23	.25*	.24*
ADF (kg)	1.35	1.39	1.37	1.36	1.40
Feed/gain	7.40	6.44*	6.55	5.88*	6.17*
Ruminal A/P	2.12	2.42	1.42	2.54	2.18

*Different from control at P<.05.

Differences in average daily feed intake (ADF) and molar ratio of ruminal acetate and propionate (A/P) were not apparent among the treatments. Significant improvements in average daily gain (ADG) and feed/gain were seen for Na-, Acid-, and Mn-lysocellin.

The Effect of the Polyether Antibiotic Lysocellin on the Performance of Growing Finishing Lambs - M. C. Calhoun, B. C. Baldwin, Jr. and G. W. Wolfrom, Texas Agricultural Experiment Station, Texas A&M University System, San Angelo and International Minerals & Chemical Corporation, Terre Haute, IN.

One-hundred eighty white-faced wether lambs were used in a 56-day growing-finishing experiment to evaluate two salts of the new polyether antibiotic lysocellin, and monensin-Na. The dietary treatments were:

1. negative control
2. positive control, monensin-Na, 16.5 mg/kg
3. lysocellin-Na, 16.5 mg/kg
4. lysocellin-Na, 33.1 mg/kg
5. lysocellin-Mn, 16.5 mg/kg
6. lysocellin-Mn, 33.1 mg/kg

Overall, feed intake was not decreased by monensin or lysocellin-Na. Feed intake was reduced during the first 14-day period when lysocellin-Na was fed at 33.1 mg/kg and when lysocellin-Mn was fed at either 16.5 or 33.1 mg/kg. Lysocellin-Mn at 33.1 mg/kg reduced feed intake throughout the 56-day study. Cumulative live weight gains were significantly greater than the negative control for both levels of lysocellin-Na, and for the 16.5 mg/kg level of lysocellin-Mn. Monensin-Na and the 33.1 mg/kg level of lysocellin-Mn did not significantly increase gains. During the first 14-day period, there were no significant effects of polyether antibiotics on

feed efficiency (feed:gain ratio). Subsequently, with the exception of lysocellin-Mn for the period from 1 to 28 days, cumulative feed:gain ratios were significantly less than the control for all polyether treatments. The lysocellin salts increased the molar percentages of propionic acid and decreased butyric acid at 21 and 49 days. Monensin-Na produced the same changes, but the magnitude of the responses tended to be less. There were no treatment effects on total rumen volatile fatty acid concentrations. All polyether antibiotic treatments decreased fecal coccidial oocyst numbers. However, there were no signs of clinical coccidiosis in any of the groups during the experiment.

Effects of the Antimicrobial Agent Teichomycin A_2 on Rumen Fermentation - A. V. Brondani, R. M. Cook and D. J. Phillips, Department of Animal Science, Michigan State University, East Lansing and Dow Chemical Company, Midland, MI.

Two rumen-cannulated cows were fed either a high alfalfa (HA) or a high grain (HG) ration, supplemented sequentially with 0, 5, 25, or 100 mg/cow/day of the glycopeptide Teichomycin A_2 (TE). Each level of TE was fed for a period of 4 weeks. Cows were allowed 1 week to adapt to the new TE level and then sampled once a week. Rumen fluid samples were taken at hourly intervals for a period of 8 hr. Blood samples were collected every 2 hr. In general, interaction between type of ration and level of TE was not statistically significant, and therefore means were pooled across rations. Results were as follows.

	Teichomycin A_2 (mg/day)			
	0	5	25	100
Acetate (mmoles/dl)	5.98^{A}	6.79^{AB}	8.31^{B}	7.86^{AB}
Propionate (mmoles/dl)	1.65^{A}	1.97^{AB}	2.53^{B}	2.35^{AB}
Butyrate (mmoles/dl)	0.85^{A}	1.13^{AB}	1.48^{B}	1.17^{AB}
Total VFA (mmoles/dl)	8.85^{A}	10.28^{AB}	12.96^{B}	11.83^{AB}
Soly protein (mg/dl)	28.19^{A}	27.35^{A}	25.16^{A}	36.74^{B}
Bacteria protein (mg/dl)	52.56	55.34	44.51	64.15
Protozoa protein (mg/dl)	114.30	152.40	136.30	157.60
a-amino-N (mg/dl)	0.99^{A}	1.68^{AB}	1.98^{AB}	2.89^{B}
NH_3-N (mg/dl)	9.99^{A}	13.59^{AB}	15.78^{AB}	18.64^{B}
Rumen turnover (l/h)	3.25^{A}	3.69^{AB}	3.71^{AB}	3.98^{B}
Plasma urea-N (mg/dl)	14.44	11.42	14.02	14.69
Plasma NH_3-N (mg/dl)	0.248^{A}	0.183^{B}	0.209^{A}	0.221^{A}
Plasma glucose (mg/dl)	65.62^{A}	64.30^{A}	65.92^{A}	78.46^{B}

AB = means within a row without common superscripts differ ($P<.05$).

Rumen concentrations of all major VFA and of total VFA were increased by TE. The magnitude of this increase was slightly greater for propionate and butyrate than for acetate. This trend resulted in a minor shift in the molar proportion of the acids favoring propionate and butyrate. Alpha-amino-N and ammonia-N were also increased by TE. In a series of 5-hr in vitro incubations, the effect of 0, 0.2, 2 and 10 ppm TE on fermentation of concentrates (casein, glucose, soluble starch, cellulose) by rumen inocula obtained from the treated cows was also examined. Individual and total VFA were increased by TE in HA but decreased in HG. Increased doses of TE tended to decrease both proteolysis and deamination, which resulted in lower ammonia production. The fermentation rate, as measured by total gas production, was not affected by TE. The results obtained in this study suggest that TE may increase digestion in the rumen and consequently could improve productive performance in ruminants.

Lasalocid and Monensin Effect on Beef Cattle Performance - B. J. Krick and T. W. Perry, Department of Animal Sciences, Purdue University, West Lafayette, IN 47907.

Sixty-five crossbred feeder steer calves averaging 246 kg were used in a 91-day growing trial followed by a 91-day finishing trial to compare monensin and lasalocid as feed additives. Steers were allotted to 6 pens in a 2 x 3 factorial design. The treatments were: (1) control, (2) monensin (200 mg/head/day), (3) monensin (300 mg/head/day), (4) control, (5) lasalocid (200 mg/head/day), and (6) lasalocid (300 mg/head/day). For the growing period, results for average daily gain (kg) dry matter intake (kg), and dry feed/kg gain (kg), respectively, were: (1) 1.41, 7.9, 5.7; (2) 1.14, 5.9, 5.1; (3) 1.12, 5.5, 4.8; (4) 1.27, 6.9, 5.5; (5) 1.37, 6.6, 4.8; and (6) 1.26, 6.3, 5.0. The feeding of 200 and 300 mg monensin decreased average daily gain ($P<.05$) while the feeding of lasalocid tended to increase it. Lasalocid and monensin tended to decrease dry matter intake and improve feed efficiency. For the finishing period, results for average daily gain (kg), dry matter intake (kg), and dry feed/kg gain (kg), respectively, were: (1) 1.20, 9.7, 8.4; (2) 1.22, 8.2, 6.8; (3) 1.29, 8.8, 6.8; (4) 1.20, 8.5, 7.1; (5) 1.30, 8.4, 6.5; and (6) 1.24, 8.1, 6.7. There was no significant effect of monensin or lasalocid on steer performance, but both ionophores tended to increase rate of gain, reduce feed intake and improve feed efficiency. Rumen fluid samples obtained on day 91 of the growing period were analyzed for volatile fatty acids and ammonia while blood samples were analyzed for glucose and BUN.

Both ionophores were effective in decreasing the molar proportion of acetate ($P<.05$) and butyrate ($P<.05$) and increasing the molar proportion of propionate ($P<.05$). Both monensin and lasalocid decreased NH_3-N ($P<.05$). Steers fed 300 mg lasalocid showed the highest level of glucose. Steers fed lasalocid presented a higher BUN level ($P<.05$). There was also a difference between levels, the steers receiving 300 mg/head/ionophere showing the lower level ($P<.05$). None of the carcass characteristics was significantly different, but carcass of steers fed lasalocid tended to grade higher and have lower liver abscesses and rumen wall damage scores than steers fed monensin.

Influence of Sarsaponin on Ruminant Protein Nutrition - R. A. Zinn, University of California, Imperial Valley Field Station.

Two trials were conducted to evaluate the influence of supplemental sarsaponin on digestive function in the ruminant. Trial 1 involved 4 steers (144 kg) equipped with rumen and proximal duodenal cannulas, in a crossover design experiment. Treatments consisted of an 80% corn-based concentrate diet (2.72% N) supplemented with 0 or 66 ppm sarsaponin. Feed intake was limited to 2.2% of body weight. Rations were meal-fed at equal intervals twice daily. Experimental periods lasted 14 days with 4 days collection during which duodenal and fecal samples were obtained twice daily at 6-hour intervals. Estimates were based on composites of spot samples using CR_2O_3 as a marker.

Trial 2 involved 3 steers (282 kg), with rumen and proximal duodenal cannulas, in a 3 x 3 Latin square design experiment. Treatments consisted of a 70% corn-based concentrate diet supplemented to contain: TMT 1) 2.24% N; TMT 2) 2.24% N plus 66 ppm sarsaponin; and TMT 3) 2.72% N. The remaining protocol was similar to that of Trial 1 except that feed intake was limited to 1.9% of body weight. Sarsaponin supplementation resulted in small and statistically non-significant differences ($P > .10$) in both ruminal and total tract digestion of organic matter and fiber. The result of sarsaponin supplementation on N metabolism in the rumen for Trials 1 and 2 is shown below.

