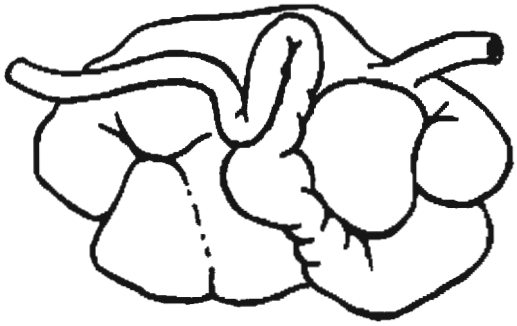
Abstracts and Program

Conference on Rumen Function

Volume 21



40 Years of Interaction

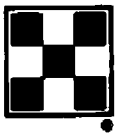
1951-1991

21st Biennial Conference on Rumen Function
Chicago, Illinois
November 12-14, 1991

The proceedings publication is a courtesy of

Purina Mills, Inc.

St. Louis, Missouri



21st BIENNIAL
CONFERENCE ON RUMEN FUNCTION
1951-1991

Welcome to the 21st Biennial Conference on Rumen Function. The Rumen Function Conference has been meeting in the Congress Hotel since November 1951. The discussions originally focused on the problem of bloat, and this aspect of rumen function was a central theme until 1961. Since this time, the Conference has broadened its program to other factors which influence rumen fermentation and physiology.

In the early days, the panel discussions were informal presentations of recent observations and theories. As the Conference grew in attendance, the participants were asked to deliver more formal podium presentations. A poster session was added in 1987.

H. W. Marston, ARC/USDA, served as Conference Chairman from 1951 until 1957 and from 1961 to 1965. N. R. Ellis, ARC/USDA, was Chairman of the 1959 meeting. C. R. Richard, CSRS/USDA, assumed the Chairmanship in 1967 and served until 1983. M. J. Allison, ARS/USDA then served as Chairman from 1985 to 1989.

I hope that this current Conference will provide a stimulating and interesting forum .

Sincerely,

James B. Russell
Research Microbiologist
ARS/USDA

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH ADMINISTRATION
WASHINGTON 25, D.C.

OFFICE OF ADMINISTRATOR

November 1, 1951

For a number of years, the Agricultural Experiment Stations and the Department of Agriculture have been advocating the improvement of pastures. The program of pasture improvement has presented many problems which require research to furnish the solutions. Among these problems is the area concerned with the effective utilization of pasture by livestock.

In many parts of the country improved pastures involve the inclusion of legumes not only for the increased nutritive value of the herbage but also for the improvement of the soil. In recent months we have received many requests for information on what can be done to assist in preventing the cases of bloat that frequently occur among cattle and sheep grazing upon such pastures. I am sure that you have also received numerous requests for similar assistance.

It seems desirable, therefore, to ask research workers who are working on rumen function to get together and plan a program that will assist the livestock producers in utilizing such improved pastures with less danger to the health of their animals.

Because many workers who would be able to contribute to such a program will be in attendance at the meeting of the American Society of Animal Production and the Conference of Research Workers in Animal Diseases in North America, we feel it would be advantageous to get the interested workers together at that time. Accordingly, we are arranging for a meeting to be held at the Congress Hotel, Chicago, Illinois, on November 27 and 28, 1951. The meeting to open at 9 AM on November 27. This would serve to reduce the cost of travel for those participating.

To be most effective a program of the type described should be developed as a result of broad participation by all of the talent available. In order to achieve this wide base of scientific background, the Department plans to have representatives of our pasture, livestock and veterinary research programs at the meeting. It is hoped that those members of your staff who are interested in this subject may also attend.

SOME HIGHLIGHTS FROM THE 1ST CONFERENCE

RUMEN PHYSIOLOGY

Dr. R. W. Dougherty opened the discussion by stating that, in his opinion, the toxic gas theory as related to bloat was a misnomer. He is at present working with unknown substance found in the ingesta and the plant to ascertain the possible relation to bloat. Some of the characteristics of the material have been determined but no substances has been identified. Dogs are being used as test animals. It is assumed that the active substance of the material affects the nervous system. He has not been able to produce bloat in cattle with the material.

Dr. W. E. Thomas reported that a juice had been extracted from alfalfa and ladino clover which will produce bloat regularly, especially if animals have been on pasture for 24 hours. A pint of the juice will produce bloat in cattle. One pint of the extract will kill sheep in from 1 to 1 1/2 hours, while one pint of extract made from birdsfoot trefoil will kill sheep (70 pounds) in about 10 minutes. Sodium thiosulfate, 20 to 25 grams, will save the sheep. The reaction of the animal appears similar to HCN poisoning. Approximately 30 ounces of the extract can be prepared from 10 pounds of alfalfa. The extract was prepared by running the alfalfa through a cane mill.

Dr. A. T. Phillipson raised the question as to whether animals died as a result of gas pressure or does the gas pressure excite some other factor to produce death. No answer.

PHYSIO-PATHOLOGY

Dr. Dougherty pointed out that under normal conditions an animal was able to get rid of all gas produced by eructation.

Dr. Burroughs found that the surface tension of the ingesta of chronic bloaters was lower than that of non-bloaters. It has been difficult to produce gas in test tubes from the fermentation of certain types of plant materials. The glucose content of young alfalfa plants was higher as compared to more mature plants. It also appeared to be higher in the afternoon than in the morning. This condition is apparently related to the amount of sunlight for on cloudy days the glucose content does not rise in the afternoon.

Dr. Dougherty summed up the discussion by saying it was apparent that not too much is known about the fundamentals of bloat and the cause of death in cases of bloat. Aside from mechanical treatment, knowledge on treatment of bloat was also deficient.

AGRONOMIC

Dr. Phillipson called attention to the fact that bloat does occur in England on strictly grass pastures. Prof. Baskett stated that rapid growing rye gasses will produce bloat.

Mr. Sullivan raised the question as to whether the amount of readily fermentable carbohydrates in pasture forage might be a factor in producing bloat. The amount of fermentable carbohydrates available varies with the type of forage. Carbohydrates are higher in grasses than in legumes while the reverse is true in the case of nitrogen. Grazing

permits animals to ingest living cells that are still active and to add them to the system already present in the rumen.

Dr. Gall pointed out that bacteria require both sugar and nitrogen for growth. This may be a factor in producing bloat on legumes.

MANAGEMENT

Dr. Cole stated that the water content of the soil, and presumably that of the plant growing on that soil, influences palatability of alfalfa. He pointed out that we have to deal with two types of bloat – chronic and acute bloat. The former condition does not depend upon the nature of the feed ingested. He feels that it would be desirable to differentiate the types of bloat in any discussion. He also pointed out that any condition which interrupts the mechanism of belching will produce bloat. Many cases of death, which have been attributed to bloat, were not actually caused by bloat.

Dr. Cole reported that grazing alfalfa alone will produce bloat in sheep. However, when they grazed alfalfa at night and Sudan during the day, or the reverse, there was no evidence of bloat. This system of grazing, however, cut the consumption of alfalfa from 10 to 15%. It was necessary to feed 10 pounds of Sudan hay to prevent bloat on alfalfa pasture. Barley straw was ineffective for this purpose as the animals would consume only 5 pounds. Alfalfa hay was of little or no value in preventing bloat when fed to animals grazing alfalfa. Cattle fed the top 3 inches of cut alfalfa bloated but when fed the whole plant no difficulties were encountered.

MICROBIOLOGY

Dr. Pounden opened this section of the conference with the observation that we need to know what causes bloat in order to determine whether organisms as such are associated directly or indirectly with bloat. He raised the question as to whether some organisms produce toxins that may be in part responsible for bloat. He suggested the possibility of using organisms in test tubes to determine the bloatability of feeds.

Dr. Gall pointed out that any work on the microbiology of the rumen prior to the last four or five years is obsolete. While bloat is a complex problem certain advances are being made that should aid in solving the causes of bloat.

In studies in an artificial rumen, Dr. Burroughs found that the following factor had to be present in order to stimulate the proliferation or organisms at a rapid rate – a readily available carbohydrate, a simple form of nitrogen, phosphorus, various iron salts (at a high P level), and unknown substances or factors occurring in young growing plants and molasses. A processed rumen juice was of only temporary value in treating chronic bloaters.

Dr. Gall has been able to grow from 25 to 30 varieties of organisms in the test tubes without rumen juices. These organisms digest the same material in approximately the same time in the artificial rumen as in the true rumen. She has found as many as 10 million aerobic organisms per gram of rumen content after feeding. Aerobic organisms can live but not metabolize at an Eh of -250 mV.

NUTRITION PANEL

November 13, 1991

9:00 BRIEF INTODUCTION. J. B. Russell, ARS/USDA and Section of Microbiology, Conrell University, Ithaca, NY 14853.

9:15 #1 RUMINAL ESCAPE PROTEIN REQUIREMENTS OF LIGHT-WEIGHT FEEDLOT CALVES. R.A. Zinn, Dept. Anim. Sci. University of California IVAC, El Centro, CA 92243 (619 352 0111)

One hundred-forty crossbred calves were used to evaluate the ruminal escape protein (REP) requirements of feedlot steers during an 84-d growing period (198 to 317 kg live weight). Dietary treatments were as follows: 1) basal diet; 2) basal diet plus 2% of a high bypass protein blend (HBP; 1/3 blood meal, 1/3 meat and bone meal and 1/3 feather meal); 3) basal diet plus 4% HBP; and 4) basal diet plus 6% HBP. The basal diet contained 18% alfalfa hay, 10% sudangrass hay, 61% steam flaked corn, 2.5% yellow grease, 6% molasses and 2.5% supplement. The diets were formulated to roughly contain 4.2, 5.2, 6.2 and 7.2% REP, respectively. There was a quadratic effect ($P<.05$) of REP on ADG and DM conversion. The greatest response was with treatment 2 (2% HBP), which increased ADG and DM conversion 13.4 and 8.4%, respectively, over that of the basal diet. There was also a quadratic effect ($P<.05$) of protein supplementation on the NE value of the diet. The addition of 2% HBP increased the NEm and NEg of the diet 5. 6 and 7.5%, respectively. Characteristics of digestion of the dietary treatments was evaluated using 4 Holstein steers with cannulas in the rumen, proximal duodenum and distal ileam. There were no treatment effects on ruminal and total tract digestion of OM. HBP increased linearly ($P<.01$) the passage of NAN and AAN to the small intestine. Based on plasma amino acid profiles of steers in Trial 1, the limiting amino acids (ranked in probable order of first most limiting) were lysine, isoleucine and methionine.

9:30 #2 EFFECT OF MONENSIN ON THE COMPOSITION AND QUANTITY OF AMINO ACIDS ESCAPING RUMINAL DEGRADATION IN VITRO. C-M. J. Yang and J. B. Russell, Department of Animal Science and Section of Microbiology, Cornell University and ARS/USDA, Ithaca, NY 14853 (607-255-4508)

When enzymatic hydrolysates of casein (Trypticase) and gelatin (0.5 g N/liter) were incubated <u>in vitro</u> with mixed rumen bacteria (50 mg N/liter) in the absence of carbohydrates, there was little increase in microbial protein and virtually all of the peptide utilization could be accounted as ammonia. More Trypticase was converted to ammonia than gelatin, but neither hydrolysate was completely utilized even after 96 h. The residual material was mostly free amino acids. With Trypticase, valine, leucine and isoleucine were the predominant amino acids, but glycine, alanine, and proline accounted for most of the residual gelatin hydrolysate. Monensin (5 uM) caused a decrease in ammonia production from Trypticase and gelatin hydrolysate (33 and 55%, respectively), and much of the residual amino acid nitrogen was peptide (43 and 62%, respectively). When monensin was added, the residual Trypticase had an abundance of proline, glutamine or glutamate, alanine, phenylalanine, and glycine as well as branched amino acids. The residual gelatin hydrolysate was still mostly glycine, proline and alanine. Based on these results, it appears that monensin may affect the composition as well as total amount of amino acid nitrogen escaping ruminal degradation.

9:45 #3 THE EFFECT OF SUPPLEMENTARY PEPTIDES OR BRANCHED-CHAIN VOLATILE FATTY ACIDS ON THE EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS BY RUMEN BACTERIA GROWING ON ALKALINE HYDROGEN PEROXIDE-TREATED WHEAT STRAW IN CONTINUOUS CULTURE. B. L. Kernick[1], J. B. J. van Ryssen[2] and R. I. Mackie[3], Animal and Dairy Science Research Institute, Irene, 1675, South Africa. [1]Present address: Kynoch Feeds (Pty) Ltd., P O Box 4880, Randburg, 2125, South Africa. (011 2711 7870419) [2]Department of Animal and Poultry Science, University of Natal, Pietermaritzburg, South Africa. [3]Department of Animal Sciences, University of Illinois, Urbana-Champaign, USA.

Microbial protein synthesis by rumen bacteria grown on purified substrates in vitro has been increased by the supply of peptides in a number of studies. Supplementation of ruminant diets, particularly low-quality roughages, with rumen-degradable protein sources has however yielded inconsistent results in this regard. The object of this experiment was to determine whether microbial protein synthesis by mixed rumen bacteria growing on alkaline hydrogen peroxide-treated wheat straw (AHPWS) was affected by supplementation with peptides or branched-chain VFA (BCVFA). Media based on AHPWS which was supplemented on an isonitrogenous basis with either ammonium salts alone, ammonium salts plus BCVFA or a combination of ammonium salts and peptides in the form of casein hydrolysate, were incubated in continuous culture with mixed rumen bacteria at a dilution rate of $0.09h^{-1}$. Both peptide and BCVFA supplementation significantly increased total amino acid concentrations and reduced NH3-N concentrations in culture effluent flowing from the fermentors, relative to that of the treatment supplemented with ammonium salts alone ($P<0.05$). Bacterial N synthesis was increased by BCVFA and peptide supplementation, however this was only significant ($P<0.05$) in the case of the peptide-supplemented medium. Despite the increase in bacterial protein synthesis, supplementation of AHPWS with peptides resulted in the lowest overall efficiency of amino acid synthesis due to extensive deamination of the amino acids supplied by the casein hydrolysate.

10:00 #4 IN VIVO EFFECTS OF SODIUM SUCCINATE ON RUMINAL pH AND LACTIC ACID IN A SUBACUTE AND ACUTE MODEL OF GRAIN INDUCED ACIDOSIS IN BEEF CATTLE. W.J. Smolenski*, J.A. Robinson and M.L. Ogilvie. The Upjohn Company, Kalamazoo, MI. 49001 (616 385-6596)

Sodium succinate hexahydrate (SSH) was tested for effects on ruminal pH and lactic acid in two in vivo acidosis models (subacute and acute) using fistulated beef cattle. When included in the diet at 0.4-1.0 g/kg BW, SSH prevented subacute acidosis. For three separate subacute studies the mean minimum pH values for control animals was 5.2, 5.8 and 5.1 whereas SSH treated cattle had significantly ($P<0.1$) elevated pH values, 6.0, 6.5 and 5.7, respectively. The mean maximum lactic acid concentrations reached in control animals was 18, 4 and 55 mM for the three respective studies. In SSH treated cattle, the mean maximum lactic acid concentration was 0.4, 0.3 and 7.6 mM, respectively. Although SSH reduced the concentration of lactic acid compared to controls in all three studies, only study three had significantly different ($P<0.1$) maximum lactic acid concentrations. No differences were observed for total ruminal volatile fatty acids; however, SSH treatment did decrease the acetate/propionate ratio. In the acute acidosis model SSH increased ruminal pH and decreased lactic acid accumulation when compared to controls, however, this numeric trend was not statistically significant. The pH buffering observed when SSH is added to the rumen may be accounted for by proton consumption as succinate is decarboxylated to propionate by ruminal bacteria.

10:15 BRIEF BREAK

10:30 #5 Effects of ruminal inoculation of *Megasphaera elsdenii* strain 407A on ruminal pH and organic acids in beef cattle. J.A. Robinson, R.C. Greening, W.J. Smolenski, K. Barsuhn, M.M. Johnson and R.L. Bell. Performance Enhancement Research, The Upjohn Company, Kalamazoo, MI.

A strain of Megasphaera elsdenii (407A) was tested for prevention of acute ruminal acidosis in fistulated cattle. Forty cattle in blocks of 10 each were allotted to four treatments: (i) uninoculated controls (n=2), (ii) inoculation with isolate 407A (n=2) 8 hours prior to induction of acidosis (-8 h), (iii) inoculation with 407A (n=4) at the start of acidosis induction (0 h) and (iv) inoculation with 407A (n=2) 2 hours after initiation of acidosis induction (+2 h). The inoculum size was 2-5 x 10^{12} CFU per head across blocks. Ruminal fluid was collected from -8 to 16 h for measurement of pH, total lactate and volatile fatty acids (VFA). The pH, lactate and VFA time course data for each heifer were converted to single degree-of-freedom summary variables. Mean minimum pH values for the control, -8, 0 and +2 h treatments were: 4.7, 4.7, 5.5 and 5.3, respectively. Mean maximum lactate concentrations were 120, 120, 50 and 46 mM, respectively. Mean areas under the total VFA curves for the respective treatments were 470, 510, 910 and 870 mM·h. Differences between the summary variables for the control versus -8 h groups or for the 0 h versus +2 h groups were not significant (P>0.1). Differences between the pooled control and -8 h versus the pooled 0 and +2 h least squares means were highly significant (P<.01). Our data support that 407A may allow simplification of the feedyard adaptation period.

10:45 #6 EFFECT OF LIVE YEAST CULTURES ON FEED DEGRADATION IN THE RUMEN AS ASSESSED BY *IN VITRO* MEASUREMENTS. J.P. Jouany[1], G. Fonty[2], Bernadette Lassalas[1], J. Dore[2], Ph. Gouet[2], G. Bertin[3], Unites de (1) Digestion Microbienne et (2) Microbiologie, INRA, 63122 Saint-Genes-Champanelle (73624000), (3) OHF-Santel, 92300 Levallois-Perret (147906509), France.

The effect of three strains of live yeast cultures (A,B,X) has been compared using rumen microbial inocula maintained in RUSITEC. Rumen contents were sampled from animals adapted (YA+) or not (YA-) to the yeasts (diet : roughage 50, concentrate 50). Microbial populations were followed, and fermentation products were monitored as well as dry matter (DM), organic matter (OM) and plant constituants' degradation. In RUSITEC, the greatest part of autochtonous microbial flora as well as yeast populations appear bound to the particle fraction. With (YA-) inocula, yeast strains A and B doubled the total microflora while strain X had no effect on this parameter. None of the strains affected cellulolytic, methanogenic or fungal populations. Strain A and even more so strain B significantly increased degradation of DM, OM, NDF, ADF, hemicellulose and cellulose. Organic matter fermentation was increased by strain B only; while strain X had no effect at all. With (YA+) inocula, no effect could be observed on microbial populations. Strain B nevertheless still exerted a clear positive effect, more important than that of A and X, on microbial activity: significant increase of DM, OM and plant constituants' degradation. Indeed for strain B, in vitro digestibilities were greatly increased for cellulose (+70%) and hemicelluloses (+39%) and VFA production was greater by 11,1%. In conclusion, live cultures of yeasts can exert a probiotic effect towards microbial activity in the rumen, with is highly dependent on the strain used.

11:00 #7 EFFECT OF SUPPLEMENTAL CORN GLUTEN MEAL OR COTTONSEED MEAL PROVIDED TO CATTLE CONSUMING RANGE HAY. T. M. Hill, B. R. Carmean and D. E. Johnson. Metabolic Laboratory, Colorado State University, Fort Collins, CO 80523 (303-491-1239).

The objective of this study was to determine if corn gluten meal (CGM), a presumed ruminally undegraded intake protein (UIP) source, can be used as effectively as cottonseed meal (CSM) for cattle consuming range hay (6.1% CP; 68% NDF). Four Hereford steers (470 kg) fitted with ruminal and duodenal cannulae were fed either 100% hay (CON), 95:5 hay to CGM (5CGM), 90:10 hay to CGM (10CGM) or 90:10 hay to CSM (10CSM) at near maintenance (2.2 kg as-fed twice daily). Ruminal and total tract OM digestion, fraction of total tract OM digestion occurring in the rumen, flow of total protein (TP), MP, UIP and microbial efficiency increased (P<.01) with increasing levels of CGM supplementation and there was no difference between 10CGM and 10CSM treatments, except for TP and UIP (10CGM > 10CSM; P<.01). Fractional UIP was .39 and .17 for CGM and CSM, respectively, and .28 for hay (based on CON). These data suggest that CGM can be as effective as CSM at increasing digestion and protein supply to the duodenum, possibly due to the small UIP fraction of all supplements, when cattle consume poor quality forage.

11:15 #8 RETICULAR MOTILITY AND ESTIMATED RUMEN WALL TENSION IN DAIRY COWS TREATED WITH INERT RUMEN BULK AND FED DIETS DIFFERING IN FORAGE CONTENT. T.R. Johnson* and D. K. Combs, Dept. of Dairy Sci., Univ. of Wisconsin-Madison, 53706 *Dept. Animal Sci., Purdue University, West Lafayette, Indiana, 47907(317-4-4-4805).

Eight rumen cannulated multiparous cows were used in two 4x4 Latin squares with a 2x2 factorial treatment arrangement. Factors were feeding 74:26 F:C, 33% NDF (HF) or 50:50 F:C 27% NDF diets with (+) or without (-) ruminal water filled bladders. Periods were 21d. Reticular contractions were recorded during eating, resting, and rumination with a closed tipped catheter attached to a strain gauge transducer. Intake and milk yield were greater for cows fed the LF than HF diet. Bladders increased total rumen volume (P<.01) but had no effect on intake or mean contraction frequency (FRC), duration (DUR), or amplitude (AMP). The law of Laplace T= Pr; where T = organ wall tension, P = baseline intraluminal fill pressure and r = hollow organ radius was applied. The mean tension estimate was $6.4x10^5$ dyne/cm_2 and was not affected by treatment. We propose that compensation for HF and bladders occurred by an expansion of rumen volume with a change in tonus of rumen wall smooth muscle. This may allow rumen volume expansion without increased wall tension. Expansion with minimal tension receptor exitation could result. Bladder main effects are in table. Interactions were NS (P>.05).

	Bladders	
Item	-	+
DMI,kg	25.0	24.8
FCM,kg	32.4	32.9
Rumen Vol., l		
Total	102	119**
Digesta only	102	96
Reticular motility		
FRQ, / h	74.3	76.5
DUR, sec.	7.1	7.3
AMP, cm H20		
Base line	17.8	17.5
Peak	18.4	16.9
Tension, Dyne/cm_2		
	$6.3x10^5$	$6.5x\ 10^5$

**P<.01

AGRONOMY PANEL

2:00 INVITED SPEAKER #9 Polysaccharide Interactions That Restrict Plant Cell Wall Digestibility.R. D. Hatfield USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI 53706

Forge cell walls are organized into complex composites of polysaccharides, lignin, phenolic acids, proteins, water and ions. The composition of individual walls vary depending upon the developmental stage of the cell and its structural/functional role. Interactions among individual components of the cell wall are key to the formation and structural integrity of the total wall matrix. These include bonding interactions that range from weak hydrogen bonds to covalent cross-link involving phenolic acids, uronic acids, and ether linkages to lignin. Wall carbohydrates represent a potential energy source for ruminants that are not completely utilized. Lignin is most often correlated with decreases in cell wall digestibility although the responsible mechanism has not been elucidated. The amount of lignin may not be the most critical aspect but rather the type and degree of cross-links between lignin and other wall components. Substitution patterns on individual polysaccharides and the degree of cross-linking among polysaccharide components may also play an important role in regulating wall degradation.

2:30 #10 RUMINAL PROTEIN AND CELL-WALL DIGESTION OF GRASS SILAGE TREATED WITH ADDITIVES. E. Thorstensson, D.R. Buxton, and P. Lingvall. Dept. Agronomy, Iowa State University; USDA-ARS, Ames, IA. 50011; Dept. Animal Nutr. and Manag., Swedish University Agric. Sci., Uppsala, Sweden (ph 515-294-3886).

This study was conducted to compare the effects of formic acid, lactic acid bacteria (LAB), and cell-wall (CW) degrading enzymes on silage protein and CW digestion. Orchardgrass (*Dactylis glomerata*)-timothy (*Phleum pratense*) silage was treated with formic acid (4 L/Mg fresh herbage), Siloferm (*Lactobacillus plantarum* and *Pediococcus acidilactici*, 10_6 LAB/g fresh herbage) Pediococcus (*Pediococcus acidilactici*, 10_6 LAB/g fresh herbage), Econase (endo-1,4-beta- glucanase, 2 hydroxyethylcellulose units (HECU)/g fresh herbage), or Siloferm combined with Econase (10_6 LAB+2 and 4 HECU/g fresh herbage). Formic acid, which rapidly decreased silage pH to 4.2, had less proteolytic activity during ensiling and therefore 44% less non-protein-nitrogen (NPN) than the other treatments. After 4 h ruminal digestion, formic acid treated silage had 26% greater undegradable protein concentration than that of inoculants, Econase, or control. Formic acid and Econase alone and combined with Siloferm had 5% less neutral detergent fiber than Siloferm and Pediococcus, which behaved similarly to the control. Siloferm, both alone and combined with Econase, and Pediococcus had 24% lower NPN than the control because of extensive homofermentation of sugars and fiber degradation by Econase. Fiber digestion rates ranged from 0.096/h to 0.126/h and were faster for Formic acid, Pediococcus, and Siloferm+Econase (4 HECU) compared with untreated silage. Protein digestion rates ranged from 0.025/h (Econase) to 0.041/h (Siloferm). Total ammonia-N and alpha-amino-N (- blanks) ranged from 2.9 (Formic acid) to 4.5 mM (control) after 4 h incubation. Associated ruminal net production of ammonia-N and alpha-amino-N was 1 and 0.5 mM, respectively.

2:45 #11 Effect of environment on fiber components and fiber digestibility of corn forage. M. S. Allen and K. A. O'Neil. Department of Animal Science, Michigan State University, East Lansing, MI 48824 (517- 336-1386)

Corn hybrids were grown in replicate plots (n=2) at 2 locations in each of 2 maturity zones (95-110 d, 16 hybrids; 110-120 d, 19 hybrids) in MI in 1988 (drought year) and in 1989. Hybrids were evaluated for yield and quality traits. *In vitro* NDF digestibility was measured by fermentation in buffered rumen fluid for 30 h.

	1988		1989	
	MEAN	SEM	MEAN	SEM
Growing degree days (5/1-9/1)	2387	37	2072	36
Precipitation (cm; 5/1-9/1)	21.3	1.9	41.3	1.9
Dry matter yield (Mg/ha)	9.0	0.2	21.3	0.2
NDF (% DM)	40.8	0.4	42.2	0.3
ADF (sequential, % DM)	19.4	0.2	21.8	0.2
LIGNIN (sequential, 72% H_2SO_4, %DM)	2.44	0.02	2.96	0.03
LIGNIN (sequential, 72% H_2SO_4, %NDF)	6.02	0.04	7.01	0.05
NDF digestibility (%)	50.3	0.3	42.0	0.3

Effect of year and year by hybrid interaction were significant (P<.01) for all traits. NDF digestibility was significantly (P<.01) correlated to LIGNIN (% NDF) across years (r=-.73) and within 1989 (r=-.60) but not within 1988 (P=.65, r=-.04). NDF and NDF digestibility were not highly correlated (r^2 <.09) within or across years. Environmental conditions can have a large effect on NDF digestibility and lignification of DM and NDF.