		Trial 1		Trial 2		
		Control	Sarsaponin	2.24% N	2.24% N+ Sarsaponin	2.72% N
Calves		4	4	3	3	3
Weight (kg)		144	144	282	282	282
N Intake (g/d)		84	82	118.5	118.1	154.4
N Reaching S.I. (g/d)						
Total NAN	x	71.1	86.1	137.1[a]	161.6[b]	166.0[b]
Microbial	y	36.4	43.4	82.1	91.7	89.8
Feed		34.7	42.7	55.0	69.9	76.2
Ruminal Efficiency*						
Total N	x	.84	1.05	1.16[a]	1.37[b]	1.07[a]
Microbial N	x	23.8	29.2	29.6	33.2	33.4
Feed N Bypass %		41.1	51.8	46.4	59.2	49.3

x means in Trial 1 differ significantly ($P < .01$)
y means in Trial 1 differ significantly ($P < .10$)
* Total N = non ammonia N leaving abomasum/N intake
Microbial N = g microbial N/kg organic matter fermented
ab means in a row with different superscripts differ significantly ($P<.05$, Trial 1)

Effect of Oral or Abomasal Glucose Addition on Energetic Efficiency of Wethers - Steven R. Rust, Montana Agricultural Experimental Station, Huntley, Montana 59037, and F. N. Owens and L. J. Walters, Oklahoma State University, Stillwater, OK 74078.

Fermentation of simple sugars and starch in the rumen is theoretically 20-40% less efficient than enzymatic digestion. To date, no long term feeding trials have demonstrated this reduced caloric efficiency. Therefore a feeding study with 10 wether lambs (21 kg) was conducted over 165 days to evaluate the caloric efficiency of ruminal versus intestinal degradation of corn sugar. Wethers were divided into three groups and received one of the following three treatments: 1) control, 2) oral consumption, or 3) abomasal infusion. Four wethers served as an initial slaughter group. Thirteen lambs received abomasal cannulas of which eight were used on the infusion treatment. Digestibility, rumen, and blood samples were collected three times during the trial. At time of slaughter, carcass characteristics and organ weights were obtained. Fat and lean tissue were physically separated for each carcass.

Average daily gain and feed to gain estimates were similar for the oral and infused treatments. Digestibility of organic constituents was similar for all treatments. Ash digestibility tended to increase with the addition of glucose ($P<.15$) however abomasal sugar infusion increased digestibility to a greater extent ($P<.11$). Rumen propionate and valerate were slightly higher in lambs consuming glucose. Butyrate isobutyrate and isovalerate levels were lower with the oral group. Blood glucose levels tended to be higher with the abomasally infused sugar treatment. Oral consumption of the sugar reduced blood urea levels.

Carcasses from wethers infused with glucose were heavier and contained more fat. Lambs which consumed the sugar had larger livers. Intestinal and bone weights ($P<.05$) were greater for lambs infused with sugar. Every gram of additional carcass gain over the control group required 16 and 10 g of sugar respectively for the oral and infused treatments. Infused sugar was utilized more efficiently for fat deposition than consumed sugar.

Results from this study provide further evidence for the 20-40% energetic advantage of intestinal digestion over rumen fermentation.

Feeding Particle Board Waste to Growing Heifers - Amy Duffield, R. S. Emery and J. W. Thomas, Michigan State University, East Lansing.

Forest products are a major agriculture industry in Michigan with particle board manufacturing being a part of that industry. Large amounts of water with a high content of dissolved organic material are being produced during the manufacturing of particle board. This water must undergo treatment prior to discharge. A product of the waste treatment consists of the fibrous solids combined with the microbial mass formed during the aerobic degradation of the dissolved organic waste where nitrogen and phosphorus are added.

This material is dried to 60% dry matter containing protein (40.71% DM) and acid detergent fiber (16.06% DM). The ADIN content, however, represents approximately 23% of the nitrogen. Our objectives were to compare the byproduct to SBM as a source of supplemental protein for growing heifers.

This material was fed to 28 heifers (284 kg body weight) in an 8-week feeding trial. Equal numbers of animals were assigned to each of the four pens, with 2 pens per treatment. The dietary treatments for the duration of the experiment consisted of: corn silage - 84% ration DM, straw - 9.9%, mineral supplement - .7% and either soybean meal - 4.9% or byproduct - 5.5%.

The ration DM contents were similar (34.5%), but the CP differed (11.33%) CP SBM vs. 9.63% CP byproduct.

The average daily gains were significantly higher for SBM vs. byproduct ration (.67 vs. .55 kg/day, P<.20). Dry matter intakes did not differ between rations (6.30 kg per day); however, feed conversion ratios showed soybean meal ration as being used more efficiently than the byproduct ration (9.19 vs. 11.38 kg DM/kg gain). Based on the feed conversion ratios, feeding value of the byproduct was estimated to be about 80% relative to soybean SBM.

	Animal Performance		
	Soybean Meal	Byproduct	S.E.
Body weight, start (kg)	284.3	284.7	
Average daily gain (kg)	0.67	0.55	.07*
Dry matter intake/day (kg)	6.31	6.32	1.16 NS
Dry matter/gain	9.19	11.38	.66 NS
Relative value of byproduct to soybean meal = .81			

*P<.20

The byproduct proved resistant to ruminal degradation as demonstrated by the low disappearance of DM (13.5%) and N (12%) following 12 hr incubation in a ruminally suspended nylon bag. Byproduct protein was also resistent to bacterial protease degradation (76% undegradable) but was susceptible to mammalian pepsin degradation (91% IVDMD).

This product has potential for use as a protein supplement in growing heifer rations and as a source of bypass protein.

Utilization of Corn Crop Residues in Diets of Growing Dairy Heifers - Jesus Lopez-Guisa and Larry D. Satter, U.S. Dairy Research Center, University of Wisconsin, Madison, WI. (No abstract received.)

Comparisons Between Sheep and Goats in Utilization of High Fiber Diets - W. L. Johnson, Ederlon Oliveira, T. W. Robb, J. E. J. van Eys and M. Rangkuti, Department of Animal Science, North Carolina State University, Box 5127, Raleigh, NC 27650.

Since 1979, North Carolina State University has been involved in collaborative research in small ruminant nutrition with the National Goat Research Center of EMBRAPA in Sobral, Ceara, Brazil, and the Animal Production Research Institute in Bogor, West Java, Indonesia. One of the objectives is to design improved feeding systems for village small ruminant producers. To improve the precision of extrapolation of experimental data, it has been of interest to compare the utilization of diets based on locally available feedstuffs, by local breeds of sheep and goats.

Previous literature is equivocal on whether sheep or goats have higher intake and/or digestibility of medium and high fiber diets. In spite of a lack of solid supporting evidence, however, there exists a popular notion that the goat has superior fiber-utilizing abilities.

A series of digestibility trials was conducted at Raleigh with goats and sheep of European extraction. In a trial with 3 diets incorporating 35, 50, and 65% wheat straw (WS), voluntary DM intake per $W^{.75}$ decreased less in goats than sheep as fiber concentration increased. DM digestibility, however, decreased more in goats, being equal at 35% WS but 5 percentage units less by goats than sheep at 65% WS. NDF digestibility decreased in goats but not sheep as dietary fiber increased. In a second trial with tall fescue and bermudagrass hays, DM intake per $W^{.75}$ was higher and DM and NDF digestibility lower in goats than sheep. In a third trial the same relationships were observed with coastal bermudagrass as in trial 2, but with alfalfa hay the rankings of goats and sheep reversed. Mean retention time of Cr-mordanted NDF from coastal or alfalfa was shorter in goats than sheep.

Meanwhile, in Brazil, native tropical goats and hair sheep were fed diets with 50% maize crop residues, with ADG's of 29 to 37 g for goats and 98 to 149 g for sheep. Voluntary DM intakes were also higher for the sheep. Also, in Indonesia, in 3 trials with combinations of napier grass, cassava leaves, and a tropical legume, DM intake by goats was consistently lower than sheep while DM and NDF digestibilities were either higher by goats or not different between animal species.

We conclude that factors such as breed type, dietary fiber concentration, selectivity and mastication may influence comparative differences between goats and sheep in DM intake, digestibility, and mean retention time.

Interaction of Dietary Phosphorus and Roughage Levels on Phosphorus Utilization by Sheep - H. O. S. Lopes and T. W. Perry, Department of Animal Sciences, Purdue University, West Lafayette, IN.

Metabolism trials were conducted with sheep to study the effects of phosphorus and roughage dietary levels upon phosphorus, calcium, magnesium,

potassium, and nitrogen balance. Thirty-six crossbred intact male lambs were assigned to nine treatment groups in a 3 x 3 factorial arrangement of treatments with three levels of phosphorus (0.120, low; 0.240, medium; 0.480%, high) and three levels of roughage as corn cobs (25%, low; 50%, medium; 75%, high). The experiment was carried out in three successive periods. Each period utilized 12 animals assigned randomly to nine treatment combinations plus three additional treatments also assigned at random. Animals fed the high phosphorus (HP) diets had higher fecal ($P<0.05$) and urinary ($P<0.01$) phosphorus excretion than the animals fed the low phosphorus (LP) and medium phosphorus (MP) diets. Phosphorus balance in grams per day, percent of dietary phosphorus retained, and apparent phosphorus digestibility were higher ($P<0.01$) for the lambs receiving the MP and HP diets as compared with the lambs receiving the LP diet. Blood serum phosphorus levels increased ($P<0.01$) with each increment of dietary phosphorus. Phosphorus balance in grams per day, percent of dietary phosphorus retained, and apparent phosphorus digestibility decreased significantly as the roughage dietary levels increased. Urinary phosphorus as percent of total excretion was higher ($P<0.05$) for the animals fed the low roughage (LR) diet. Calcium balance in grams per day and apparent calcium digestibility were lower ($P<0.01$) for the lambs fed the HP diet as compared with the lambs fed the LP and MP diets. Lambs receiving the diet adequate in phosphorus (MP) retained more calcium ($P<0.05$) as percent of dietary intake than lambs receiving the diet deficient in phosphorus (LP). Blood serum calcium levels decreased significantly as the dietary phosphorus levels increased. Roughage dietary levels exerted no significant effect on any of the calcium balance parameters studied. Apparent magnesium digestibility was lower ($P<0.05$) for the lambs fed th HP diet as compared with the lambs fed the LP and MP diets. Blood serum magnesium levels decreased significantly as the dietary phosphorus levels increased. Nitrogen retained as percent of dietary intake for the animals fed MP levels was higher ($P<0.05$) than for the animals fed HP levels. Apparent digestibility of nitrogen was lower ($P<0.05$) for the lambs receiving the HR diet as compared with the lambs receiving the LR and MR diets. When expressed as percent of total excretion, urinary nitrogen was lower ($P<0.05$) and fecal nitrogen was higher ($P<0.05$) for the animals fed the HR dietary level as compared with the animals fed the LR and MR dietary levels. Acid detergent fiber digestibility (ADF) for the animals fed the LP diet was lower ($P<0.01$) than for the animals fed the HP diet, whereas dry matter digestibility was not affected by the phosphorus dietary levels.

Effect of Selenium Administration Method to Pregnant Cows on Deposition in the Fetus - L. R. A. Toledo and T. W. Perry, Department of Animal Sciences, Purdue University, West Lafayette, IN 47907.

Thirty pregnant Holstein cows, seven months into pregnancy, were allocated to six groups. Five treatments were randomly assigned to each cow in each group: control (C); 1 mg Se/day orally ingested (Na_2SeO_4) (1-0); 2 mg Se/day orally ingested (Na_2SeO_4) (2-0); 5 ml Mu-Se intramuscularly injected 40 and 20 days prepartum (Mu-Se - 5 mg Se [Na_2SeO_4] and 68 I.U. d-alpha-tocopherol/ml) (2-I); 5 ml Mu-Se intramuscularly injected 60, 40, and 20 days prepartum (3-I). The basal diet consisted of corn silage (.05 ppm Se)

ad libitum, 0.5 kg ground corn/day, 40,000 I.U. vitamin A, 4,000 I.U. vitamin D, and iodized salt with trace minerals except Se. Selenium treatments 2-0, 2-I, and 3-I increased (P<.05) cow serum Se concentration. The cows on the 1-0 treatment showed serum Se concentration significantly different (P<.05) from the cows on the control treatment only in samples taken 10 days pre-parturition. Treatments did not increase (P>.10) cow colostrum Se. The cow hair Se was significantly increased (P<.01) by treatments 2-I and 3-I and (P<.05) by treatment 2-0. Calf hair Se was significantly increased (P<.01) by all treatments. Calf serum Se was increased by treatments 2-I and 3-I; therefore, the selenium transfer through the placenta was demonstrated. The data did not allow any conclusions regarding retained placenta. There was no difference (P<.05) in effectiveness between the intramuscular injection or the oral methods of administration of Se treatments. The response of the treatments increased accordingly with increase in dosage.

Proposed Modes of Action for Methionine Hydroxy Analog in Ruminants - C. D. Knight and L. Kung, Jr., Nutrition Chemicals Division, Monsanto Company, St. Louis, MO 63167.

Several in vitro and in vivo studies have demonstrated positive effects of mha supplementation on growth rate of rumen microorganisms, and their rate of carbohydrate utilization and lipid synthesis. Rumen bacteria do not use mha as an energy source nor is the effect of mha due solely to its sulfur content. Methionine has been shown to have effects similar to mha but of shorter duration.

In support of its effect on rumen function, mha has been shown to improve dietary fiber and fat digestibilties, improve the efficiency of metabolizable energy conversion to milk and shift rumen VFA profiles to a higher acetate to propionate ratio.

Data have demonstrated lower rates of rumen degradation and greater recovery of radioactivity in milk and tissues for mha than methionine. This led to the hypothesis that mha provided its benefit to the ruminant by escaping rumen degradation and providing a direct source of methionine to the animal via the small intestine. Attempts to directly measure abomasal flow of mha have not detected measurable rumen by-pass of the compound and production responses to mha have not been well correlted with plasma methionine values.

There is ample evidence in the literature demonstrating that mha increased fat corrected milk production either by increasing milk fat and/or overall milk volume. Although the exact mechanism of activity in the ruminant is not know, mha has been shown to impact rumen function and affect methionine sensitive tissues and processes outside of the rumen.

Metabolism of Fats in the Rumen - D. L. Palmquist and T. C. Jenkins, Department of Dairy Science, The Ohio State University, Wooster, and William Chalupa, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

The lipid intake of ruminant animals consuming forage diets is relatively low (fatty acids equal 1-2% of feed dry matter) and occurs predominantly as mono- and digalactosyl diacylglycerols and phospholipids. Moderate amounts of fatty acid (2-3% of DM) are provided by cereal grains mainly as triacylglycerols. The predominant fatty acid in forages is linolenic acid (18:3), whereas it is linoleic acid (18:2) in cereals.

Intake of fatty acids from forages and grains by lactating cows rarely exceeds 400 g/day (2% of DM). Fatty acids are readily released by lipases, followed by their biohydrogenation, mainly to stearic acid (18:0), with formation of small amounts of various monoenoic isomers.

Polyunsaturated fatty acids (18:2 and 18:3) required by the animal are preserved from biohydrogenation by engulfment of chloroplasts by rumen protozoa on high forage diets. With feeding of high cereal diets, protozoa numbers are often reduced; however, lipolytic bacteria numbers are also decreased, permitting rumen throughpass of esterified 18:2.

With higher energy requirements of highly productive animals, supplementation with fats has drawn increased interest. Such fats may be provided in free form as tallow or vegetable oils, or in whole seeds, as cottonseed, sunflower, or soybean.

It has long been popularly believed that supplemental fatty acids lowered rumen digestibility by physical coating of feed particles, thus, interfering with microbial attachment and/or enzymatic attack. However, the evidence is much stronger for direct inhibitory effects on microbial growth and survival. Indeed, inhibition is minimized when forage particles are present; fatty acids adsorb to the feed particles, decreasing association with the microbial cells. It has been demonstrated that inhibition occurs only when fatty acid concentration exceeds adsorption capacity on the microbial cell surface. Saturates adsorb more strongly and readily than do unsaturates; however, unsaturates are more toxic than saturates. Whether this is caused by specific structural characteristics affecting the microorganisms, by high water solubility of unsaturates, or tendency of saturates to precipitate as insoluble salts, is undetermined.

In general terms fats fed as free fatty acids are more toxic than as triglycerides. Fats provided as free oils (triglycerides) have been shown to associate predominantly with feed particles, where they are hydrolyzed to free fatty acids by an extracellular lipase. About 25% of triglyceride is taken up intact by the organisms and hydrolyzed and biohydrogenated intracellularly. Free fatty acids associate with microbial cells and feed particles. Biohydrogenation occurs extracellularly in association with feed particles. Biohydrogenation (BH) of fatty acids fed as unsaturated free fatty acids often results in accumulation of large amounts of trans Δ-11 18:1, whereas BH of fatty acids fed in esterified form may be more complete, resulting in formation of predominantly 18:0.

Very few rumen microorganisms capable of lipolysis and/or BH have been identified. Characterization of BH in detail is known only for Butyrivibrio fibrisolvens. It seems that to fully capitalize on rumen BH as a means of detoxifying fatty acids and maximizing fat feeding that we need to examine rates of lipolysis and BH in more detail, especially with regard to use of seed oils. It is known, for example, that equivalent amounts of soy oil are much less toxic when fed in unextracted crushed seeds as compared to feeding the free oil.

Long chain fatty acids are most toxic to protozoa and to methanogenic and cellulolytic bacteria; thus fat feeding results in lowered methanogenesis, fiber digestion and rumen acetate:propionate. At moderate levels of fat intake, protozoa numbers and fiber digestion are decreased, without decreasing bacterial numbers, resulting in net increases in efficiency of microbial protein synthesis (g microbial N/kg organic matter truly digested in the rumen). This is due to decreased futile cycling of bacterial N through protozoa, and possibly to increased outflow of organic matter from the rumen (due to lowered rumen dry matter digestibility).

Recent research has been directed to minimizing toxic effects of fatty acids on rumen microorganisms. It has long been known that saturated fats were less toxic and that supplemental calcium decreased toxicity. Providing slower release of fatty acids (as from whole seeds) is helpful. High forage diets reduce toxicity by providing absorptive sites for long chain fatty acids and by promoting normal rumination. Blended fats (commercial mixtures of esterified and unesterified fats) are less inhibitory to fiber digestion in vitro than either ingredient provided alone.

High-melting fats (combinations of palmitic, stearic, and oleic acids) are supplied by some manufacturers in finely-granulated form to minimize rumen activity. Calcium salts of long-chain fatty acids have been shown to have no inhibitory effects on rumen microbial activity. However, they are sensitive to low rumen pH (pKa ≈ 4-5), so that proper feeding practice must be followed.

Continued research with fats in rumen studies should prove useful. The mechanism of fatty acid toxicity remains to be identified. Many aspects of association of fatty acids with microorganisms and feed particles, and competition for association, remain to be characterized. Optimum chemical composition of feed particles for maximum association is unidentified. Factors influencing total capacities and rates of lipolysis and biohydrogenation are unknown. Opportunities for maximizing microbial protein synthesis exist but have not been realized. Optimum balance of fermentable carbohydrate and nonprotein nitrogen sources for maximum microbial synthesis in presence of added fat have not been determined.

Many other problems and opportunities exist with regard to increasing fat content of ruminant diets. These remarks have been limited to some of the important aspects of the rumen. REFERENCE

1. Harfoot, C. G. 1981. Lipid metabolism in the rumen. In: W. W. Christie, ed. Lipid Metabolism in Ruminant Animals. Pergamon Press.

Effect of Forage:Concentrate Ratio on Kinetics of Forage Fiber Digestion in Situ - Bryan G. Miller and Russell B. Muntifering, University of Kentucky, Lexington.