3:00 #12 FORAGE PROTEIN AND CARBOHYDRATE UTILIZATION BY RUMINAL MICROORGANISMS IN VITRO. D. J. R. Cherney, J. H. Cherney, and J. B. Russell, Department of Animal Science, Department of Soil, Crop, and Atmospheric Sciences, and Section of Microbiology, Cornell University, and ARS/USDA, Ithaca, NY 14853 (607-255-0604)

When alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), 20 mg/ml, at various ages (20, 30, and 40 d regrowth) were incubated in vitro with mixed ruminal microorganisms (300 mg bacterial protein/l), initial rate of volatile fatty acid production was proportional to rate of soluble carbohydrate utilization. Soluble protein concentration of freshly prepared material remained relatively constant over 11 h incubation, even though ammonia concentration increased by as much as 12 mM. Birdsfoot trefoil at all ages produced less ammonia than alfalfa. The greatest accumulation of lactate was seen with birdsfoot trefoil, the forage containing the most soluble carbohydrates at 20 d, but lactate accounted for less than less than 10% of total fermentation acids. By 30 and 40 d, the soluble carbohydrate content of birdsfoot trefoil was comparable to alfalfa. Ammonia/total fermentation acids ratios were lower with birdsfoot trefoil (.118, .133, .066 at 20, 30, and 40 d) than alfalfa (.198, .184, and .181 at 20, 30, and 40 d). Based on these results, it appears that more alfalfa protein will be lost as ruminal ammonia than birdsfoot trefoil protein. This difference is primarily due to the low level of soluble carbohydrates in alfalfa. Podium

3:15 BRIEF BREAK

PHYSIOPATHOLOGY PANEL

3:30 #13 EFFECT OF ELEVATED INTRARUMEN PRESSURE ON ERUCTATION IN CATTLE. R. C. Backus and H. W. Colvin, Jr. Dept. of Animal Physiology, University of California, Davis, CA 95616 ((916) 752-0203)

The effect of nitrogen gas insufflation on rumen motility and eructation was studied in seven calves (241 ±16 Kg) . Rumen motility was evaluated from recordings of intrarumen pressure (IRP) and myoelectric activities of the reticulum, ventral sac, and posterior ventral blind sac. Eructation was determined by using the transected tracheal technique. Most eructated gas (>90%) was passed into the trachea rather than the face mask. Elevation of IRP from resting level, to 20 cm H_2O increased eructation frequency from 0.37 ±0.02 to 1.11 ±0.10 min^{-1} and volume from 1.39 ±0.16 to 7.57 ±0.74 l. The increase in eructation frequency was due to an IRP-dependent increase in secondary rumen contraction frequency. Eructation during primary contractions rarely occurred. Elevation of IRP from 5 to 20 cm H_2O prolonged the time required for rumen deflation from 3.4 ±1.0 to 8.8 ±4.1 mins. These data indicate that eructation in cattle relative to that in sheep is influenced less by changes in IRP.

3:45 #14 METABOLISM IN SHEEP OF GALLIC ACID, TANNIC ACID AND HYDROLYSABLE TANNIN FROM *Terminalia oblongata*. T.B. Murdiati, C.S. McSweeney and J.B. Lowry, Division of Tropical Animal Production, CSIRO, Davies Laboratory, Townsville, Qld. 4814, Australia (07 3770820)

Hydrolysable tannin (HT) is present in a variety of tropical browse plants, some of which poison ruminants. In an attempt to clarify the toxic action, we investigated the major urinary metabolites resulting from dosing of sheep with the commercial HT, tannic acid; its simplest and major phenolic component, gallic acid; and the HT-containing and toxic *Terminalia oblongata* (yellow-wood). Phenolic metabolites were separated by HPLC and their structures investigated by proton and ^{13}C NMR. Gallic acid was less toxic (w/w) than tannic acid. Comparison of urinary metabolites from rumen and abomasal administration indicated that decarboxylation and reductive dehydroxylation of phenolics occurred principally in the rumen and a significant proportion was totally degraded. Rumen metabolism prevented toxicity at dose rates < 0.4g phenolics/kg liveweight. Resorcinol glucuronide and the glucuronide of 2-carboxy-2'4'4,6,-tetrahydroxy diphenyl 2,2' lactone glucuronide were the major urinary metabolites derived from tannic acid and yellow-wood HT while resorcinol glucuronide was the major product of gallic acid metabolism. Minor urinary metabolites included unconjugated pyrogallol, resorcinol and phloroglucinol. Toxicity appeared to correlate with the passage of the diphenyl lactone metabolite, presumably arising from the degradation of the hexahydroxydiphenic acid moiety in HT.

4:00 #15 SULFIDE TOXICOSIS AND POLIOENCEPHALOMALACIA (PEM) OF RUMINANTS. D.H. Gould, M.M. McAllister, D.W. Hamar, Department of Pathology, Colorado State University, Fort Collins, CO 80523 (303 491-6144)

PEM is a naturally occurring disease of ruminants that has been associated with high sulfur diets. It has been induced experimentally in calves with a semipurified diet containing added sulfate, readily fermentable carbohydrate, and low long fiber. This experimental, diet-induced form of PEM is associated with elevated concentrations of ruminal sulfide but no alterations of thiamine status. In order to study more directly the roles of sulfide and sulfate in PEM we investigated the effect of 1) oral administration of sodium sulfide to sheep and 2) deletion of added sulfate from the PEM-inducing diet fed to calves. Esophageal administration of sodium sulfide solution to sheep resulted in clinical neurologic signs in 10 of 10 sheep. Four of these developed lesions of PEM. When the unaltered PEM-inducing diet was fed to calves, ruminal sulfide concentrations increased and 4 of 7 calves developed PEM, whereas if the added sulfate was deleted, ruminal sulfide was decreased and none of 6 calves developed PEM. These results demonstrate that sulfide can directly induce PEM in ruminants and that decreasing dietary sulfate can prevent excessive ruminal sulfide formation and PEM. (Supported by USDA/CSRS, 87-CRSR-2-3208).

4:15 #16 ISOLATION OF A RUMINAL BACTERIUM CAPABLE OF GROWTH ON TANNIC ACID J.D. Brooker, D.K. Lum and S. Miller#. Department of Animal Sciences, Waite Agricultural Research Institute, Glen Osmond, S.A. 5064. #Queensland Department of Primary Industries, Charleville, Qld.

Tannins that occur in plants at concentrations exceeding 8-10% dry weight have significant anti-nutrient effects on domestic ruminants (sheep, cattle) that consume them. Condensed tannins form insoluble protein-tannin complexes that are not readily degraded by ruminal organisms. We have isolated ruminal organisms capable of degrading tannin-protein complexes from rumen microbial populations of goats browsing *Acacia* in the Northern Flinders Ranges of South Australia. Two isolates have been selected on tannic acid-based plates and they have been characterised as strains of *Streptococcus*, but differ from *S. bovis* in their capacity to clear tannin-protein plates and total DNA-DNA homology. With one isolate, conjugation from *Enterococcus faecalis*:Tn916 was demonstrated, but the transposon was not stable and was lost in the absence of selection. A stable population of the organism could be established in sheep when a tannin-rich *Acacia* diet was supplied. Crude rumen transfers from goats to sheep produced an increase in *Acacia* digestibility of 8-10%. Experiments are in progress to identify and isolate genes involved in tannin-degrading activity.

4:30 #17 EFFECTS OF ANTIBACTERIAL AGENTS ON IN VITRO, OVINE RUMINAL BIODEGRADATION OF JACOBINE, A HEPATOTOXIC PYRROLIZIDINE ALKALOID. A.M. Craig and D.E. Wachenheim.* Col. of Vet. Med., Oregon State University, Corvallis, OR 97331-4802. Phone (503)737-2872.

Many domestic animals in the Northwest develop hepatotoxicity and cirrhosis after consuming *Senecio jacobaea*, which contains hepatotoxic pyrrolizidine alkaloids (PAs). Ruminal microorganisms can prevent the toxicosis by biodegrading the PA; this protective phenomenon varies among animals. Antibacterial agents (AAs) could explain some of this variability. AAs can also be used to characterize PA degrading consortia. Jacobine is a predominant, relatively biodegradation resistant, PA of *S. jacobaea*. The objective was to evaluate in vitro inhibition of ovine ruminal jacobine biodegradation, using 22 AAs. Erythromycin, ionophores, penicillin G, rifampin, and tetracyclines severely inhibited jacobine biodegradation. Bacitracin, chloramphenicol, crystal violet, gramicidin, kanamycin, nalidixic acid, polymyxin B, streptomycin, and vancomycin were less inhibitory to jacobine biodegradation. Sodium azide, sulfisoxazole, and brilliant green had little or no effect on jacobine biodegradation.

4:45 #18 PREGASTRIC FERMENTATION IN Ophistocomus hoazin, A FOLIVOROUS BIRD. Dominguez-Bello, M. G.; Michelangeli, F., Ruiz, M.C.; Suarez, P. Laboratorio de Fisiologia Gastrointestinal, CBB. Instituto Venezolano de Investigaciones Cientificas. Apdo. 21827, Caracas 1020A, Venezuela. (58-2-5011396).

Known as hoatzin in english or locally as chenchena, this bird lives on young leaves and sprouts of plants. The crop (already developed at birth) with its contents represents about 12% of body weight (of 700g in adults). It is linned internally with stratified epithelium, and constitutes a chamber where pregastric fermentation takes place. Crop pH is about 7.5, and VFA concentrations of C2, C3 and C4 were (mM) 76.8, 14.4 and 8.8 respectively. Anaerobic bacteria have been isolated from agar roll tubes, mainly G- sporeformers, (Clostridia) and G+ coccobacilli (Peptrostreptococci, Ruminococci). Counts of viable bacteria in the crop were in the order of 10^9 in adults and 10^{12} in chicks. Protozoa (Isotricha) are also present in the crop in numbers of 10^4 in adults and 10^5 in chicks. The cellulolytic activity of crop contents is low, although bacteria digesting cell walls have been observed in electron microscopy of crop contents. As some plants from the diet of the chenchena are known to contain toxic compounds, we postulate detoxification as the main role of crop fermentation. Growth of crop bacteria was not affected by some phytotoxins such as canavanine and mimosine, that have been shown to depress growth or viability of rumen bacteria or protozoa. The study of gut ecology of this and other wild herbivores may help to understand the rumen function and open novel possibilities regarding microbial degradation of toxic compounds.

MICROBIOLOGY PANEL

November 14, 1991

8:00 Invited Speaker: A BRIEF REVIEW AND LESSONS OF 44 YEARS OF RUMEN BACTERIOLOGY. M. P. Bryant, University of Illinois, Champaign-Urbana, IL

8:30 #19 Protein and DNA "Fingerprinting" of Rumen Fungi. Roger E. Calza, Animal Sciences, Washington State University, Pullman, WA 99164, (509) 335-7051.

The presence of rumen fungi in animals fed high fiber diets and the close coupling of their life cycles to *in vivo* colonization of plant materials justifies their study. Our research objective is to develop nonclassical tools which can unambiguously identify fungal species. Microscopic or fermentative observations used to speciate fungi are subjective. Methods of species identification using protein and DNA "fingerprinting" are under investigation in our laboratory. *In situ* assays of electrophoretically separated cellulases have been used to distinguish morphologically identical fungi. Progress using restriction fragment length polymorphism mapping (RFLP) has been slow since adequate DNA probes are unavailable. Satellite DNA sequences from rumen fungi may prove useful as probes. Randomly amplified polymorphic DNA analysis (RAPD) using genomic DNA and PCR amplifications may prove useful as an identification tool. Success in the extraction of high molecular weight DNA from zoospores of small (i.e. 1ml) cultures should aid in these efforts. Objective methods which accurately and rapidly assign species identities will aid the study of rumen fungi. The 21st Biennial Conference on Rumen Function should promote colloborative research efforts.

8:45 #20 THE ENERGETICS OF LYSINE AND ARGININE FERMENTATION BY STRAIN SR, A MONENSIN-SENSITIVE, AMINO ACID-FERMENTING RUMINAL BACTERIUM. J. S. Van Kessel and J. B. Russell, Department of Animal Science and Section of Microbiology, Cornell University and ARS/USDA, Ithaca, NY 14853 (607-255-4508)

Strain SR, a recently isolated, monensin-sensitive, ammonia-producing ruminal bacterium, grew rapidly on arginine and lysine, but only if sodium was present. The arginine carrier had an absolute requirement for sodium, and either an electrical potential or chemical gradient of sodium could serve as the driving force for high affinity arginine transport. The Eadie-Hofstee plot was biphasic, and arginine was also be taken up by a diffusion mechanism. Arginine was converted to ornithine and citrulline, and it appeared ornithine and citrulline efflux created a sodium gradient which could drive arginine transport. Arginine transport was strongly inhibited by low pH and lysine. Since the rate of lysine fermentation was always proportional to the extracellular lysine concentration, lysine was only taken up by facilitated diffusion. The lysine carrier also required sodium. Based on substrate level phosphorylation, arginine and lysine fermentation produced 1 mol ATP/mol substrate. When SR was grown in continuous culture on arginine or lysine, the theoretical maximum growth yield was similar (13 g cells/mol ATP), but the apparent maintenance energy requirement for arginine was greater than lysine (9.4 versus 4.4 mmol ATP/g cells/h). The difference in maintenance could largely be explained by the cost of arginine transport. Since affinity of SR for lysine is very low, it is unlikely that monensin would have a significant impact on lysine degradation.

9:00 #21 ADHESION STUDIES USING *Ruminococcus albus* AND BACTERIAL CELLULOSE. A.N. Pell and P. Schofield, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876)

Bacterial cellulose (CELLULON, Weyerhauser Corp.) has recently become available commercially. We have studied the binding of ^{14}C-labelled *Ruminococcus albus* 8 (grown on ^{14}C-cellobiose) to this substrate in order to better define the fundamental parameters of the adhesion process. A new filtration assay has been developed which permits kinetic studies of the adhesion process. Adhesion was kinetically biphasic with a fast initial phase in which most of the binding occurred within the first minute of cell-fiber contact followed by a slower secondary phase in which a proportionally small increase in binding occurred. Maximum levels of 70-80% of the input bacteria were bound when excess sites were available. Binding was substantially reduced when the bacteria were heated to 100°C before binding or if methylcellulose was added to the assay mixture. Binding was decreased only slightly by 30% ethanol and was stimulated about 10% in 0.5 M NaCl. The binding process appears to be irreversible since unlabelled *R. albus*, added after labelled bacteria had bound to a limiting number of fiber sites, did not significantly displace the labelled cells.

9:15 #22 ISOLATION OF A FLOCCULANT, SHEATHED BACTERIUM FROM RUMEN FLUID. M. J. Fron & D. M. Schaefer. Dept. of Meat & Animal Sci., Univ. of Wisconsin, Madison, WI. 53706. (608-263-4314).