Five rumen-fistulated Holstein steers (320 kg) were used in a 5 x 5 Latin square design to determine the effect of dietary grain level on kinetic parameters determining ruminal forage fiber digestion. Steers were limit-fed 7 kg/day of a ground (2.54-cm screen) fescue hay-cracked corn diet containing 0, 20, 40, 60, or 80% corn. Each period in the Latin square consisted of a 2-wk dietary adjustment followed by a 10-day collection phase. A single pulse dose of Cr-mordanted fescue NDF was administered via cannula on day 0 of each collection period; fecal grab samples were collected every 6 hr for 120 hr and then every 8 hr through 168 hr (day 7). Rate of passage from the rumen (Kp, hr^{-1}) was defined as the slope of the descending portion of the fecal Cr excretion curve obtained by regressing ln fecal [Cr] vs time. Nylon bags (7.5 x 15 cm) containing 4 g ground (2-mm screen) fescue hay were placed in the ventral sac of the rumen immediately prior to the morning meal on day 7 of each collection period; duplicate bags were removed at 6, 12, 18, 24, 48, and 72 hr (day 10) and assayed for residual NDF. The potentially digestible fiber fraction (PDF, %) was obtained by assuming that the potential extent of digestion (PED, %) was complete at 72 hr. Digestion rate constants (K_D, hr^{-1}) were determined by regressing ln PDF remaining vs time; solution of the first-order kinetic equation to equal 100% PDF remaining yielded estimates of discrete lag time (L_D, hr). The following equation was used to predict apparent extent of forage fiber digestion (AED) in the rumen:

$$AED = PED \cdot \frac{K_D}{K_D + K_p} \cdot e^{-K_p L_D}$$

Increasing concentrate level tended to increase (P>.05) lag time and decrease (P>.05) potential extent of forage fiber digestion in the rumen. Concentrate level (%), L_D (hr) and PED (%) were: 0, 9.1, and 54.0; 20, 8.7, and 54.8; 40, 9.8, and 48.8; 60, 10.5, and 47.0; and 80, 11.5, and 28.7. Rate of digestion ranged from .039 to .062 hr^{-1}, and was unaffected (P>.05) by concentrate level. Ruminal passage rate was greater (P<.05) for the 20% (.027 hr^{-1}) compared to the 80% (.019 hr^{-1}) concentrate level. Apparent extent of ruminal forage fiber digestion was greater (P<.05) for the 0% (32.4%) compared to the 80% (16.7%) concentrate level. Stepwise multiple regression analysis indicated potential extent of digestion to be the primary determinant of decreased forage fiber digestion as concentrate levels increased.

Evaluation of Dual Flow Continuous Culture for Estimating In Vivo Ruminal Digestion - Scott M. Hannah and Marshall D. Stern, Department of Animal Science, University of Minnesota, St. Paul.

Continuous culture (CC) devices which permit differential removal rates for liquid and solids were used in a completely randomized block design to determine ruminal digestion of four soybean products. Soybean meal (SBM),

whole soybeans (RAW) and whole soybeans extruded at 132C (132) and 149C (149) provided 50% of the protein in diets which were comprised of 52% grain, 36% corn silage and 12% alfalfa hay (dry matter basis). These same diets were previously studied in vivo using lactating dairy cows fitted with rumen cannulae and t-type cannuale in the duodenum and ileum. Based on these results, liquid and solids dilution rates in continuous culture were maintained at .096 h^{-1} and .055 h^{-1}, respectively, while pH was maintained at 6.25. Diaminopimelic acid (DAP) was used to estimate the relative proportions of bacterial and dietary nitrogen in the CC effluents and in duodenal digesta of cows. Estimates of in vivo and in vitro ruminal digestion are shown in the following table:

	SBM	RAW	132	149	Overall means
True OM digestibility, %					
in vivo	57.0	58.8	53.0	54.4	55.8
CC	60.4	56.8	55.9	57.4	57.6
Degradability of CP, %					
in vivo	73^{ab}	80^{a}	66^{ab}	60^{b}	70
CC	77	80	71	75	76
Bacterial synthesis, g N/kg OM truly digested					
in vivo	44	39	44	42	42
CC	32	37	34	36	35

^{a,b,c}Means in the same row with different superscripts differ ($P<.05$).

Results from the in vivo study indicate that protein degradation in the rumen was lower for the diet containing soybeans extruded at 149C compared to raw soybeans while continuous culture results showed no significant difference among treatments. Average values for nitrogen (%), organic matter (%), and DAP (mg/g N) content of bacteria for all diets were 7.8, 83.9, and 32.3 in the rumen of cows and 7.7, 80.0, and 41.4 in continuous culture. The percentages of total N in duodenal digesta as ammonia, bacterial and dietary for th SBM, RAW, 132, and 149C diets were 4.5, 74.1, 21.4; 4.0, 78.4, 17.6; 4.2, 69.3, 26.5; and 4.6, 64.9, 30.5, respectively. Comparative values from CC effluent were 17.0, 57.7, 25.2; 15.8, 62.3, 21.9; 12.1, 56.4, 31.5; and 13.8, 60.0, 26.2, respectively.

Utilization of Nitrogen from Alfalfa, Birdsfoot Trefoil and Smooth Bromegrass in Continuous Culture of Rumen Contents - B. J. Barno, M. D. Stern, and F. R. Ehle, Department of Animal Science, University of Minnesota, St. Paul, and USDA ARS, U.S. Dairy Forage Research Center.

Dual flow continuous culture fermenters were used to determine nitrogen utilization and fiber digestion of alfalfa, birdsfoot trefoil, and smooth bromegrass by rumen microbes. This technique was also compared with rumen

digestibilities of the same forages estimated by in vitro batch culture and dacron bag techniques. Digestibilities were determined for all three techniques at a mean solids retention time of 25 hours. Forages were ground through a 2 mm mesh screen. Observations were duplicated for each forage within technique. Nitrogen utilization by the rumen microbes in continuous culture is shown in the following table:

	Alfalfa	Birdsfoot trefoil	Smooth Bromegrass
NH_3-N, mg/100 ml	8.3	9.3	7.0
Degradability of CP, %	55.7	43.4	40.9
Bacterial N synthesized, g N/kg OM truly digested	36.9	23.0	28.9

Results from this experiment indicate that protein degradation and synthesis by the rumen microbes were higher for alfalfa. Mean protein degradation determined by continuous culture (46.7%) was higher ($P<.05$) than that determined by the dacron bag technique (62.4%). The dacron bag technique also estimated dry matter digestibility to be higher ($P<.05$) and cellulose digestibility lower ($P<.05$) than the in vitro batch culture and continuous culture techniques. Dry matter digestibility of birdsfoot trefoil was higher ($P<.05$) and cellulose digestibility of alfalfa lower ($P<.05$) compared to the other forages.

Potential Problems When Using Liquid:Solid Marker Ratios to Correct Duodenal Digesta Flow Measurements in Dairy Cows - D. K. Combs, R. C. Kellaway, B. D. Siebert, and L. D. Satter, U.S. Dairy Forage Research Center-Madison, WI.

The behavior of various digesta markers (lanthanum, ytterbium, chromium mordanted fiber, ADL (72% H_2SO_4 and cobalt-EDTA) was evaluated in duodenal digesta and feces of dairy cows. Five cows fitted with ruminal and T-type duodenal cannulae were fed *ad libitum* a diet of corn silage (35%), alfalfa silage (35%) and ground corn grain mix (30%) (DM basis) for two 14 day periods. Solutions of La, Yb and Co-EDTA were sprayed onto the grain mix to provide 40, 40 and 20 mg marker/kg diet, respectively. Chromium was mordanted to alfalfa NDF residue, ground through a 2 mm screen and mixed with the grain to provide 20 mg Cr/kg diet. Duodenal and fecal samples were collected six times daily during the last three days of each period, composited volumetrically, dried and analyzed for Co, Cr, La and Yb by neutron activation analysis. Ruminal and total tract digestion of OM and ADF was then calculated from each marker and apparent digestion coefficients compared.

Apparent total tract digestion of OM and ADF based on lignin was higher than digestion estimates based on the other markers which gave similar estimates of digestibility. Apparent ruminal digestion coefficient for OM and ADF were 40 to 50% higher when Cr-M or lignin was the marker than when La, Yb or Co-EDTA was used.

Estimates of ADF digestion based on Cr-M or lignin suggest that nearly all of the potential ADF digestion occurs within the rumen. Only 59 to 68% of total ADF digestion appears to occur within the rumen when La, Yb or Co-EDTA is the marker.

Individual duodenal samples from four of the cows utilized in this study were dried and analyzed for Cr, La, Yb and Co. Ratios of the liquid phase marker (Co-EDTA) to the solid phase markers (Cr-M, La, Yb) were calculated and compared (Table). Co-EDTA to Cr-M ratios were affected by changing dry matter composition of the duodenal samples more than ratios of Co-EDTA to La or Co-EDTA to Yb. This suggests that La and Yb may actually behave more like a liquid phase marker at the duodenum than a solid phase marker and indicates that reconstitution techniques based on La or Yb as solid phase markers may not be appropriate.

Relative Liquid to Solid Marker Ratios in Duodenal Digesta Samples of Various Dry Matter Compositions

Dm content of duodenal digesta, difference from mean[b]	Number of samples	Co-EDTA:Cr-M	Co-EDTA:La	Co-EDTA:Yb
Percentage Units		Relative Marker Ratio		
-3 to -2.1	4	137[a] 15	120 10	115 7
-2 to -1.1	20	115 31	108 9	116 22
-1 to -0.1	21	88 15	103 11	111 21
0 to 1	10	89 27	98 9	101 14
1.1 to 2	9	74 14	96 13	103 16
2.1 to 3	5	62 8	99 12	96 9
Composites	10	76 16	96 5	95 3

[a](Co:Cr in digesta sample/Co:Cr in ration) x 100
[b]Mean dry matter composition of duodenal digesta from each cow was calculated from individual samples. Individual samples were then grouped by dry matter content.

The Effect of Frequency of Feeding on Rumen Outflow Patterns in Steers Fed High Grain-Corn Silage Based Rations - W. V. Rumpler, G. M. Weber, W. G. Bergen, and J. C. Waller, Department of Animal Science, Michigan State University, East Lansing, MI.

The effect of feeding frequency on duodenal digesta flow and indirectly rumen outflow was studied with four Holstein steers (500 kg BW) fitted with abomasal and duodenal T type cannulas. The study was conducted as a 4 x 4 latin square with feeding frequencies of either 1, 2, 6, or 12 times daily as treatments. Irrespective of feeding frequency, each steer consumed a total of 7.5 kg DM daily. The diet consisted of 65 percent high moisture corn, 30 percent corn silage, and 5 percent supplement (86% soybean meal,

limestone, TM salt). All animals were adapted for 10 days on the new feeding schedules before digesta sample collection. Duodenal digesta samples were collected at 6 hour intervals over a 3 day period, such that each of the 12 samples obtained represented an odd hour sample in a reconstructed, theoretical day. Duodenal digesta flow was measured by infusing Cr · EDTA at a constant rate into the abomasum and determining the Cr concentration in the duodenal sample. Flow rate then equals infusion rate divided by Cr concentration in the duodenal sample. Regardless of feeding frequency, duodenal dry matter flow patterns were similar. High flow rates occurred between 3-5 am and low flow rates occurred between 1-3 pm. Duodenal dry matter flows for individual 12 intervals (e.g. a 2 hour period) in the reconstructed day ranged from 2-15% of the totaly daily (or 24 hour) dry matter flow. The data showed that fluctuations in duodenal dry matter flow (and hence rumen outflow pattern) during the day were not reduced by increasing the feeding frequency from 1-12 per day.

MICROBIOLOGY

Sensitivity and Resistance of Lactate- and Propionate-Producing Rumen Bacteria to Antimicrobial Feed Additives - M. B. Taylor and T. G. Nagaraja, Dept. of Animal Sciences and Industry, Kansas State University, Manhattan.

Sensitivity and resistance of Lactate- and Propionate-producing rumen bacteria to Avoparcin, Lasalocid, Monensin, Narasin, Salinomycin, Thiopeptin, Tylosin and Virginiamycin were determined. The minimum inhibitory concentration (MIC) was determined by inoculating anaerobically prepared media with pure cultures of rumen bacteria plus antibiotic concentrations ranging from 0 to 48.0 µg/ml. Cultures were incubated at 39°C for four days with growth measured by absorbance. Selenomonas ruminantium was the only Lactate-producing rumen bacteria resistant to all eight antibiotics. The MIC of all other lactate-producers ranged from .09 to 12.0 µg/ml. Streptococcus bovis and strains of Lactobacillus ruminis and L. vitulinus were less sensitive to Avoparcin and Tylosin. Both succinate-producing and lactate-fermenting rumen bacteria were resistant to all the antibiotics. Only Veillonella alcalescens was sensitive to high concentrations of Tylosin and Virginiamycin.

The effects of antibiotics on lactate inhibition and propionate enhancement were tested in vitro. Lactate inhibition was tested by incubating glucose as a substrate with rumen fluid from a high-roughage fed rumen-fistulated cattle. Propionate enhancement was tested by incubating glucose cellobiose, maltose, xylose, casein hydrolyzate, urea and B vitamins with rumen fluid from cattle on a 50% grain diet. Concentrations of antibiotics tested were 0, 3, 6 and 12 µg ml^{-1}. All incubations were at 39°C for 12 hours, and concentrations of total lactate and propionate were measured.

Among the antibiotics tested, Virginiamycin was the most effective inhibitor of lactic acid (90% inhibition). Narasin and Salinomycin were more effective than Lasalocid, Monensin and Thiopeptin. Avoparcin and Tylosin were least effective. Salinomycin was the most effective

propionate enhancer of the antibiotics tested. Narasin, Lasalocid, and Avoparcin were slightly more effective than Monensin and Thiopeptin. Tylosin and Virginiamycin increased the molar proportion of propionate at 3.0 μg concentration, but at higher concentrations (6 and 12 μg ml^{-1}) molar proportion of propionate decreased. Although succinate producers and lactate fermenters were resistant to all antibiotics tested, the extent of propionate enhancement obtained in vitro was different.

Effects of Sodium and Potassium Ion Concentrations on the Antimicrobial Activity of Ionophores Against Rumen Anaerobes - K. A. Dawson and J. A. Boling, University of Kentucky, Lexington, KY 40546.

Cultures of Butyrivibrio fibrisolvens, Eubacterium ruminantium, Ruminococcus albus, Ruminococcus flavefaciens, and Bacteroides succinogenes were inhibited by lasalocid and monensin at concentrations of 2.5 μg/ml. However, responses to these antimicrobial agents was influenced to a large extent by the potassium ion concentration in the media between 1.3 and 12.3 mM. The minimum inhibitory concentrations of monensin ranged from $\leq$.08 μg/ml to .62 μg/ml in low potassium medium while they ranged from $\leq$.08 μg/ml to .31 μg/ml for lasalocid in the low potassium medium. As much as 32 times more antibiotic was required to bring about similar inhibition in media containing high potassium concentrations.

Cultures of Bacteroides ruminicola strain GA33 and Selenomonas ruminantium strains D and GA192 were found to be resistant to monensin and lasalocid concentrations of up to 40 μg/ml. However, growth yields were decreased and lag times increased at high concentrations of ionophores. In all cases, increases in the potassium concentration in the media resulted in increased cell yield and decreased lag times in cultures grown in the presence of monensin and lasalocid. Growth responses suggested increased potassium requirements in the presence of the ionophores.

Bacteroides ruminicola strain 23 was initially found to be sensitive to monensin and lasalocid. Growth was inhibited by monensin concentrations of .62 μg/ml and lasalocid concentrations of .16 μg/ml in low potassium (1.3 mM) medium and by monensin and lasalocid concentrations of 10 μg/ml in the high potassium (12.3 mM) medium. This organism was adapted to increased monensin concentrations by passage through a medium containing .62 μg monensin/ml and then through a medium containing 10 μg monensin/ml. The adapted strains were resistant to 10 μg of monensin/ml and 5 μg of lasalocid/ml in the low potassium medium and to antibiotic concentrations greater than 40 μg/ml in the high potassium medium. Addition of monensin (10 μg/ml) to growing cultures of both the unadapted and the adapted strains resulted in decreases in the levels of potassium associated with the cells. However, monensin adapted strains continued to grow in this potassium depleted state.

Data from this study suggest that increased potassium concentrations can decrease the antimicrobial and selective activities of monensin and lasalocid against rumen bacteria and indicate that potassium plays a key

role in the antimicrobial activities of the ionophores. However, depletion of cellular potassium can not in itself account for the antimicrobial activities because resistant strains continue to grow even when potassium levels are depleted by monensin.

Metabolism of Mercapturic Acid Pathway Metabolites of Xenobiotics in the Gastrointestinal Tract - G. L. Larsen and J. E. Bakke, USDA, ARS, Metabolism and Radiation Research Lab., Fargo, ND 58105.

To study the metabolism of mercapturic acid pathway (MAP) metabolites of xenobiotics in the gastrointestinal (GI) tract, MAP-metabolites of propachlor were incubated with GI contents from the rat and pig and with pure cultures of GI bacteria. Results from these studies indicate that MAP-metabolites of propachlor were catabolized to the cysteine conjugate in the GI tract and that a bacterial enzyme cleaved the cysteine conjugate of propachlor to 2-mercapto-N-isopropylacetanilide. This enzyme has been isolated from Fusobacterium necrophorum, partially purified and characterized as a cysteine conjugate β-lyase. The enzyme has also been isolated from other GI bacteria. The cysteine conjugate β-lyase was shown to cleave the thioether linkage in cysteine conjugates of S-alkyl (e.g. 2-S-cysteinyl-N-isopropylacetanilide and 1,2-dihydro-1-hydroxy-2-S-cysteinylnaphthalene) and S-aryl [e.g., S-benzothiazolylcysteine] linked xenobiotics. This bacterial enzyme is different than the liver cysteine conjugate β-lyase because the liver enzyme reportedly only cleaves the thioether linkage in cysteine conjugates of S-aryl linked xenobiotics. The cleavage of biliary MAP-metabolites of xenobiotics results in the formation of thiols in the GI tract. These thiols are precursors for the formation of nonextractable fecal residues or are excreted as metabolites of xenobiotics containing methylthio, methylsulfinyl or methylsulfonyl groups. The toxicology of this pathway is not known.

Factors Influencing the Production of p-Cresol and Skatole by Lactobacillus Isolated from the Rumen and Pig Feces - Melvin T. Yokoyama, Kristen A. Johnson, and James R. Carlson, Department of Animal Science, Michigan State University, East Lansing, and Department of Animal Science, Washington State University, Pullman.

In attempting to elucidate the physiological significance for the decarboxylation of indoleacetic acid (IAA) to skatole (3-methylindole) and p-hydroxyphenylacetic acid (pHPAA) to p-cresol (4-methylphenol), we have studied the effects of pH (4-9), energy level (0% and 1% glucose) and ionophores (monensin and lasalocid) in two Lactobacillus isolates from the rumen (SpC) and pig feces (pC), which possess the reaction capability. We have previously shown that SpC will decarboxylate IAA to skatole, and will also decarboxylate pHPAA to p-cresol. pC can only decarboxylate pHPAA to p-cresol. From a mechanistic viewpoint, the similarity of the reaction in these bacteria suggest a common function. Optimum growth of SpC and pC occurred at pH 8 and pH 5. p-Cresol was produced in the pH range 5-8, but was not produced at either pH 4 or pH 9. Skatole production by SpC was

inhibited when the growth medium was modified, except at pH 8. Skatole and p-cresol production by SpC and pC occurred in both 0% and 1% glucose supplemented cultues, but higher decarboxylase activity was observed in non-glycolyzing, resting cultures than in growing cultures. Although growth was drastically inhibited by monensin and lasalocid, low concentrations (.04-.4 ppm) of the ionophores stimulated skatole production by SpC. Higher concentrations (1-4 ppm) of the ionophores did not appreciably decrease skatole production. Lasalocid depressed p-cresol production by SpC, but decarboxylase activity remained high relative to control cultures. Growth of pC was stimulated by monensin and low concentrations of lasalocid, but higher concentrations (1-4 ppm) were inhibitory. The data suggest that skatole and p-cresol production by SpC and pC is not a function of growth, but rather of maintenance, and that the action of the ionophores is not directly on the activity of the decarboxylases.

Metabolism of Trans-Aconitic Acid by Mixed Rumen Bacteria: Possible Implications in Grass Tetany - J. B. Russell and P. J. Van Soest, USDA and Cornell University, Ithaca, NY.

Trans-aconitate can account for as much as 4.6 percent of the dry matter in forages, and high concentrations of this acid have been associated with hypomagnesemia known as "grass tetany." Stout et al. (1967) suggested that trans-aconitic acid formed complexes with magnesium and decreased the availability of dietary magnesium. Subsequent experiments by Bohman et al. (1969) indicated that oral administration of trans-aconitate and KCl could induce hypomagnesemia. A one percent concentration of trans-aconitate is generally considered toxic if magnesium status is low.