A starch-associating rumen bacterial strain was required to study the effect of aggregate growth on nitrogen utilization. Serial anaerobic roll tube enumerations repeatedly included a unique, filamentous colony morphology, resembling the *Lachnospira* genus, which was 0.05 to 1.80% of the total viable colony forming units. Negative staining, phase contrast microscopy, SEM and TEM suggested that chains of cells were surrounded by a hyalin sheath similar to that of *Sphaerotilus* and *Leptothrix*. Growth was supported by a minimal defined medium containing glucose, NH_4Cl, minerals and B vitamins. Fermentation products were identified as lactate and formate by HPLC analysis, however, closer scrutiny revealed co-elution of fumarate with formate. Products were analyzed after growth on defined medium containing cytochrome precursors to encourage the reduction of fumarate to succinate. Lactate was the primary fermentation product along with trace amounts of acetate, formate and fumarate. Characterization results did not conform to known rumen bacterial strains suggesting the recovery of a novel sheathed organism from the rumen.

9:30 #23 PROPIONATE PRODUCTION BY *Prevotella (Bacteroides) ruminicola* 23: VITAMIN B_{12}-DEPENDENT CHANGES IN METABOLIC PATHWAYS AND CELL YIELD. H. J. Strobel, Dept. of Animal Sciences, Univ. of Kentucky, Lexington, KY 40546 (606-257-7554)

Propionate is not normally a major fermentation product of ruminal *Bacteroides* species, but propionate is produced by certain strains if the growth medium is supplemented with rumen fluid. When *Prevotella (Bacteroides) ruminicola* 23 was grown in a defined medium containing a mixture of vitamins and micro-minerals, significant amounts of propionate were formed. Succinate and acetate were the only fermentation acids produced when vitamins were omitted, and further experiments demonstrated that propionate formation was dependent on the inclusion of vitamin B_{12}. When the organism was grown in continuous culture at dilution rates less than 0.20 per h, propionate and acetate were the major fermentation products and little succinate was produced. A shift in metabolism occurred at higher dilution rates and this change resulted in succinate accumulation. Since cell protein yields were reduced 15 to 25% in the absence of vitamin B_{12}, it appeared that the pathway for propionate formation may contain an energy conserving step. Previous work has indicated that propionate formation by *P. ruminicola* proceeds via a non-randomizing pathway not involving succinate. However, propionate production at the expense of succinate suggested that other metabolic pathways might be employed.

9:45 #24 *IN VITRO* RUMINAL EFFECTS OF SUCCINATE AND OTHER SUBSTRATES THAT UNDERGO MICROBIAL DECARBOXYLATION. M.L. Ogilvie, W.J. Smolenski, C.P. Cornell and J.A. Robinson. The Upjohn Company, Kalamazoo, MI 49001 (616 385-6562)

In a series of in vitro experiments, sodium succinate, salts of other tricarboxylic acid cycle intermediates and several amino acids were evaluated for their effect on ruminal fermentation characteristics. Ruminal contents were obtained via esophageal tube 2 h after feeding and incubated in batch culture for 24 hours at 39° C. In the initial experiment, sodium succinate hexahydrate was added to 25 ml of 10-fold diluted ruminal contents and 0.5 g of feed. In subsequent experiments, 75 ml of undiluted ruminal contents were incubated with the test compounds plus 1.5 g of feed. Amounts of propionate recovered from the incubation with diluted ruminal fluid indicated an equimolar conversion of added succinate to propionate (100.5% of theoretical). Succinate treatment reduced ($P < .01$) the decline in pH compared to the control incubations. Final 24 h pH values were 5.24, 5.46, 5.70, 6.00 and 6.27, respectively, for 0, 15, 30, 45, and 60 mM concentrations of sodium succinate. Total VFA, gas and methane production was increased ($P< .01$) at all concentrations of succinate compared to the control incubations. When added to undiluted ruminal fluid, 60 mM succinate reduced ($P < .01$) the rate of pH decline and increased ($P < .01$) the final 24 h pH values. In an additional experiment with undiluted ruminal fluid, addition of 60 mM alpha-ketoglutarate, aspartate, citrate, fumarate, malate, maleate, pyruvate or succinate reduced ($P < .01$) the rate of pH decline. Incubations containing 60 mM alpha-ketoglutarate, arginine, arginyl-glutamate, aspartate, citrate, fumarate, glutamate, malate, maleate, pyruvate, succinate or threonine had higher ($P < .01$) 24 h pH values. Although the precise mode of action of these carboxylates is unknown, we postulate that the observed effects on pH are due to proton consumption occurring during decarboxylation.

10:00 BRIEF BREAK

10:15 #25 ISOLATION AND PARTIAL CHARACTERIZATION OF A PLASMID FOUND IN A HYDROGEN-UTILIZING, ACETATE-PRODUCING RUMINAL ISOLATE. R. S. Pinder[1], L. R. Steenson[2] and J. A. Patterson[1], Department of Animal Sciences[1] and Food Science Department[2], Purdue University, West Lafayette, IN 47907 (317-494-4826)

Two of nine non-methanogenic hydrogen utilizing ruminal isolates screened for plasmids contained plasmid DNA. Five plasmids, ranging in size from 3.2 to 41 kilobase pairs (kb) were observed in isolate H3HH while a single 35 kb plasmid was observed in isolate H4. The 3.2 kb plasmid from H3HH was isolated using gel electrophoresis followed by electro-elution. Of twelve restriction endonucleases tested, EcoRV, SinI, and HindIII cut the plasmid once and BglII cut twice. The plasmid was cloned into pBR322, using the EcoRV recognition site, to obtain large amounts of plasmid DNA and as a preliminary step in constructing a shuttle vector. The recombinant plasmid was electroporated into *E. coli* LA6 and into H3HH. The recombinant plasmid conferred ampicillin resistance in both *E. coli* LA6 and H3HH. In conclusion, two isolates of acetogenic bacteria have been shown to contain plasmids and a plasmid in one of the isolates is currently being characterized.

10:30 #26 USE OF A TRANSPOSON FOR GENE TARGETTING IN RUMEN BACTERIA J.D. Brooker*, M. Bachleitner, J.A. Hackett, and D.K. Lum. Department of Animal Sciences, Waite Agricultural Research Institute, Glen Osmond, S.A. 5064. Australia.

Transformation of ruminal bacteria is a key step in the genetic manipulation of the rumen. However, physical techniques such as electroporation, protoplasting or other chemical methods have, with a few exceptions, been largely unsuccessful. An alternative approach is to establish gene transfer procedures based on mobilisable transposons. We have previously established conjugal transfer and chromosomal integration of transposon Tn916 carrying tetracycline resistance, from *Enterococcus faecalis* to gram-positive ruminal species, including *Streptococcus bovis* and *Butyrivibrio fibrisolvens*. Tn916 integrated into *S. bovis* was mobilisable to *B. fibrisolvens*, although the reverse was not possible. In both ruminal hosts, the transposon was stable. A recombination plasmid vector has been constructed using a fragment of Tn916 cloned into a broad host range gram positive-gram negative shuttle vector (pMU 1328). Transformation of *S. bovis* :Tn916 with this construct resulted in activation of a promoterless CAT gene by chromosomal integration of the plasmid into the Tn-homologous region of the transposon. These results suggest that self-mobilisable transposons may be appropriate vectors for gene movement between ruminal bacteria and targets for gene insertion by homologous recombination .

10:45 #27 *Bacteroides thetaiotaomicron* BTX: A GENETICALLY MODIFIED BACTERIUM CONTAINING A XYLANASE GENE FROM *Bacteroides ruminicola*. Michael A. Cotta* and Terence R. Whitehead. USDA/ARS, Natl. Ctr. Agric. Utilzn. Res., Peoria, IL 61604, 309-685-4011.

Degradation of forage in the rumen is inefficient. Efforts to improve the utilization of the cellulose and hemicellulose fractions of feeds have largely centered on physical and chemical treatments of feedstuffs. Another approach for altering rumen function is to genetically modify bacteria for more efficient degradation of xylans. The xylanase gene from the ruminal bacterium *Bacteroides ruminicola* 23 was introduced into the chromosome of the colonic organism *B. thetaiotaomicron* 5482, where the gene was highly expressed. The gene was found to be stable in continuous culture in the recombinant strain BTX. Furthermore, BTX was able to effectively compete in coculture with the parent strain and against *B. ruminicola* strain D31d. BTX was able to degrade xylan to oligosaccharides, but could not use these products for growth. Xylanolytic ruminal organisms could utilize the xylooligosaccharides produced by BTX (>80%), and strains of the nonxylanolytic bacterium *Selenomonas ruminantium* could also use these hydrolysis products (approx. 40%).

11:00 #28 TRANSFER OF ANTIBIOTIC RESISTANCE ELEMENTS BETWEEN HUMAN COLONIC *Bacteroides* AND THE RUMINAL ANAEROBE *Prevotella ruminicola*. N. B. Shoemaker, G. Wang and A. A. Salyers, Dept. of Microbiology, University of Illinois, Urbana, IL 61801 (217-333-7378)

Recently, we showed that a self-transmissible chromosomal element originally found in the human colonic *Bacteroides* (Tc^rEm^r 12256), could mobilize a specially constructed shuttle vector (pRDB5) from *Bacteroides uniformis* to the ruminal anaerobe *Prevotella* (formerly *Bacteroides*) *ruminicola* B_14. We have now shown that the Tc^rEm^r 12256 element can transfer itself to *P. ruminicola* B_14. Moreover, this element was re-transferred from *P. ruminicola* to *B. uniformis*. It was not clear if this transfer was mediated by the Tc^rEm^r 12256 element, however, because we found evidence for the existence of a cryptic transfer element in P. ruminicola B_14.

Flint et al. (Appl. Environ. Microbiol. 54: 855) described a plasmid, pRRI4, which was transferred between strains of *P. ruminicola*. We have shown that this plasmid is also transferred from *P. ruminicola* B_14 to *B. uniformis*. It did not retransfer itself back from *B. uniformis* to *P. ruminicola* B_14, but it could be mobilized from *B. uniformis* to *P. ruminicola* B_14 by the Tc^rEm^r 12256 element. Thus, naturally occurring plasmids and chromosomal elements can transfer between human and rumen normal microflora. Evidence that such events may have occurred in nature comes from the observation that the Tc^r gene on pRR14 is highly homologous to the Tc^r gene on the *Bacteroides* Tc^rEm^r 12256 element. The similarity of the two genes suggests that horizontal transfer has occurred.

11:15 #29 THE DEVELOPMENT OF A GENE TRANSFER SYSTEM FOR *Ruminococcus*. M. Morrison, T. May, R. I. Mackie, and B. A. White. Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 (217-333-2091)

The lack of a gene transfer system in *Ruminococcus* is indicative of a lack of knowledge concerning the genetics and nucleic acid biochemistry of the genus. DNA entry into *Ruminococcus* has been shown by using FITC-labeled dextrans that approximate the size of supercoiled plasmid DNA. These molecules can be introduced into *Ruminococcus* by electroporation. Restriction-modification systems are also important factors affecting DNA transfer. *R. flavefaciens* FD-1 possesses no less than 2 endonuclease activities. One activity, *Rfl* FI, is an isoschizomer of *Sal* I, and the second activity, *Rfl* FII, appears to be an isoschizomer of *Sca*I. *R. albus* 8 apparently possesses a single activity, *Ral* 8I, which is sensitive to *dam* methylation and recognizes the sequence 5'-GGATC-3'. These studies have provided the information necessary to develop methylation strategies for protection of plasmid DNA prior to electroporation of these strains. A cryptic plasmid has been identified in *R. flavefaciens* R13c2. This plasmid, pBAW301, is approximately 3.5 kb in length, contains two *Hinc*II and three *Taq*I sites, is not cut by the restriction endonucleases of *R. flavefaciens* FD-1, and can be protected against the restriction enzyme from *R. albus* 8 by *in vitro* methylation. Endogenous tetracycline and erythromycin antibiotic resistance markers from *Ruminococcus* are currently being investigated for their potential use as selectable markers. Moreover, constructs of pBAW301 with the streptococcal *tet* M gene are underway to eventually develop a system of gene manipulation for these ruminal bacteria.

11:30 #30 THE INTEGRATION SYSTEM OF PHAGE phiAR29 OF *Bacteroides ruminicola*. A.V. Klieve and K. Gregg. Institute of Biotechnology, Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, N.S.W. 2351, AUSTRALIA ((067) 73 2942)

One possible means of incorporating DNA permanently into the chromosome of rumen bacteria is to use the integration systems of temperate bacteriophages. A temperate phage (phiAR29) of *Bacteroides ruminicola* was isolated and shown to exist in the host bacterium as an integrated prophage. The section of DNA containing the phage integration system was subsequently isolated and mapped with restriction endonucleases (*Dra*I, *Hind*III, *Eco*RV, *Hinc*II and *Xba*I). A 3 kb *Eco*RV - *Hind*III fragment was cloned in *E. coli* and the DNA sequence was determined. From DNA-DNA hybridization the attachment site of the phage (*AttP*) was localized to the vicinity of an internal *Dra*I site. Sequence data located an inverted repeat downstream of this region which has similarities to the *sib* retroregulation region of the lambda phage integration system. Upstream of *AttP* are two open reading frames. The putative proteins encoded by these open reading frames have structural similarities with known excisionases and integrases respectively. The structure of the integration module of phiAR29 therefore appears to have all the required components for integration, but the positions of the *Xis* and *Int* genes are reversed in relation to *AttP* in comparison to other known phage integration systems.

Currently, phiAR29 integration determinants are being developed for use in integrative vector systems.

11:45 #31 CHARACTERIZATION OF A TEMPERATE BACTERIOPHAGE FOR A WILD STRAIN OF *Ruminococcus albus*. T. Tadese, M. E. Benson, S.R. Rust and M.T. Yokoyama. Dept. of Animal Science, Michigan State University, East Lansing, MI 48824, (517)353-2299.