Mixed rumen bacteria from either timothy hay or 60% concentrate-fed cows were incubated with 7.5 mM trans-aconitate. In both cases, trans-aconitate was rapidly fermented and acetate was the primary product. Examination of high pressure liquid chromatography (HPLC) traces revealed a peak that did not have the same retention time as either aconitate or volatile fatty acids. Concentration of this unidentified peak increased during periods of rapid aconitate fermentation and decreased slightly thereafter. After partial purification, a methylated derivative was subjected to chemical ionization mass spectrometry. The protonated, methylated derivative had a molecular weight of 219 and the most probable molecular weight for the original compound was 176 [219 - 1 - 3 (14)]. This molecular weight corresponded to tricarballylate and further HPLC, gas chromatography, electron mass spectrometry, and chemical ionization mass spectrometry indicated that the unknown compound and tricarballyate were identical.

Tricaballylate was metabolized very slowly by mixed rumen bacteria. Magnesium binding studies have not been performed, but the three carboxylic acid groups suggest it would be an effective chelator of magnesium. Given its slow rate of metabolism and its potential as a magnesium chelator, tricarballylate formation in the rumen from trans-aconitate could be an important factor in the hypomagnesemia that leads to the clinical symptoms of grass tetany.

Detoxication of 3-Hydroxy-4-(1H)-Pyridone, the Goitrogenic of Mimosine, by Rumen Bacteria from Hawaiian Goats - M. J. Allison, H. M. Cook, National Animal Disease Center, ARS, USDA, Ames, IA 50010; and R. J. Jones, CSIRO, Davies Laboratory, AITKENVALE, Townsville, Qld. 4814, Australia.

Leucaena leucocephala is a leguminous shrub with considerable potential for forage production in the tropics. At present, however, the value of leucaena as a feed for ruminants is limited by the prsence in both leaves and seeds of the toxic amino acid, mimosine. In northern Australia, prolonged feeding of leucaena to cattle has led to low weight gains, hair loss, goiter, and esophageal ulceration. These problems are a result of the toxicity of 3-hydroxy-4(1H)-pyridone (3,4-DHP), which is produced from mimosine by enzymes present in both ruminal microbes and leucaena leaves.

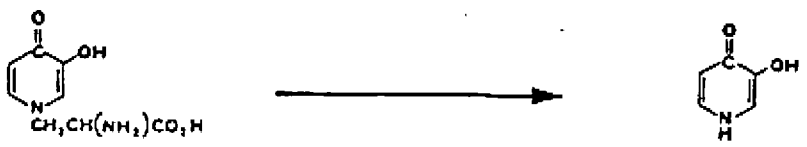
Cattle and goats in Hawaii, however, consume large amounts of leucaena without showing the signs of toxicity noted above. Recognition of this difference between toxicities in Australia and Hawaii led R. Jones to propose that the absence of leucaena toxicity in cattle and goats in Hawaii is due to degradation of DHP by microbes that are present in the rumens of Hawaiian animals, but are not present in Australia (R. Jones, 1981, Aust. Vet. J. 57:55).

Mixed cultures that degraded DHP were obtained when medium 98-5 (Bryant and Robinson, 1961) containing .01% DHP and 40% rumen fluid was inoculated with rumen contents from Hawaiian goats. Similar cultures inoculated with rumen contents from cattle in Iowa and Texas did not degrade DHP. Serial transfers of the Hawaiian mixed populations retained DHP-degrading capacity, and these cultures are being studied both in Australia and in Iowa.

In Australian experiments, the mixed DHP-degrading population has become established in the rumens of inoculated goats and steers. These animals subsequently have recovered from DHP toxicity and, furthermore, there is evidence for animal-to-animal transmission of the active organism.

In both Australia and Iowa, isolates that degrade DHP have been obtained from the serially transferred mixed culture. Relatively little is yet known about the isolates. Preliminary results indicate the active agent is an obligately anaerobic, gram-negative rod that ferments some amino acids but does not ferment mimosine or carbohydrates.

Changes in populations of ruminal microbes that are important for detoxication of other substances (e.g., oxalate, nitrate) have been described. These changes, however, involve selections or changes in proportions of indigenous organisms. In contrast, the detoxication of DHP clearly involves introduction of a non-ubiquitous geographically limited organism,and thus appears to be a rather unique phenomenon. The practical implications of introduction and colonization of the rumen by these DHP-degrading microbes are perhaps great.



Mimosine ⟶ 3–Hydroxy–4(1H)–Pyridone
(3,4–Dihydroxypyridine)
3,4–DHP

The Biodegradation of Vitamin D by Rumen Microbes - R. M. Gardner, R. L. Horst, P. A. Hartman, and M. J. Allison. National Animal Disease Center, ARS, USDA, and Iowa State University, Ames, IA 50010.

Metabolism of naturally occurring steroids by the gut microflora has been extensively documented. Recently vitamin D (a 9-10 seco-steroid) has been shown to be metabolized to trans-10-keto-19-nor vitamin D (10-keto) by a mixed population of rumen microbes. Both dietary D_2 and endogenous D_3 can be substrates for this transformation. Data obtained from centrifugated rumen fluid samples suggest that bacteria and not protozoa are primarily responsible for this metabolism.

In an early attempt to maximize the *in vitro* production of the 10-keto derivative, it was discovered that molecular oxygen was a prerequisite. The 10-keto metabolite has been detected in the plasma of D-toxic cows, and there is evidence that low levels of dietary 10-keto D_2 are present in the rumen. If oxygen is obligately linked to the *in vitro* formation, it would represent an unusual phenomenon given the anaerobic nature of the rumen. The optimal concentration of oxygen is not know, since the *in vitro* incubation system relies on the establishment of an oxygen gradient. The value is thought to approach *in vivo* values since bubbling with 2 to 0.01% oxygen completely inhibited all vitamin D metabolism.

This metabolite provides evidence for a unique pathway of vitamin D metabolism, and is the first characterized vitamin D metabolite of microbial origin. The conversion of vitamin D to its 10-keto form probably represents a detoxicifaction mechanism. It would be logical to expect the presence of these organisms in monogastric animals since the rumen serves as a model for studying the microflora of the colon.

Enumeration of Anaerobic Oxalate-Degrading Bacteria from the Sheep Rumen - S. L. Daniel, H. M. Cook, and M. H. Allison, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA 50010.

Ingestion of large quantities of high oxalate-producing plants such as *Halogeton glomeratus* (halogeton) by ruminants can result in oxalate poisoning. Oxalate intoxication involves hypocalcemia, formation of calcium oxalate crystals in kidney and rumen wall, and may lead to death. Oxalate is degraded by ruminal microbes and adaptation to high oxalate diets leads to an increased ruminal oxalate-degrading capacity. A selective enrichment of anaerobic oxalate-degrading bacteria in the rumen probably accounts for the increased oxalate degradation rates.

Anaerobic oxalate-degrading bacteria were isolated from the rumen of an adapted sheep using enrichment culture techniques (Dawson et al., 1980, Appl. Environ. Microbiol. 40:833). Isolates were gram-negative, nonmotile, slightly curved rods which degraded oxalate to CO_2 and formate. One isolate, designated OxB, was used to develop a direct isolation medium. This medium contained minerals, acetate, 0.1% yeast extract, 7 mM $CaCl_2$, 40 mM oxalate, and agar. The presence of calcium oxalate made the medium

slightly opaque. Colonies of oxalate-degrading bacteria were detected by the production of clear zones around colonies.

In the present study, a sheep was changed gradually from a diet of ground hay and oats to a diet that contained 32% halogeton (5% oxalic acid). Enumeration of oxalate-degrading bacteria in rumen contents was not possible with the 40 mM oxalate medium. When the oxalate concentration in the medium was reduced to 20 mM, counts of oxalate-degrading bacteria ranged from 1.5 to 5.3 x 10^5/g rumen contents. Oxalate degradation rates decreased by about one-half when he sheep was changed to diet containing 16% halogeton; however, counts of oxalate-degrading bacteria decreased by much more than one-half. This medium, therefore, appeared to be underestimating the number of oxalate-degrading bacteria.

Next, a sheep was gradually adapted to a diet that contained 3.0% technical grade sodium oxalate (2.0% oxalic acid). Enumeration of oxalate-degrading bacteria was improved when the $CaCl_2$ level in the medium was doubled (14 mM) and when yeast extract concentrations were increased. Counts of oxalate-degrading bacteria were 1.3 and 2.7 x 10^5/g rumen contents with 0.1 and 0.3% yeast extract in the medium, respectively. In another experiment with 0.3, 0.5, and 1.0% yeast extract in the medium, counts of oxalate-degrading bacteria were 5.5, 4.3, and 3.7 x 10^6/g rumen contents, respectively. Thus a medium containing 0.3% yeast extract, 20 mM oxalate, and 14 mM $CaCl_2$ appears to be better for enumeration of oxalate-degrading bacteria, but further tests to verify this are needed.

Results of this study suggest that: (1) enumeration of oxalate-degrading bacteria in the rumen is possible, (2) oxalate-degrading bacteria represent only a small portion of the total bacterial population in the rumen, and (3) in contrast to OxB, most oxalate-degrading bacteria demonstrate different nutrient requirements and various degrees of tolerance to oxalate.

Effect on Ammonia Treatment on Straw Digestion by Rumen Bacteria - N. Kolankaya, C. S. Stewart, and J. W. Costerton, Haceteppe University, Ankara, Turkey; Rowett Research Institute, Aberdeen, Scotland; and University of Calgary, Alberta, Canada.

The treatment of barley straw with gaseous ammonia (3 parts NH_3:100 parts dry straw) at 90°C for 15 h increased the susceptibility by the predominant cellulolytic rumen bacteria Ruminococcus albus, R. flavefaciens, and Bacteroides succinogenes. Under the conditions used, with inocula grown on soluble substrates and sterility of the straw ensured by autoclaving R. albus solubilized straw more rapidly than the other bacteria tested. Ammonia treatment of straw enhanced growth of the bacteria as measured by the accumulation of cell protein in the culture liquid, and the production of fermentation acids (acetic, succinic, formic) was also increased relative to the amounts detected when untreated straw was used as a substrate. The bacteria was unable to significantly reduce the particle size of either the untreated or ammoniated straw. Colonization of straw particles, measured by assay of ATP associated with straw, was enhanced by ammonia treatment.

Use of a Multi-Compartment Continuous Culture System to Examine Interactions Between Rumen Microbes and Fibrous Substrates - J. L. Jeraci and Peter J. Van Soest, Department of Animal Science, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca. (No abstract received)

Diurnal Carbohydrate and Bacterial Profiles in Rumen Digesta Samples From Steers Fed High or Low Forage Diets - Jane A. Z. Leedle, Karen Barsuhn, Microbiology and Nutrition, The Upjohn Company, and Robert B. Hespell, Department of Dairy Science, University of Illinois.