A temperate bacteriophage for a wild strain of *Ruminococcus albus* designated Rum-phage, has been isolated and its morphological and physiological properties characterized. Its icosahedral head (50 nm) and non-contractile, flexible tail (120 nm) indicates that it belongs in Group B of the classification scheme of Bradley. The phage exhibits a wide range in host specificity. Sensitive ruminal anaerobes include the genus, Butyrivibrio, Selenomonas, Bacteroides and Ruminococcus. Storage for 6 months at -135 C results in 20% loss of the initial phage titer, and almost a 50% loss at 4 C for the same period of storage. The phage is inactivated by incubation at 70 C (1 h), but unaffected by 50 C (1 h). The phage is tolerant to a pH range of 6.8 to 10.0 (2 h), but 80% inactivated at pH 3.0 (2 h). The total DNA of the phage is 48.5 Kb as determined by pulsed field gel electrophoresis. A probe developed by random priming of the phage DNA and used in hybridization studies demonstrates that the phage is harbored in Ruminococcus albus

POSTERS
(November 13, 8:00 - 11:00 P.M.)

NUTRITION PANEL

#32 INFLUENCE OF OILSEEDS ON FERMENTATION AND MICROBIAL POPULATIONS IN RUMEN-SIMULATING FERMENTERS. R. J. Fisher, G. A. Harrison, W. W. Foster, and R. A. Sporleder. Dairy Science Dept., South Dakota State University, Brookings 57007.

Effects of physical form and level of oilseeds on fermentation and microbial populations of mixed ruminal microorganisms were examined using six rumen-simulating fermenters. Inoculum donor was fed a diet of 50% alfalfa hay and 50% concentrate (corn/soybean meal), DM basis. Fermenters were fed 10 g twice daily and dilution rate was .05 h^{-1} during the 7 d run with sampling 2 h postfeeding on d 4-7. Treatments were control (C), raw soybeans (SB), steam-flaked soybeans (EN), and whole cottonseeds (WCS). Runs 1-3 tested physical form (oilseeds ground through 1 mm screen versus masticated seeds collected via esophageal cannula) within oilseed type (C=2.5% EE; SB,EN,WCS=4.4% EE). Physical form of oilseeds had little effect on microbial numbers or fermentation. Runs 4-6 compared C diet against two levels of masticated oilseeds (low=3.7% EE; high=5.0% EE). Microbial numbers were not greatly affected by oilseeds. Compared to control, cultures fed SB diets had higher pH and decreased TVFA concentrations, while fermenters fed EN diets had lower pH and higher TVFA concentrations. Fermenters fed WCS diets had higher pH and increased acetate:propionate ratios compared to fermenters fed C diet. Inclusion of oilseeds in diets of 50% alfalfa hay and 50% concentrate altered fermentation in rumen-simulating fermenters but had negligible effects on microbial numbers.

#33 Effect of formaldehyde-treated barley or escape protein on the rumen environment and digestion in steers. T. A. McAllister, K.-J. Cheng, L. M. Rode and J. G. Buchanan-Smith[1], Agriculture Canada, Lethbridge, Alberta and [1]University of Guelph, Guelph, Ontario.

Six Holstein steers with ruminal and duodenal cannulae were used to evaluate three diets; formaldehyde-treated rolled barley (0.1% wt wt^{-1}) with canola meal(FTB); rolled barley with maize distillers' grain and fishmeal (escape protein, EP); and untreated rolled barley with canola meal(UB). FTB did not alter rumen pH but resulted in lower ruminal ammonia and plasma urea nitrogen concentrations 1 and 2 h after feeding. FTB and EP tended to lower ($P<0.1$) the ruminal concentration of volatile fatty acids (VFA) associated with microbial amino acid metabolism. Ruminal digestion of DM, OM, starch and NDF were not influenced by diet. While EP had no effect on total tract digestion, FTB increased ($P<0.1$) the digestibility of starch and decreased ($P<0.1$) the digestibility of NDF. EP increased ($P<0.05$) the amount of dietary protein and decreased the amount of microbial protein reaching the small intestine. With the exception of increased ($P<0.05$) glutamine with FTB, the amount of individual amino acids reaching the small intestine was not effected by diet. FTB tended to increase the amount of total nitrogen reaching the small intestine by increasing escape of feed protein from the rumen without inhibiting microbial protein synthesis. Damage to cereal grain structure by mastication may account for the failure of formaldehyde to alter the digestive properties of barley starch.

#34 PEARL MILLET GRAIN DIGESTIBILITY AND UTILIZATION IN CATTLE DIETS. G. M. Hill*, W. W. Hanna, M. N. Streeter and P. R. Utley, Dept. of Animal Science, Univ. of Georgia, and USDA-ARS, Dept. of Agronomy, Tifton 31793 (912-386-3364).

Pearl millet grain [13% crude protein (CP)] was blended with cracked corn in cattle diets in two experiments (EXP). Percentages of corn, sorghum, pearl millet, soybean meal, peanut hulls, minerals, and dietary CP in metabolism and feedlot trials, respectively, by treatment were: EXPl--C=79.5, 0, 0, 4.5, 15.0, 1.0, 11.2; CS=28.0, 54.5, 0, 1.5, 15.0, 1.0, 12.2; CM=28.0, 0, 56.0, 0, 15.0, 1.0, 11.8; EXP 2--CSB=81.5, 0, 0, 3.5, 13.5, 1.5, 9.9; CPM=42.5, 0, 42.5, 0, 13.5, 1.5, 9.7. EXP 1. Apparent digestibilities (6 steers, 251 kg; replicated 3 x 3 latin squares; 4.6 kg DMI) of a dry matter (DM), crude fiber, and CP were similar (P>.10) for the diets, but dietary TDN was higher (P<.05) for C vs CS and CM. Although higher N intake (P<.Ol) and fecal N (P<.06) were observed for CS and CM vs C, N retention was unaffected (P>.10) by diet. Feedlot steers (45 steers; 396 kg initial wt, 70-d ad libitum feeding) had similar ADG and DM/gain (P>.10) for the three treatments (ADG=1.87, 1.89, 1.76 kg, SE=.08; DM/gain=6.1, 6.0, 6.4, respectively, for C, CS and CM). On d 70, ruminal fluid pH was higher (P<.05) for CS and CM vs C (6.4 and 6.6 vs 6.1, SE= l); and propionate was similar (34.0, 34.0, 32.2 mol/100 mol, SE=l.9, P>.10, respectively for C, CS and CM). EXP 2. Metabolism trial (replicated 2 X 2 latin squares; 8 steers, 213 kg; 4.6 kg DMI) apparent digestibilities for DM and CP were similar (P>.10) for CSB and CPM. Nitrogen intake and N retention were unaffected (P>.10) by CSB or CPM diets. In a 92-d feedlot trial (30 heifers; 318 kg initial wt; ad libitum intake), ADG was similar for CSB and CPM (1.28 vs 1.33 kg, SE .07, P>.10), but DM/gain was higher for CPM vs CSB (7.69 vs 6.86, SE=.l, P<.05). Pearl millet grain can be effectively utilized as a protein-energy feedstuff in beef cattle diets.

#35 RUMINAL FLOW OF NITROGEN AND AMINO ACIDS IN SHEEP FED ZINC-TREATED SOYBEAN MEAL. K.J. Karr, K.A. Dawson, R.E. Tucker and G.E. Mitchell, Jr., Dept of Animal Science, University of Kentucky, Lexington KY 40546 (606-257-2891)

Ten abomasally cannulated crossbred wethers were randomly allotted to two dietary treatments in a crossover design experiment to evaluate the effect of zinc-treated soybean meal (Zn-SBM) on ruminal fermentation, diet digestibility and N and amino acid (AA) flow to the small intestine. Diets were fed in two equal portions at 0800 and 2000 h to provide 1.5 x maintenance energy (1.28 kg DM/hd/d) and more than 50% of the dietary CP furnished by control (C-SBM) or Zn-SBM. Abomasal digesta and fecal grab samples were collected at 12-h intervals with 2-h advancements daily during a 6- d collection period. Animals consuming Zn-SBM had higher (P<.10) ruminal pH, total VFA concentrations and acetate:propionate ratios but lower (P<.10) concentrations of NH_3 N, isovalerate and anaerobic bacteria. Source of SBM did not affect apparent digestibility of dietary OM, NDF or ADF in either the rumen or the total digestive tract. Ruminal CP digestibility was lower (P<.01) in animals fed Zn-SBM (51.8 vs. 34.7%); however, total tract digestibilities were not different. Microbial N and AA flows from the rumen tended to be reduced (P<.10) while feed and subsequently total N and AA flows were increased (P<.05) in animals consuming Zn-SBM. It was concluded that zinc treatment of SBM can enhance the flow of dietary AA to the small intestine without detrimentally influencing diet digestibility.

#36 EFFECT OF RUMINAL OR ILEAL UREA INFUSIONS ON FEED INTAKE AND DIGESTIBILITY OF PRAIRIE HAY BY BEEF CATTLE. J.D. GARZA-F AND F.N. OWENS. Department of Animal Science, Oklahoma State University, Stillwater 74078.

In previous studies post-ruminal infusions of urea or casein has enhanced intake of a low quality hay diet (4.5% CP). The response may be due in part to nitrogen recycling to the rumen; however relative responses to ruminal vs postruminal infusions of nitrogen have not been tested. This study compared ruminal to ileal nitrogen infusion on forage and water intake, digestibility and site of nitrogen utilization. In a crossover experiment (42 d), 9 steers (451 kg BW) were fed ad libitum a prairie hay diet (4.3 % CP). Animals were infused (100 ml) either in the rumen or in the ileum three times (0830, 1430, and 2030) daily with a urea (50 g/d) solution. Dry matter intake was greater (7.0 vs 6.8 kg; P<.05) when urea was infused into the rumen; daily water intake was greater (33 vs 31 liters; P<.03) in steers ileally infused with urea. Fecal output tended (P<.09) to be greater (2.5 vs 2.4 kg/d) with the ileal urea infusion, but duodenal DM flow was equal (4.5 vs 4.6 kg DM/d). Ruminal (29 vs 31%) and intestinal (32 vs 33%) DM digestibilities were similar for both sites of urea infusion, although total tract DM digestibility was greater (P<.01) with ruminal urea infusion (64 vs 62 %). The fact that hay intakes were nearly similar for the two sites of infusion indicates that ilially infused urea was useful to improve N status, either through N recycling to the rumen or meeting tissue needs for non-specific nitrogen.

#37 POSTRUMINAL CASEIN EFFECTS ON VOLUNTARY ALFALFA HAY INTAKE BY STEERS. J.D. GARZA-F AND F.N. OWENS. Department of Animal Science, Oklahoma State University, Stillwater 74078.

The effect of supplemental duodenal protein on voluntary intake of alfalfa hay and water, ruminal volume and site of nitrogen digestion was studied in a crossover experiment (45 d) with 8 crossbred steers (443 kg BW). Animals had ad libitum access to alfalfa hay (17.5% CP) and received either no infusion or four daily (0830, 1240, 1630 and 2030) doses (750 ml/dose) of casein (300g + 3g of methionine). Digesta and fecal samples were taken 3 times/d on two consecutive days; ruminal contents were evacuated on d 15 of each experimental period. Infused duodenal casein did not influence intake of hay or water. Steers dosed with casein tended to have larger ruminal liquid volume (47.5 vs 43.51) and to have more free liquid (17.8 vs 17.51) than the control steers. Casein infusions depressed (16%) daily duodenal DM flow and fecal output (11%), but enhanced ruminal DM digestibility (50.3 vs 42.1%; P<.05). Regardless of treatment, ruminal NH_3-N concentration peaked at 4 h postfeeding (P<.01) with higher NH_3-N values for the casein infusion. Total tract protein digestibility tended to be increased (67.7 vs 64.4%) by infused casein. Additional duodenal protein appeared to reduce flow to the small intestine and increase extent of ruminal and total tract digestion of the alfalfa hay. Protein levels above 17.5% may increase extent of ruminal and total tract digestion of alfalfa.

#38 RELATIONSHIP BETWEEN SODIUM BICARBONATE AND MONENSIN ON UTILIZATION OF A HIGH-ENERGY FINISHING DIET BY FEEDLOT STEERS
R.A. Zinn and J.L. Borquez, University of California IVAC, El Centro, CA 92243 (619 352 0111)

Two feedlot growth-performance trials and a metabolism trial were conducted to evaluate the relationship between monensin (MON) and sodium bicarbonate (SB) supplementation on utilization of a high energy finishing diet by feedlot steers. Two levels of MON supplementation (0 and 33 mg/kg diet) and two levels of SB supplementation (0 and . 75% DM) were compared in a 2x2 factorial arrangement of treatments. The basal diet contained 75% steam-flaked corn, 4% yellow grease and 12% forage (DM basis). There were no interactions (P>.10) between MON and SB. Neither MON nor SB influenced (P>.10) feedlot growth-performance. There was an interaction between cattle breed and SB on carcass yield. With brahman crossbred steers ribeye area was smaller and carcass yield was lower with SB (P<. 05), while with Holstein steers ribeye area and carcass yield was greater (P<.01). SB also reduced fat thickness in Holstein steers (P<.01), but not in crossbred steers (P>. 10) . MON decreased (P<. 05) marbling score and tended (P<.10) to increase KPH in crossbred steers. MON increased fat thickness and decreased carcass yield in Holstein steers (P<. 05) . There were no treatment effects on ruminal Ph (P>.10). However, MON tended to increase propionate (P<.10) and decrease butyrate (P<.05) and methane (P<. 10) . SB did not influence site or extent of digestion. However, MON tended to decrease (P<. 10) ruminal digestion and increased (P<. 05) postruminal digestion of OM. There were no treatment effects on total tract digestion of OM, ADF, N or DE.

#39 Variation in feeding behavior characteristics measured continuously among dairy cows in early lactation. R.G. Dado and M.S. Allen, Department of Animal Science, Michigan State University, East Lansing, MI 48824 (517-336-1386)

Measurements of within day feeding activity are valuable for understanding relationships among diet, rumen function, and animal performance. A computerized data acquisition system was used to continuously monitor feed disappearance, water intake, and chewing activity of 12 Holstein cows (63 d postpartum; 34.4 kg milk/d) for 21 days (11 d adaption, 10 d collection) to measure within and among cow variation and to estimate animal numbers required for feeding behavior studies.

			Variance component	
Variable	Mean	SD	Cow	Day
Meal size (kg DM)	2.2	0.7	0.26**	0.03*
Eating bouts (/d)	11.0	2.4	2.88**	0.33
Eating time (min/d)	301	52	2200**	59.6
Ruminating bouts (/d)	14	2.5	3.92**	0.02
Ruminating time (min/d)	457	74	542	840*
Chewing rate(min/kg NDF)	110.1	18.7	215**	16.2
Drinking bouts (/d)	14.0	5.6	27.2**	0.89*
Drinking rate (l/mill)	4.3	1.0	0.79**	0.01

*(P<0.05); **(P<0.01)

Blocking cows by parity was not effective in lowering cow variation for most variables. Contrast differences of 10% of mean values for all variables tested have an 80% probability of being detected in future experiments (P<0.05) with Latin square designs utilizing twelve animals.

#40 Effect of rumen inocula preparation on proteolytic activity *in vitro*. R.A. Kohn and M.S. Allen, Department of Animal Science, Michigan State University, East Lansing, MI 48824 (517-336-1386)

Six inocula preparation treatments were compared on different days (n=4) for initial enzymatic activity on azocasein (AZO), and for protein degradation of soybean meal (SBM) by rumen bacteria. Sulfur-35 was used as a microbial marker. Strained rumen contents were stored for 2h at 4, 24 or 38 °C before inoculation (treatments 1-3). The filtrand was divided and rinsed with one of three solutions: cold (4° C) aerobic, cold anaerobic reduced, or warm (38° C) reduced media. Rinses were added to rumen inocula stored at 38°C (treatments 4-6). Incubation times were 1 or 2 h for AZO and 3, 9, 16 or 48 h for SBM. Adding rinses increased AZO degradation at 1 and 2 h ($P< .01$), but decreased SBM degradation at 9 and 16 h ($P<.01$). Storage temperature decreased (4°C) and increased (24°C) AZO degradation compared to 38°C ($P< .05$) but did not affect SBM degradation. AZO degradation (2 h) was negatively correlated ($r^2>(0.10)$ with SBM degradation (all times). Rinsing rumen digesta increased proteolytic activity of rumen inocula on AZO, but is not recommended for systems using growing organisms. Azocasein degradation should not be used to standardize rumen inocula.

#41 EFFECT OF SLAFRAMINE ADMINISTRATION ON SITE AND EXTENT OF STARCH DIGESTION IN BEEF STEERS. M.N. Streeter and M.A. Froetschel. Dept. of Animal and Dairy Science. The University of Georgia, Athens 30602. (404-542-0944) and W.J. Croom, Jr. and W.M. Hagler, Jr. Dept. of Animal Science, North Carolina State University, Raleigh 27650.

Four steers (228 kg) equipped with double L type duodenal and ileal cannulas were fed a 77% corn diet in 12 equal portions with a total dry matter intake of 2.25% of body weight (BW). Slaframine (SLAF) was infused into the duodenum every 12 hours at levels of 0, 15, 30 and 45 ug.kg BW^{-1} $dose^{-1}$ for the 12d experimental period. A 4x4 Latin square design was employed with responses to SLAF determined by linear, quadratic and cubic contrasts. Starch excreted in the feces decreased 50.5% and total tract starch digestibility increased 5.6% as SLAF dose increased (linear; $P<.10$). Starch flow to the duodenum (g/d) increased 26.4% for 30 vs 0 SLAF (quadratic; $P<.16$). Elevated starch flow to the small intestine was partially compensated for by a 46.3% increase in starch disappearance (g/d) and an 18.4 % increase in fractional starch digestibility within the small intestine for 30 compared to 0 SLAF. Small intestinal responses to increasing SLAF tended to be cubic ($P<.20$). Starch flow to the cecum and starch disappearance from the large intestine were increased by SLAF administration (quadratic; $P<.10$). Slaframine increased total tract digestibility but also increased the amount of starch fermented in the large intestine. A SLAF dose of 30 tended to increase starch digestion in the small intestine; however, the small response observed could be explained by increased starch flow to the duodenum.

#42 THE EFFECT OF UREA-TREATMENT OF RICE STRAW ON THE PHYSIOLOGY OF DIGESTION IN SWAMP BUFFALO. F. J. Hart and M. Wanapat*. Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

This experiment was designed to provide a comparison of the effect of urea treatment (5%, w/w) of rice straw with untreated rice straw. A 4X4 latin square design using four rumen fistulated swamp buffalo (~140 kg. liveweight) was employed. Prior to the experiment they were vaccinated and drenched against contageous diseases, liver, fluke, intestinal parasites, and injected with vitamin A, D, and E. Nitrogen, mineral and trace elements were supplied at adequate levels to both diets in order to overcome deficiencies which may have otherwise confounded a direct comparison. There was a 46% increase in the intake of digestible organic matter (OM) with the urea treated diet. This was contributed by a 17% increase in the digestibility of OM and a 25% increase in digestibility of hemicellulose increased by the greatest amount (26%). There was an increased rate of passage of particulate matter out of the rumen for the treated straw, which along with the increased rate of OM fermentation resulted in a 9% decrease in the amount of digesta dry matter (DM) contained in the rumen. The volatile fatty acids (VFA) pool in the rumen was 24% higher for the treated diet as compared to the untreated straw.

#43 THE EFFECT OF YEAST (*Saccharomycese cerevisiae*) ON RUMEN FERMENTATION IN CROSSBRED DAIRY HEIFERS FED ON UNTREATED AND UREATREATED RICE STRAW IN THE TROPIC. M. Wanapat*, K. Sommart, W. Wongsrikeao, C. Wachirapakorn, S. Chanthai, and C. Watanachant. Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

The experiment was designed according to a 2X2 factorial arrangement in a CRD using four rumen fistulated crossbred dairy heifers. Factor A was type of rice straw (untreated VS urea-treated, 5% w/w/) and factor B was unsupplemented and supplemented with yeast (at 10 g/hd/d) while feeding trial was carried out for 120 days during the hot summer months (35-40°C) in Thailand. Rumen fermentation parameters as well as intake were measured. In addition OM degradabilities of feeds using in sacco technique were evaluated. It was found that yeast supplementation significantly enhanced the voluntary intake of rice straw from 2.13 to 2.53% BW ($P<.002$) (19% increase) and total VFA production from 96 to 104 mM ($P<.01$) on the untreated rice straw group. Meanwhile, the values of pH (6.16, 5.75) and ruminal NH_3-N (6.1, 4.5 mg%) were slightly reduced. The intake of urea-treated straw was slightly increased by yeast supplementation (2.30 to 2.35% BW). The in sacco OMD of the protein and energy sources were found to be lowered in the yeast supplemented group which may have enhanced the rumen escape nutrient potential. According to the exponential equation, c-rates of degradation/h of protein and energy sources, were found to be .279 and .156 for unsupplemented and supplemented with yeast groups while the values for untreated rice straw and urea-treated rice straw were .273 and .150, respectively.

#44 Effect of NaOH treatment of rice straw on the rumen environment in Korean Native Goats. H. D. Bae, K.-J. Cheng, J. Y. Kim[1], K. H. Chung[1] and H. T. Shin[1], Agriculture Canada, Lethbridge, Alberta and [1] Sung Kyun Kwan University, Suwon Republic of Korea

Rice straw is highly resistant to microbial fermentation and is considered to be a poor quality forage. However, due to its availability rice straw is utilized extensively in ruminant diets in Korea. Research with dairy cows has shown that NaOH increases feed intake and milk fat. Six goats (24.5 kg, 3 per treatment) with ruminal fistula were fed concentrate at maintenance twice daily at 0600 h and 1800 h. Untreated rice straw (US) and rice straw treated (TS) with 4 % NaOH were provided ad libitum. US and TS straw were ground, placed in nylon bags and incubated in the rumen for 72 h. Rumen fluid samples were collected at 0, 2, 4, 6, 8 and 10 h post-a.m. feeding, pH was measured and samples were analyzed for NH_3, VFA's and microbial protein. The results were as follows:

Item		US	TS
Ruminal pH		6.7	6.9
Ruminal NH3 mg dL^{-1} RF		7.0	3.4*
VFA mM L^{-1}	Acetate	42.5	57.7*
	Propionate	11.7	21.1*
	Butyrate	1.4	6.4*
	Total	55.6	85.4*
Microbial protein mg dL^{-1} RF		77.6	82.5*
DMD (72 h)		38.6	48.9*

* significantly different from untreated straw at $P<0.05$.
These data indicate that NaOH treatment of rice straw increases the susceptibility of rice straw to microbial digestion.

#45 The Capacity of Ruminal Fermentation to Detoxify Endophyte-Infected Tall Fescue. M.A. Rasmussen, National Animal Disease Center, USDA-ARS, Ames, IA 50010. (515) 239-8200.

The ability of ruminal microbes to degrade toxins contained in endophyte-infected tall fescue seed (Kentucky-31) was determined. Using a rat bioassay procedure, preincubation of fescue seed with rumen contents collected from fescue-fed (20 day adaptation period) and fescue-naive sheep did not eliminate those seed-borne factors which reduce feed intake and weight gain in rats. Weight gain in weanling rats (90 g, initial weight), on diets containing infected-fescue seed (40% of the diet) was reduced 50% compared to diets containing noninfected seed (Mozark). With diets containing fescue seed previously incubated with rumen contents, weight gains were 71% and 67% of controls (noninfected fescue seed similarly treated) for the fescue-fed and fescue-naive ruminal fermentations, respectively. Parallel experiments evaluating the in vitro degradation (24 hour incubation period) of ergonovine and lysergic acid by ruminal microbes were also conducted. No microbial degradation (determined by HPLC analysis) of these ergot alkaloids was observed when ruminal inocula from fescue-fed and fescue-naive ruminants were used. It is concluded that microbial activity in the rumen shows little capacity to detoxify tall fescue when previous dietary exposure is limited. Ruminal capacity after longer term exposure to fescue is unknown.

#46 GRAIN SORGHUM DIGESTION BY RUMINAL MICROFLORA. K. K. Thurn and S. F. Kotarski, Performance Enhancement Research, The Upjohn Company, Kalamazoo, MI. 49001 (616)-385-6578; and R. D. Waniska, Texas A & M University, College Station, TX. 77843 (409)-845-2985.

An in vitro ruminal starch disappearance assay was used to compare the initial starch digestion rates of sorghum cultivars differing in endosperm type and texture. Whether grits (large particles from the corneous endosperm) or whole grain flours were substrates, digestion rates were fastest for the floury and slowest for the intermediate- and vitreous-textured cultivars ($P<.05$). However, the digestion rates of starch granules purified from these cultivars did not differ. When digestion rates of whole grain flours from 22 sorghum cultivars were compared, differences were not detected between intermediate- and vitreous-textured, yellow and white or homozygous waxy and
homozygous nonwaxy endosperms. In contrast, floury- and intermediate-textured endosperms differed in rates both across and within genetic backgrounds ($P<.05$) suggesting that the endosperm matrix may have an impact on in vitro ruminal starch digestion. Grits from a vitreous cultivar treated with cellulase or pronase had digestion rates higher then untreated controls. These data support that protein and structural carbohydrates may limit starch digestion in sorghum grain by ruminal microflora. However, there were no strong correlations between protein content, pepsin digestibility and starch disappearance rates of the 22 sorghum cultivars.

MICROBIOLOGY PANEL

#47 GROWTH OF *Fibrobacter succinogenes* S85 IN CELLULOSE-LIMITED CONTINUOUS CULTURE. P.J. Weimer, USDA-ARS Dairy Forage Research Center, Madison, WI 53706 (608-264-5408)

Fibrobacter succinogenes S85 was grown in continuous culture using a segmented slurry delivery system to dispense a medium containing cellulose as growth-limiting nutrient. Fermentation data were obtained at steady-state for six dilution rates within the range 0.016-0.076/h; steady-state growth was not achieved at dilution rates outside this range. At D=0.016/h, succinate was the major fermentation product, and smaller amounts of acetate, formate, and hydrogen were produced. Increasing dilution rate resulted in a progressive shift away from succinate production and toward acetate production. Cell yields increased with increasing dilution rate, and reached a maximum of 0.22 g cells/g cellulose consumed. A Pirt plot of the cell yield data was nonlinear, which precluded direct determination of maintenance energy requirements, and which suggests that the biochemical pathways that produce acetate and succinate in this organism proceed with different ATP yields.

#48 EFFECT OF pH ON GROWTH YIELD, FERMENTATION PRODUCTS, AND CELLULOSE CONSUMPTION OF *Ruminococcus flavefaciens* FD-1 AT TWO FIXED DILUTION RATES IN CONTINUOUS CULTURE. Y.Shi and P.J. Weimer, Department of Bacteriology, University of Wisconsin-Madison, and USDA-ARS Dairy Forage Research Center, Madison, WI 53706 (608-264-5408)

The ruminal cellulolytic bacterium *Ruminococcus flavefaciens* FD-1 was cultured in cellulose-limited, pH-controlled continuous culture at dilution rates (D) of 0.02 and 0.06 /h. The organism grew on cellulose within the pH range about 5.90 - 7.05, which is somewhat wider than that previously reported. At both dilution rates, growth yield and cellulose conversion were optimal at pH 6.6. There was a significant change ($p < 0.001$) in the relative proportion of acetate and succinate (the two major fermentation products) at both dilution rates when the culture pH was shifted from the optimum, suggesting a shift of pathways involved in the metabolism of three-carbon intermediates. Interestingly, the relative proportion of acetate at D=0.02 /h increased when the pH was shifted away from pH 6.6, while at D=0.06 /h the relative proportion of acetate decreased following a similar shift. An analysis of variance show that there is a significant interaction between the pH and dilution rate ($p < 0.0002$). The results suggest that both pH and dilution rate are important environment factors which regulate cellulose consumption and endproduct formation in this organism.

#49 SIGNAL PHOSPHOLIPID COMPONENTS AS MARKERS FOR THE STUDY OF INTERACTIONS BETWEEN *Ruminococcus flavefaciens* AND *Fibrobacter succinogenes*. [1]Liliana Saluzzi, [2]Alastair Smith & [1]Colin S. Stewart, [1]Rowett Research Institute, Aberdeen, UK, (224 712751) and [2]Macaulay Land Use Research Institute, Aberdeen, U K.