A method to sequentially extract the five (5) major carbohydrate types from feed and rumen digesta samples was developed. It was based on accountability of total dry matter (chemical determination), minimal carryover between extracts, solvent choice, simplicity, and compatibility with work day/work week scheduling. The analysis was limited to carbohydrate components in order to complement existing bacterial data which were in terms of carbohydrate fermenting groups.

Our objective was to discover whether the trends observed in bacterial group numbers in the rumen contents samples from steers fed maintenance diets (IX metabolizable energy requirement for the animal) once daily were supported by the types and amounts of carbohydrate in the digesta over time.

We found that carbohydrate fermenting bacterial groups were related to the distribution of digesta carbohydrates with time after feeding. Specifically, the complex polysaccharide degrading bacterial groups followed diurnal patterns similar to those detected in the digesta. The disappearance of hemicellulose and cellulose materials could be detected at the time(s) predicted by the bacterial data and the theoretical fermentation curves.

These data support the theory that feed carbohydrates are fermented at different times (and apparently at different rates) by different groups of bacteria. Development of these two methods to measure changes in bacterial "functional" groups and changes in digesta carbohydrate distribution represents a first step toward quantification of mixed bacterial group interactions in the rumen.

Microbiology and Ration Digestibility in the Hindgut of the Ovine - Sherry M. Lewis and Burk A. Dehority, Department of Animal Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691.

Contents from the terminal ileum, cecum-proximal colon and terminal rectum were obtained from a total of nine sheep, three each fed 100% orchardgrass hay (OG); 60% cracked corn-40% OG; and 80% cracked corn-20% OG. Digestibility of dry matter in the cecum was greatest when the all-hay diet

was fed. On the other hand, cellulose digestion increased with increasing levels of concentrate. Total VFA concentration was greatest in the cecum for all diets and cecal pH decreased with increased corn in the diet. Total microbial numbers in the ileum and cecum increased in response to feeding concentrate; however, across all diets, ileal counts were 2% or less of the cecal counts. In contrast, cellulolytic numbers in the ileum were 50% or more of those in the cecum, and were highest with the all-hay diet. Bacterial numbers in the cecum were equivalent to those in the rumen on a per gram basis and produced similar concentrations of end-products. A total of 16 cellulolytic cultures were isolated and characterized from ileal and cecal contents of animals on all three diets. Seven Gram-negative rods were classified as Butyrivibrio fibrisolvens. Capacity of all strains to digest cellulose exceeds that of several rumen strains. Guanine plus Cytosine content of DNA for one strain was 38.8% which compares to the only previous report for that species of 41.2%. Nine cocci were classified as Ruminococcus flavefaciens, based upon morphology, end-products and nutrient requirements. All cellulolytic organisms isolated in this study appear to be closely related to rumen strains of these species.

Rumen Fungi: Cell Wall Degradation and Influences on Growth on Forage Fiber - D. E. Akin and R. H. Brown, Russell Research Center, ARS-USDA, Athens, GA and Agronomy Department, University of Georgia, Athens, GA.

Recent research has established that anaerobic fungi are part of the microbial population responsible for fiber degradation in the rumen. The rumen fungi attack certain lignified tissues (i.e., chlorine-sulfite positive types) and cause extensive disruption of plant tissues. In the presence of penicillin (1.25 mg penicillin G per ml broth) and streptomycin (0.2 mg per ml broth) rumen bacteria were inhibited while the number of sporangia developing on the cut edge of forage leaves was higher (29.0 ± 27.6) when compared to similar leaves incubated without those antibiotics (4.3 ± 4.1). Cyclohexamide (0.5 mg per ml broth) inhibited the development of sporangia on fiber. Sporangia representing rumen fungi from a single source of inoculum developed to different extents on different forage grasses; higher sporangial numbers developed on bermudagrass compared to other species, but variations occurred among bermudagrass cultivars. Sulfur fertilization tended to stimulate greater numbers of sporangia on forage leaves in vitro, but variations were high and differences between sulfur-fertilized and unfertilized forages were not great. Elemental sulfur increased the number of sporangia developing on forage leaves in vitro by about 3 times over that in control experiments, whereas no increases occurred with methionine, cysteine, or sodium sulfate. Rumen fungi occupy a niche in the rumen by attacking resistant fiber, but specific factors related to their colonization and development need further research.

The Attachment of Butyrivibrio fibrisolvens and Other Ruminal Bacteria to Cellulose - M. A. Rasmussen, B. J. Paster, and R. B. Hespell, Department of Dairy Science, University of Illinois, Urbana, IL 61801.

The attachment of Butyrivibrio fibrisolvens and other rumen cellulolytic species to cellulose was investigated. Attachment was measured with ^{14}C labeled cells or by turbidometric means. The methods yielded similar results.

The attachment of B. fibrisolvens to increasing concentrations of ball milled Whatman #1 filter paper indicated a hyperbolic function. Saturation conditions were reached at approximately 1% cellulose. Attachment to crystalline cellulose gave a linear response up to approximately 5% cellulose at which point saturation was reached. On ball milled filter paper, the attachment ability of B. fibrisolvens strains ranged from 100% attached cells for strains A38 and D_1 to 30% for strains 12 and 49. Attachment to crystalline cellulose was much less, ranging from 20% for A38 and D_1 to 5% for 49. The ability of B. fibrisolvens strains to degrade both types of cellulose was also measured. It was observed that the ability to attach was not related to the cellulolytic capacity of the strain.

Other cellulolytic species of the rumen showed greater degrees of attachment to cellulose. Strains of Ruminococcus, Eubacterium, and Bacteroides all showed 100% attachment to the ball milled cellulose. Attachment to crystalline cellulose ranged from 35% to 85%. One exception noted was ruminococcus albus strain B199. This noncellulolytic strain attached poorly to both types of cellulose.

This information indicates that the rumen cellulolytic species when grown on soluble carbohydrates, maintain their capability to attach to cellulose. Attachment was observed to be greater on ball milled cellulose. Greater concentrations of crystalline cellulose were required to reach saturation levels. This indicates a reduced number of attachment sites available per gram of crystalline cellulose when compared to amorphous cellulose.

Evaluation of Subsampling and Fixation Procedures Used in Counting Rumen Protozoa - Burk A. Dehority, Dept. Animal Science, Ohio Agricultural Research and Development Center, Ohio State University, Wooster, OH 44691.

Total protozoan numbers can be significantly lower in rumen fluid as compared to whole rumen contents, depending on the time of sampling and the procedure used to separate the fluid and solid fractions. However, generic distribution in rumen fluid was significantly affected in all cases, the percentage of Entodinium increased while Diplodinium and Ophryoscolex decreased. Microscopic observation of fresh and fixed rumen contents did not indicate any marked attachment of protozoa to particulate matter. In addition, dilution ot whole rumen contents with water, 5 mM sucrose or 0.1% Tween 80 prior to fixation did not affect total protozoan numbers or generic composition. It was thus concluded that attachment to feed paticles is probably not a problem in counting procedures. Blending of whole rumen contents to facilitate subsampling caused a decrease in protozoan numbers. The concentration of formaldehyde used for preservation of rumen contents, 4, 10, or 18.5%, did not affect total count.

Special Features of Anaeroplasmas - I. M. Robinson, National Animal Disease Center, Agriculture Research Service, U.S. Department of Agriculture, Ames, IA 50010.

The requirement for anaerobic growth condition is the single most important property distinguishing the Anaeroplasma from all other mycoplasmas. Both sterol- and nonsterol-requiring strains have been isolated from the rumens of cattle and sheep (10^5-10^8 per gram of ruminal contents). Primary cultures were obtained from colonies growing on a 40% clarified rumen fluid medium in anaerobic roll tubes.

Organisms with bacteriolytic and proteolytic enzymes were detected by observing mycoplasma-like colonies surrounded by a clear zone due to lysis of autoclaved Escherichia coli or casein suspended in the isolation medium. Lipid A, a component of lipopolysaccharide, fulfilled the lipid requirement for these organisms. This requirement could also be met by chemically synthesized phosphatidyl choline wth esterified unsaturated fatty acids. Glycerolphosphoryl compounds without esterified unsaturated fatty acids or free fatty acids in various combinations with glycerol and choline would not replace the intact phospholipid.

Cultural, biochemical and DNA hybridization data place the strains studied into five distinct genetically-related groups. Two species of Anaeroplasma have been described; both are sterol-dependent: A. bactoclasticum possessing bacteriolytic and proteolytic enzymes, and A. abactoclasticum, lacking these enzymes. A third taxonomic group which consists of nonsterol-requiring organisms needs to be established. The ecologic role of these organisms in the rumen has not been determined and their taxonomic position in the class Mollicutes has not been described.

Histochemical Localization of Urease in Rumen Bacteria - R. J. McLean, K.-J. Cheng, W. D. Gould, and J. W. Costerton, Agriculture Canada Research Station, Lethbridge, Alberta, Canada and the University of Calgary, Calgary, Alberta, Canada.

The bacterial urease activity of tissue samples from various regions of the ruminant digestive tract was determined and multiple sites in the reticulo-rumens of all cattle and sheep examined showed high activity. The abomasum, omasum, and intestine regions had very low levels of tissue-associated bacterial urease activity except in Hereford and Angus breeds where the abomasum also showed high activity of this enzyme. About 10% of the tissue-associated bacteria recovered from tissue samples in the reticulo-rumen showed significant ability to produce urease. Variable percentages of urease-producing strains were found within many of the important genera of tissue-associated bacteria including both facultative and strict anaerobic bacteria (Staphylococcus 28%, Propionibacterium 19%, Selenomonas 12%, Micrococcus 9%, Streptococcus 9%, Fusobacterium 7%, Eubacterium 5%, Corynebacterium 2%, Peptococcus 2%, and Bacteroides 2%).

A new cytochemical technique for the localization of urease-producing bacteria, based on the precipitation of ammonia by sodium tetraphenyl boron

and subsequent replacement of ammonia with silver, has served to localize urease in the cell envelope region of Staphylococcus. The use of this new technique will make possible the direct identification of urease-producing bacteria, and the ultrastructural methods will allow us to determine the morphotypes of these organisms and their spatial relationships to the contents and epithelial surfaces of the rumen.