Phospholipids were found to form 5.4 + 0.4% by weight of freeze dried cells of *Fibrobacter succinogenes* strains S85 and BL2 grown on cellobiose. Cells of *Ruminococcus flavefaciens* strains FD1 and 17 contained 2.0 ± 0.4% phospholipid. Derivatization of the phospholipids and analysis of fatty acid methyl esters (FAME) and dimethyl acetals (DMA) showed that certain components could be used as markers for the two species growing in co-culture. Markers for *F. succinogenes* were the DMA C13:0i, C13:0, C14:0i and C14:0. For *R. flavefaciens*, the markers were the DMA C15:0i, C16:0, C17:0i and C18:0, and the FAME C15:0i. When these two strains of *R. flavefaciens* and of *F. succinogenes* were incubated with barley straw or clover, analysis of the microbial phospholipids present during 7 day incubations suggested that *R. flavefaciens* rapidly outgrew *F. succinogenes*. The preponderance of *R. flavefaciens* was greatest when clover was the substrate. Degradation of the clover in cultures containing both species was less than that by *R. flavefaciens* alone, suggesting that a competitive or antagonistic relationship existed between these two species growing on this substrate.

#50 INTRACELLULAR pH OF ACID-TOLERANT RUMINAL BACTERIA. J. B. Russell. ARS/USDA and Section of Microbiology, Cornell University, Ithaca, NY 14853 (607-255-4508).

Short chain volatile fatty acids are often an end-product of anaerobic fermentations, but many bacteria (e.g. *Escherichia coli*) are unable to tolerate these acids if the pH is acidic. With the advent of the chemiosmotic theory, the idea that volatile fatty acids could act as uncouplers became fashionable. The "uncoupling" theory, however, did not explain why some bacteria tolerated volatile fatty acids even if the pH was low. Acid-tolerant ruminal bacteria (*Bacteroides ruminicola* B14, *Selenomonas ruminantium* HD4, *Streptococcus bovis* JB1, *Megasphaera elsdenii* B159 and strain F) allowed their intracellular pH to decline as a function of extracellular pH and did not generate a large pH gradient across the cell membrane until the extracellular pH was low (< 5.2). This decline in intracellular pH prevented an accumulation of volatile fatty acid anions inside the cells. Poster.

#51 Development of vectors and transformation systems for *Butyvibrio fibrisolvens*. R.G. Clark*[1], K.-J. Cheng[1], and M.F. Hynes[2]. [1]Agriculture Canada Research Station, Lethbridge, Alberta, Canada T1J 4B1, (403)327-4561 and [2]University of Calgary, Calgary, Alberta, T2N 1N4.

Seven potential shuttle vectors have been developed for *Butyvibrio fibrisolvens*. Three vectors were constructed using the cryptic 3.0kb *B. fibrisolvens* plasmid (pBF194) and other well characterized vectors. pLRS03 (7.4kb) contains pBF194 cloned into the ClaI site of pBR322 (Ap^r, Tc^r). To construct pLRS05 (8.4kb), the 3.5kb EcoRI-SalI fragment from pLRS03, containing pBF194, was cloned into the *Bacillus subtilis* vector pHP13 (Em^r, Cm^r). pLRS06 (13.3kb) consists of pBF194 cloned into the XbaI site of the *Streptococcus-E.coli* shuttle vector, pSA3 (Cm^r, Em^r, Tc^r). Four vectors were constructed utilizing a promoter probe vector, promoter fragments from *B. fibrisolvens*, and the vector pLRS03. HindIII fragments of the *B. fibrisolvens* E14 xylanase gene were cloned into the HindIII site of the promoter probe vector, pKK232-8. Three different fragments were cloned that resulted in the expression of chloramphenicol resistance. These three constructs were cloned into the SalI site of pLRS03, to yield pRC106, pRC109, pRC213, and pRC339.
Using $CaCl_2$ transformation or electroporation, all seven vectors can be introduced into *E. coli*. All attempts at transforming *B. fibrisolvens* using electroporation have so far been unsuccessful. Numerous electroporation conditions are therefore being examined.

#52 D-LACTATE UTILIZATION BY *Selenomonas ruminantium* HD4. D. J. Nisbet, Southern Plains Area Food Animal Protection Research Laboratory, ARS-USDA, College Station, TX 77845 and S. A. Martin, Dept. of Animal and Dairy Sci. and Dept. of Microbiology, The University of Georgia, Athens, GA 30602 (404-542-1065)

Growth of *Selenomonas ruminantium* HD4 in medium that contained 2 g/l D-lactate was stimulated to varying degrees by malate, fumarate, and an *Aspergillus oryzae* fermentation extract (Amaferm). Amaferm treatment caused the greatest stimulation. Initial uptake rates (30 sec) and long term uptake rates (30 min) of D-lactate by whole cells of *S. ruminantium* were increased in the presence of malate. Amaferm also stimulated long-term uptake rates of D-lactate whereas fumarate had no effect. Initial uptake of D-lactate was depressed in the presence of fumarate or Amaferm. When malate, fumarate or Amaferm were included in the D-lactate growth medium, a homo-succinate fermentation resulted and an inverse relationship was observed between growth (protein synthesis) and succinate production. Recently, it was shown that Amaferm contains malate and this dicarboxylic acid may be involved in stimulating D-lactate utilization by *S. ruminantium*. The ability of *S. ruminantium* to grow on malate or fumarate in the presence of extracellular hydrogen was also evaluated. Growth occurred on both malate (OD_{600} = .330 after 9 h) and fumarate (OD_{600} = .642 after 9 h) with the corresponding production of succinate (11.4 and 12.5 mM for malate and fumarate, respectively). These results suggest that malate may be providing an electron sink for hydrogen that allows increased lactate utilization by *S. ruminantium* HD4.

#53 METABOLISM OF NITROALKANES BY RUMINAL MICROORGANISMS. R. C. Anderson, P. A. Hartman, M. A. Rasmussen, and M. J. Allison, Iowa State University and National Animal Disease Center, USDA-ARS, Ames, IA, 50010. (515-239-8200)

Rates of degradation of the naturally occurring plant toxins nitropropionic acid (NPA) and nitropropanol (NPOH) by ruminal microbes vary with diet and time after feeding, but degradation mechanisms, products, or principal microbes involved have not been defined. Tests for products were made with bovine ruminal microbes at 3X concentration in anaerobic mineral dilution solution plus 12.6 mM NPA or NPOH. Evidence was found that nitro-groups were not split from the carbon compounds. The small increase in propionate when >9 umoles/ml NPA had been degraded accounted for <10% of the NPA degraded and no propanol was found in incubations with NPOH. Analysis for amino compounds (Dabsyl-Cl derivatives by HPLC) revealed that beta-alanine accounted for >36% of the NPA degraded and that 3-amino-1-propanol accounted for >86% of the NPOH metabolized. This recovery was explained by evidence that beta-alanine was further metabolized while 3-amino-1-propanol was not. We conclude that metabolism of NPA and NPOH involves in situ reduction of the nitro-groups without cleavage from the parent molecules.

#54 EFFECTS OF Aspergillus oryzae EXTRACT (AMAFERM) ON RUMINAL FIBROLYTIC BACTERIA AND IN VITRO FIBER DEGRADATION. A.A. Beharka* and T.G. Nagaraja, Dept. of Animal Sci., Kansas State University, Manhattan, Kansas 66506 (613-532-5654)

The effect of Amaferm on growth of pure cultures of ruminal cellulolytic, hemicellulolytic and pectinolytic bacteria (*Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Eubacterium cellulosolvens*, *Ruminococcus flavefaciens*, *R. albus*, *Prevotella* (*Bacteroides*) *ruminicola*, and *Lachnospira multiparus*) was determined. Bacteria were grown in anaerobic, complete carbohydrate rumen fluid medium with filter-sterilized Amaferm at 0, 2 or 5% of the medium. The medium was inoculated with late-log-phase culture and growth was monitored by measuring absorbance. The addition of Amaferm to the medium increased ($P<.1$) the specific growth rate of *Ruminococcus albus* (.71 vs .61) and *Fibrobacter succinogenes* (.35 vs .26). Amaferm had no effect on growth of other fibrolytic bacteria. Selective antimicrobial compounds (penicillin, streptomycin, and cycloheximide) were used to assess the influence or Amaferm on bacterial and fungal contributions to in vitro fiber degradation. A variety of ground, fibrous substrates (0.5g) were incubated with ruminal fluid inoculum (1:2 ruminal fluid to buffer). Amaferm was added at 0, .4, .8 or 1.2 g/l. NDF and ADF digestibilities were determined after 96 h incubation. Addition of Amaferm increased ($P<.1$) NDF and ADF digestion of brome, and alfalfa hay. Amaferm addition at .4 or .8 g/l, and not 1.2 g/l, increased NDF and ADF digestion of high endophyte fescue. The enhanced fiber degradation by Amaferm was attributed to its stimulation of bacterial activity. Amaferm did not appear to stimulate fungal activity. Addition of Amaferm had no effect on NDF or ADF digestion of pure cellulose, low endophyte fescue, wheat straw, corn silage and prairie hay. In conclusion, Amaferm appears to stimulate NDF and ADF digestibility of certain feedstuffs and this increase in digestibility maybe a consequence of growth stimulation of some fibrolytic bacteria.

#55 THE DETECTION OF FERMENTATION ACIDS IN RUMINAL FLUID BY ION CHROMATOGRAPHY. K. Barsuhn, C. P. Cornell and M. L. Ogilvie, Performance Enhancement Res., The Upjohn Co., Kalamazoo, MI 49001 (616-385-6768)

A Dionex high performance ion chromatographic method was used to separate and quantify two nonvolatile (lactic and formic) and six volatile fatty acids (VFA; acetic, propionic, isobutyric, butyric, valeric and isovaleric) in bovine ruminal fluid. The acids were separated by ion chromatography exclusion (Dionex AS1-ICE column) with 0.1 M octane sulfonic acid in 2% isopropyl alcohol and detected electro-chemically following chemical ion suppression with 10 mM tetrabutylammonium hydroxide. The acids were eluted within 30 min in order listed above. Standard curves for all eight acids were linear ($r^2>0.99$) from 0-150 mM. Duplicate ruminal fluid samples were collected from 84 animals over a four month period. Over 4,000 deproteinated samples were analyzed by this method. The method proved to be easy, sensitive and reliable for the analysis of fermentation acids in ruminal fluid.

#56 EFFECT OF *Propionibacterium shermanii* ON RUMINAL FERMENTATIONS
L. Kung, Jr., A. Hession, R. S. Tung, and K. Maciorowksi. Department of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717-1303 (302 451 2522)

Propionibacterium shermanii (PS) converts glucose and lactate to propionate and acetate. In pure cultures, addition of lactate improved the growth rate of PS. In batch cultures (low capacity buffer) with mixed rumen organisms fed a high concentrate diet, 10^6 cfu PS/ml increased the molar proportions of propionate from approximately 21 to 27% within 24 h of fermentation. With a high capacity buffer PS increased molar % of propionate from approximately 20 to 40%. PS organisms were fed twice daily with a 50:50 forage:concentrate diet to continuous cultures of mixed rumen organisms (pH of about 6.2, d = .0625/h). PS dose was 10^5 cfu/ml of continuous culture from days 0 to 6 and 10^6 cfu/ml from days 7 to 11. Numbers of ruminal lactic utilizing bacteria in cultures were greater than added PS organisms at time 0 (10^8/ml) and after 6 days (10^7/ml) of culture. Addition of PS had no effect on molar proportions of propionic acid: 22.1% (control) and 22.5% (PS) during low dosing and 23.5% (control) and 24.2% (PS) during high dosing. Molar % acetate and total VFA were also not affected by treatment. Addition of propionic acid producing organisms has potential for altering ruminal fermentations.

#57 LABELLING OF *Ruminococcus albus* USING ENZYMATICALLY-SYNTHESIZED ^{14}C-CELLOBIOSE. P. Schofield and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876).

Partially-purified cellobiose phosphorylase was obtained from *Clostridium thermocellum* using ammonium sulfate fractionation and DEAE-cellulose chromatography. Cellobiose (CB) labelled at either the reducing (CB*) or non-reducing (*CB) end was prepared by incubating a mixture of glucose and glucose-1-phosphate (either moiety labelled) with the enzyme. CB was separated from the monosaccharide precursors by chromatography on charcoal and was characterized by thin layer chromatography and autoradiography. The effect of limiting CB on growth and CB uptake of *R. albus* was investigated using a semi-defined medium. A CB concentration of 0.15% was chosen for labelling studies. *CB was incorporated about 50% more effectively than CB* and, for cells grown to mid-log phase, the overall incorporation level was about 10% of the input *CB. For an input of 2.4 uCi/10 mL culture, the specific activity of the washed cells was 2.6×10^{-5} dpm/cell. Somewhat greater incorporation was obtained when cells were grown in a rumen fluid medium. There are several advantages to the use of CB as a label, including: a) a relatively high labelling efficiency; b) the possibility of CB metabolism studies; c) the wide availability of specific site labelled ^{3}H- and ^{14}C-glucose precursors.

#58 ENHANCED CELLULOSE DIGESTION IN COCULTURES CONTAINING *Ruminococcus albus* AND VARIOUS STRAINS OF *Bacteroides ruminicola* AND *Fibrobacter succinogenes*. K. A. Dawson and D. M. Hopkins, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606-257- 7552)

Cellulose digestion by axenic and cocultures of *Ruminococcus albus* strain 7, *Bacteroides ruminicola* strain 23 and *Fibrobacter succinogenes* strain S85 was examined over a 144-h period in serum bottle cultures containing a rumen fluid medium and filter paper discs as a substrate. The total extent of cellulose digestion was greater in cocultures of the two cellulose degraders (strains S85 and 7) than in either axenic culture (51.7 vs. 36.7 and 21.5%, respectively). Succinate production from cellulose was only associated with cultures containing strain S85 or containing strain 23 in cultures with a cellulolytic organisms and accounted for about 53% of the metabolic activities in cocultures containing strains 7 and S85. Coculture of strain S85 with strain 23 did not result in increased cellulose digestion but resulted in a 2-fold increase in propionate production. Coculture of strain 7 with strain 23 resulted in a 2.5-fold increase in cellulose digestion over axenic cultures of strain 7 and was accompanied by increases in succinate and propionate production. This study suggests that interactions between cellulolytic and non-cellulolytic bacteria can profoundly influence the metabolism and extent of cellulose digestion.