Regulation of Exoprotease Activity in Bacteroides Ruminicola GA-33 - D. B. Bates and W. G. Bergen, Michigan State University, East Lansing.

Exoprotease activity of Bacteroides ruminicola GA-33 was assessed under a variety of conditions. An assay was used which measured hydrolysis of azocasein by whole call suspensions (WCS). The WCS were prepared using .1M potassium phosphate buffer (pH 6.7, .02% [w/v] dithiothreitol). WCS were incubated for 2 hours with 1% (w/v) azocasein. Trichloroacetic acid (TCA) was used to stop the reaction and precipitate undegraded azocasein. Dye released by enzymatic hydrolysis was measured colorimetrically at 440 nM following the addition of 1N NaOH. Degradation of the free dye was observed subsequent to TCA addition. This degradation followed 2 pool kinetics with 1 pool decaying exponentially within 24 hours leaving a 2nd, more stable, pool. Sixty % of the color intensity remained after 36 hours regardless of starting absorbance. Comparisons were made using samples that had aged at least 36 hours after TCA addition. Glucose, cellobiose, lactose, maltose, and soluble starch were tested for their effect on the production of protease. Measurements were taken over the entire growth cycle. When cells were grown on glucose or cellobiose protease activity was significantly ($p<.01$) lower than that found with other sugars. Lactose gave the highest mean activity ($p<.01$) with maltose and soluble starch cultures intermediate in activity. The influence of stage of growth was significant ($p<.01$) as most cultures showed a decline in activity when they entered stationary phase. Soluble starch activity actually increased during stationary phase yielding the highest observed specific activity expressed per mg dry matter. Thus, a significant ($p<.01$) sugar-stage of growth interaction was obtained. Monensin increased (or did not influence) activity with glucose, lactose, and soluble starch (during exponential growth), but decreased activity with maltose and soluble starch (during stationary phase). No significant overall treatment effect was observed for monensin yet a significant ($p<.01$) sugar-monensin interaction was seen. Glucose limitation increased activity ($p<.01$) and peptide limitation decreased activity ($p<.01$) relative to control values.

The Role of 3-Phenylpropanoic Acid (PPA) in the Metabolism of Ruminococcus Albus Strain 8 - Robert J. Stack and R. E. Hungate, Department of Bacteriology, University of California, Davis, CA 95616.

Phenylacetic acid is a stimulatory growth factor if PPA is supplied and is assimilated into phenylalanine. Strain 8 deprived of PPA and grown on cellobiose reaches maximum O.D. after 20 hours as compared to 12 hr with PPA, and whereas cells with PPA give a uniform turbidity in culture, those

without it clump in the botton and settle soon after vigorous shaking. TEM examination of both types of cells discloses a distinct capsule around those with PPA but absent from those with no PPA. Deprivation of PPA exerts even more striking effects when strain 8 is grown on pebble-milled cellulose. Digestion of the cellulose is delayed as much as three weeks, and the very large cellulase complex is absent, also in cellobiose-grown cultures. The quantity of the smaller cellulases is much increased. Labelled PPA is recovered only in the very large cellulase complex.

PHYSIOLOGY AND PHYSIOPATHOLOGY

Visceral Blood in the Neonatal Calf - A. Dobson, R. D. Gleed, and B. C. Tennant, Departments of Physiology and Clinical Science, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Seven calves were removed from their dams before they could suckle and were immediately fed bulk tank milk by bottle. Two catheters were implanted in each calf under halothane anesthesia. One catheter was placed with its tip in the left ventricle for injection of microspheres, and the second catheter had its tip at the bifurcation of the posterior aorta for withdrawl of a calibrating blood sample. Calves were fed every 6 hours beginning 16 hr after birth. Flow was measured using 15 micron diameter microspheres labelled with six different radionuclides. Observations were made on each calf at 3 hr and 0.5 hr before this second meal, during eating, and at 2 hr, 5.5 hr, and 11.5 hr after eating. Feeding increased blood pressure and markedly stimulated blood flow to the omasum, abomasum, and pancreas; but depressed flow to the omentum, mesentery, small intestine, and large intestine. Flow to the rumen, to the liver in both hepatic and portal vessels and to the parotid and submandibular glands was not altered by feeding. No post prandial effects were apparent. These changes with feeding are similar to those reported in man and dog, but quite different from those in the adult ruminant except for the rise in blood pressure and the depression of flow through the fatty omentum and mesentery. The small intestine of the calf contributed about two thirds of the portal flow before feeding, compared with less than one fifth in the adult sheep. Whereas the intensity of flow to the small intestine was greater in the calf than the sheep, the parotid, forestomach, pancreatic, and renal flow intensity was lower. On the basis of liver weight, the portal flow of the calf was only two thirds of that in the sheep. Nevertheless, the hepatic arterial flow was five times greater in the calf and preferentially perfused the right side of the liver.

Development and Evaluation of an Autoperfusion Method to Determine Amino Acid and Peptide Transport and Metabolism in Bovine Intestinal Epithelium - J. E. Nocek, C. G. Schwab, and W. E. Hylton, Agway Inc., Syracuse, N.Y. and University of New Hampshire, Durham.

Two Holstein bull calves (8 and 11 wks) were used to evaluate an autoperfusion method to study intestinal amino acid transport. Calves were

anesthetized with Halothan gas. An approximate 20 cm section of small intestine, 60 cm cranial to the ileocecal junction was isolated. A specific set of arcuate vessels associated with the 20 cm length of intestine was clamped at the proximal and distal ends to isolate blood circulation and intestinal flow. The isolated intestinal segment was fitted with a 3 mm ID catheter for intestinal infusion and a 6 mm ID catheter for drainage at opposite ends. A 16 gauge teflon catheter (7 cm) was inserted into the vein and secured by stay sutures. A one meter length of polyethylene tubing was attached to the catheter to allow continuous venous drainage and collection. Prior to treatment infusion, the isolated intestinal segment was rinsed with 300 ml of 39°C saline.

Experiment I: The objective was to determine if physiological conditions could be maintained over time when the intestinal segment is subjected to repeated infusions. The treatment used was 16 mM L-methionine (4 μCi [2 - ^{14}C])-L-methionine in 25 ml of distilled water incubated in the intestinal segment. Eight replications of this treatment were used (12 min. incubation, 30 min. wash with saline). Measurements: 1) Metabolite and enzymes in jugular serum samples taken initially and at the end of alternate replications. 2) Time course appearance of methionine in venous blood from the intestinal segment. 3) Recovery of radioactive marker. Jugular serum protein, urea N and creatinine were stable throughout the experimental time under anesthesia. Serum glucose was variable, and tended to decrease (avg. 64.5 mg/ml). Jugular serum alkaline phosphotase, gamma glutamyl transpeptidase, lactate dehydrogenase and glutamate-oxalacetate transaminase were also stable. Rectal temperature remained constant at 38 ± .1°C. All replications demonstrated saturation at approximately 7.5 minutes, with the same relative dpm for replicates 1 and 8 at saturation. Blood flows for the eight replications averaged 2.6 ± .9 ml/min. during the treatment incubation and 2.7 ± .8 ml/min. during the wash periods.

Experiment II: To determine whether the presence of L-methionyl-methionine would influence absorption of mono-methionine. With a second calf, 16 mM L-methionine was mixed with 0, 4, 8, or 16 mM of L-methionyl-methionine for 10 min. incubation periods. At 4 mM L-methionyl-methionine, appearance of L-methionine in the venous blood was decreased 5 fold. Increasing L-methionyl-methionine, to 8 or 16 mM did not further depress L-methionine appearance. Radioactive marker recovery was 99.7 percent.

These results demonstrate that an autoperfusion method to investigate amino acid transport can be used in the young bovine while maintaning physiological conditions over time with repeated intestinal infusions.

Increased Forestomach Epithelial Receptor Sensitivity to Grain Overload Rumen Fluids Following Forestomach Exposure to Lactic Acid - E. C. Crichlow and R. K. Chaplin, Department of Veterinary Physiological Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

In studying the responses of forestomach epithelial receptors to rumen fluids obtained from "grain overload" sheep, we have found that receptor sensitivity increased after the luminal surface of the forestomach was

exposed to mild solutions of NaOH. Since lactic acid occurs in the forestomach during "grain overload" and exerts a corrosive effect on forestomach epithelium similar to that shown by NaOH, we decided to assess epithelial receptor responsiveness to rumen fluids from "grain overload" sheep before and after exposing the forestomach luminal surfaces to a solution of 70.0 mmol/L lactic acid.

Two sheep were intra-ruminally loaded with ground wheat (40 gms/kg) and rumen fluids were collected at 6, 8, 10 and 12 hours after "loading" when reticulo-ruminal motility was decreased. These rumen fluids were applied to the receptive fields of 5 reticulo-ruminal epithelial receptors that were isolated in 2 halothane anesthetized sheep using single axon recordings from the left cervical vagus nerve. Responses evoked by these rumen fluids were assessed with respect to latency, in seconds, and number of action potentials elicited within an exposure time of 2 minutes. After each receptor was tested with the rumen fluids, their receptive fields were covered for 30 minutes with a solution of 70.0 mmol/L lactic acid and the receptors were tested again with the rumen fluids. This level of lactic acid was used since it produced increased receptor sensitivity without major pathological changes in the epithelium.

Prior to lactic acid exposure, 2 receptors responded to both the 6th and 8th hour rumen fluid samples. One was activated by both the 10th and 12th hour samples and the remaining 2 receptors responded to both early and late rumen fluid samples. The levels of undissociated volatile fatty acids were high in the early rumen fluid samples but low in the later samples. The concentration of free lactic acid, on the other hand, was low in the early samples but high in the later samples. After exposure to lactic acid 4 receptors responded to both early and late rumen fluid samples with the remaining receptor being activated only by the later rumen fluid samples. Responses to rumen fluids, after exposure to lactic acid, tended to have shorter latencies as well as a larger number of action potentials.

These findings indicate that reticulo-ruminal epithelial receptors can be activated by rumen fluids obtained from "grain overload" sheep exhibiting decreased forestomach motility and that exposure of the luminal surface of the reticulo-ruminal compartment to lactic acid enhanced the responsiveness of these receptors.

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