#59 PURIFICATION AND CHARACTERIZATION OF AN ALPHA-L-ARABINOFURANOSIDASE FROM *Butyrivibrio fibrisolvens* GS113. R. B. Hespell and P. J. O'Bryan. USDA/ARS, Natl. Ctr. Agric. Utilzn, Res., Peoria, IL 61604, 309-685-4011.

An alpha-L-arabinofuranosidase (EC 3.2.1.55) was purified from the cytoplasm of *Butyrivibrio fibrisolvens* strain GS113. The native enzyme had an apparent molecular mass of 240 kDa and was composed of eight polypeptide subunits of 31 kDa. The enzyme displayed an isoelectric point of 6.0, a pH optimum of 6.0 to 6.5, a pH stability of 4.0 to 8.0, a temperature optimum of 45°C, and was stable to 55°C. The K_m and V_{max} values for p-nitrophenyl-alpha-L-arabinofuranoside were 0.7 mM and 109 micromol/min/mg protein, respectively. The enzyme was specific for the furanoside configuration but had no activity on a variety of other nitrophenyl- or methylumbelliferylglycosides, cellulose, carboxymethylcellulose, or arabinogalactan. With oatspelt xylan, corn endosperm xylan, or beet arabinan, arabinose was found as the hydrolysis product. No activity was observed when either coumaric or ferulic acid ester-linked to arabinoxylobiose were used as substrates, but arabinoxylobiose was degraded to arabinose and xylobiose. Strain GS113 possesses no extracellular arabinofuranosidase activity, thus the major role of the enzyme is apparently in the assimilation of arabinose-containing xylooligosaccharides generated from other enzymatic activities on xylans.

#60 INFLUENCE OF VARIOUS LEVELS OF *Lactobacillus acidophilus* SUPPLEMENTATION ON FERMENTATION BY RUMEN MICROORGANISMS IN CONTINUOUS CULTURE. I. K. Yoon and M. D. Stern, Department of Animal Science, University of Minnesota, St. Paul, MN 55108 (612-624-6216)

Eight dual flow continuous culture fermenters were used to study fermentation by rumen microbes when various levels of *Lactobacillus acidophilus* were supplemented to a corn-based diet formulated for growing steers. Each fermenter was infused with one of the following doses of *L. acidophilus* (cfu/ml) per day: control-0, low-2.8 x 10^4, medium-2.8 x 10^6 and high-2.8 x 10^8. Acid detergent fiber digestion was higher (P<.10) with all levels of *L. acidophilus* treatment compared to the control. Lower (P<.10) degradability of crude protein (%) was observed with the low dose compared to the control which resulted in higher (P<.10) flow of dietary-N. Efficiency of bacterial protein synthesis (g N/kg OM truly digested) was lower (P<.10) with the high dose compared to the control. True OM digestion (%), ammonia-N concentration (mg/100 ml), nonammonia-N flow (g/d), total VFA concentration (mM) and molar proportions of individual VFA were not affected (P>.10) by supplementation of *L. acidophilus*. Various doses of *L. acidophilus* supplementation had little effect on microbial fermentation under the conditions used in this experiment.

#61 DEVELOPMENT OF SUBSTRATE MARKERS FOR DETECTING NOVEL MICROORGANISMS IN COMPLEX MICROBIAL ECOSYSTEMS. M.G. Beconi, R.I. Hollingsworth, M.T. Yokoyama, F.B. Dazzo and J.C. Mitchell, Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824, (517)353-2299

Traditional methods of detecting and isolating novel microorganisms from complex microbial ecosystems are laborious and time consuming. Intimate syntrophic interactions also add to the difficulty of isolating co-metabolizing microorganisms. Methods are being developed for the rapid detection and isolation of novel ruminal microorganisms, using fluorescently labelled and radioactively labelled substrates in conjunction with optical trapping using a LASER microscope. This method is being used to detect and isolate ruminal anaerobes responsible for the deepoxidation of deoxynivalenol (DON), a 12, 13-epoxytrichothecene mycotoxin contaminant of feed grains. Incubation of fluorescently labelled DON with mixed cultures of ruminal bacteria and examination by fluorescence microscopy detected morphologically distinct bacteria which fluoresced due to uptake of the labelled DON. These fluorescent bacteria are being checked for DON deepoxidation using radiolabelled DON in mixed cultures of ruminal bacteria.

#62 INOCULATION OF RECONSTITUTED HIGH MOISTURE CORN WITH PROPIONIC ACID BACTERIA WITH OR WITHOUT ADDITION OF LACTIC ACID BACTERIA. T. E. Dawson, L. A. Miranda, M. T. Yokoyama, and S. R. Rust. Dept. of Animal Science, Michigan State University, East Lansing, MI 48824-1225 (517-353-4866).

Dry corn (10% moisture) was cracked in a roller mill and reconstituted with sterile distilled deionized water to 30 % moisture. Treatments consisted of 1) control (no inoculum added); 2) *Propionibacterium acidipropionici* (ATCC 4965) and *Propionibacterium freudenrichii* (ATCC 6207); 3) *Lactobacillus plantarum*, *Streptococcus faecium*, and *Pediococcus acidilactici* ; 4) a mixture of treatments 2 and 3. All inocula were applied at approximately 10^7 cfu g^{-1} dm^{-1}. Approximately 1.5 kg was placed into a laboratory silo consisting of a capped PVC pipe. Silos were prepared in duplicate for each treatment day combination and were emptied for analysis on days 0, 2, 7, 14, 28, and 56. Lactic acid producing bacteria and non-mycotic lactic acid utilizing organisms were enumerated in an anaerobic glove box on MRS agar and Na-lactate agar with amphotericin-B respectively. Yeasts and molds were enumerated aerobically on rose bengal agar with chloramphenicol. The pH of aqueous extract was lowest ($p < .01$) on day 28 for treatments 3 and 4 (3.93 and 3.91 respectively) as compared to trt 1 and 2 (4.46 and 4.28 respectively). Yeasts and molds were lowest in trt 2 on day 28 ($10^{5.23}$ cfu g^{-1} dm^{-1}; $p < .01$) when compared to trts 1, 3, and 4 ($10^{6.10}$, $10^{6.05}$, and $10^{5.86}$ cfu g^{-1} dm^{-1}; respectively).

#63 DETECTION OF AMYLOLYTIC RUMINAL BACTERIA USING CHEMICALLY CROSS-LINKED STARCHES. [1]David A. Odelson and [2]Susan F. Kotarski, [1]Dept. of Biology, Central Michigan University, Mt. Pleasant, MI 48859 (517-774-3909) and [2]Department of Microbiology and Nutrition, The Upjohn Co., Kalamazoo, MI 49001 (616-385-6578).

Chemically cross-linked starches were examined for use in the characterization of starch-hydrolyzing bacteria from the rumen. These polymers are water insoluble and confer a turbidity when incorporated in both agar and broth media. Colonies of ruminal bacteria, including *Prevotella* (*Bacteroides*) *ruminicola*, *Butyrivibrio fibrisolvens*, *Ruminobacter amylophilus*, and *Streptococcus bovis*, produced clear hydrolysis zones of cross-linked starch in agar media. No hydrolysis zones were observed with *Eubacterium ruminantium*, *Fusobacterium necrophorum*, *Selenomonas ruminantium*, or *Succinovibrio dextrinisolvens*. Amylolytic ruminal bacteria were also able to ferment large polymers of alpha-1,4 and alpha-1,6 glycans, including amylopectin, amylose, pullulan, soluble starch, and starch granules. Cross-linked starch media were used for estimation of ruminal amylolytic bacterial populations. Thus, these starches offer an alternative to iodine-staining methods for detection and recovery of viable amylolytic ruminal bacteria.

#64 Hydrogen Threshold Values for One Methanogenic and Four Acetogenic Isolates From the Rumen. P. Boccazzil[1]* , J. A. Patterson[1] and B. J. Wilsey[2] and D. M. Schaefer[2]. Purdue University, West Lafayette, IN[1], 317-494-4826[1] and University of Wisconsin, Madison, WI, 608-263-4317[2]

Four acetogenic bacterial isolates were obtained from a H_2 limited chemostat using a semidefined medium. The inoculum was from the rumen of a lactating dairy cow fed a corn silage:corn grain (60:40%) diet once daily. All isolates stained Gram$^+$ and grew on glucose. Isolates BA2 and BA10 were coccoid and isolates BA4 and BA9 were rods. A methanogenic isolate, N1.4a, was obtained from a batch culture of rumen contents collected from a steer fed ad libitum a 90% corn grain diet. The isolate was similar in morphology to *Methanobrevibacter ruminantium*. Acetogenic isolates were grown in serum bottles to stationary phase with glucose and then adapted to a H_2:$C0_2$ (80:20) gas phase for 24 h. Bottles were then flushed and pressurized to 1.2 atm with H_2:N_2:CO_2 (1:74:25) and incubated for 3 to 6 days. Headspace gas volume was measured by syringe displacement and H_2 and CH_4 were measured using thermal conductivity. The methanogen was treated similarly except initial growth was on H_2. Thresholds for H_2 utilization by the acetogenic isolates BA2, BA10, BA4 and BA9 were (ppm): 2516, 1222, 8061 and 4911, respectively. The threshold value for the methanogenic isolate, N1.4a, was 120 ppm. Based upon H_2 threshold values alone, the data would suggest that these acetogenic isolates would not effectively compete with methanogens for H_2.

#65 ENUMERATION OF HYDROGEN UTILIZING ACETOGENIC, METHANOGENIC AND SULFATE REDUCING BACTERIA IN THE HINDGUT OF MAN AND PIG. J. Dore, B. Morvan, F. Rieu-Lesme, P. Pochart*, G. Fonty and P. Gouet. laboratoire Microbiologie, INRA, 63122 Saint-Genes-Champanelle (73624000) and * INSERM, Hopital Saint Lazare,75008 Paris, France.

Three microbial groups can contribute to hydrogen utilization in the rumen and hindgut: methanogenic bacteria (MB), sulfate reducing bacteria (SRB) and acetogenic bacteria (AB). If MB and SRB are commonly enumerated, the importance of acetogenesis has mainly been assessed by labeling techniques. We have applied selective enrichment techniques to the enumeration of $H_2/C0_2$-utilizing acetogens. Based on differential production of acetate under $H_2/C0_2$ versus $N_2/C0_2$ gas phase, the method proposed allows to apply most probable number statistics to the evaluation of population levels of the selected group. Stools from two healthy human volunteers of opposite status towards breath methane excretion were compared, as well as pooled samples of caecum and colon contents of six growing-finishing pigs. Total anaerobes, MB, SRB and AB were evaluated in all cases. Population levels of AB were the lowest of all three H_2-utilizing groups in pig gut contents (logN/g below 5.5) while SRB levels were stable between caecum and colon (logN/g= 7.2 to 7.4) and MB predominant only in the colon (logN/g= 8.8 versus 6.2 in the caecum). In human feces, SRB levels were similar for the two volunteers studied while MB were predominant in the methane-excreting subject (logN= 8.9). Interestingly, the levels of population of AB were higher than those of the SRB in both subjects (logN= 6.5 and 7.9) and highest of all three groups in the feces of the non-methane excreting subject. This approach should prove useful in the study of the mechanisms regulating population levels of H_2-utilizing bacterial groups in the digestive tract.

#66 EFFECT OF GLYCEROL ON RUMEN CELLULOLYTIC BACTERIA AND ANAEROBIC FUNGI. G. Fonty, V. Roger, C. Andre, F. Bonnemoy, and Ph. Gouet, Lab. de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand - Theix, 63122 St Genes Champanelle, France (73-62-40-00) and Lab. de Biologie Comparee des Protistes, CNRS URA138, Universite Blaise Pascal, 63170 Aubiere, France.

The production of engine fuel from plant oils releases large quantities of glycerol as a by-product. This glycerol is not up to pharmaceutical standards but is amenable to incorporation in ruminant diets, since it can be metabolized by the rumen microbes to produce volatile fatty acids. The aim of this work was to study the effect of various concentrations of glycerol *in vivo* on the cellulolytic bacterial and fungal populations in the sheep rumen and *in vitro* on the growth and the ability of pure cultures of these microorganisms to digest cellulose. *In vivo*, daily quantities of glycerol less than or equal to 180 ml affected neither the numbers of cellulolytic bacteria nor those of anaerobic fungi in the rumen of sheep fed maize silage or meadow hay. Higher quantities of glycerol led to acidosis. *In vitro*, at low quantities (0.1 - 2%) the glycerol had no effect on growth, adhesion and cellulolytic activity of *Fibrobacter succinogenes* S85 and *Ruminococcus flavefaciens* 007. In presence of 5% glycerol, the growth of *F. succinogenes* S85 was partly inhibited and the amount of cellulose degraded was greatly decreased. At the same concentrations *R. flavefaciens* was less sensitive to this molecule than *F. succinogenes*. Glycerol also affected the growth of *Neocallimastix frontalis* MCH3 and its ability to digest filter paper. The fungal cellulolytic activity was greatly inhibited by 2% and completely by 5% glycerol.

#67 NMR STUDY OF GLUCOSE METABOLISM BY *Neocallimastix frontalis*. A. Bernalier, G. Bielecki, G. Fonty, J.P Renou, and Ph. Gouet, Lab. de Microbiologie and Station de Recherches sur la Viande (Unite RMN), INRA, Centre de Recherches de Clermont-Ferrand - Theix, 63122 St Genes Champanelle, France (73-62-40-00) and Lab. de Biologie Comparee des Protistes, CNRS URA 138, Universite Blaise Pascal, 63170 Aubiere, France.

The rumen anaerobic fungi produce formate, acetate, lactate, ethanol, CO_2, H_2 and sometimes succinate from glucose. In these microorganisms, the metabolic pathways leading to the formation of these compounds are still not well defined. Therefore, we studied by NMR spectroscopy the metabolism of ^{13}C-1 and ^{13}C-2 glucose by *Neocallimastix frontalis* MCH3. The classical metabolic pathways for these metabolites were recovered : formation of ^{13}C-2 acetate, ^{13}C-3 lactate, ^{13}C-2 ethanol from ^{13}C-1 glucose and formation of ^{13}C-1 acetate, ^{13}C-3 lactate, ^{13}C-3 succinate and ^{13}C-1 ethanol from ^{13}C-2 glucose. The production of acetate labelled both on the methyl and carboxyl groups as the observation of doubly-labelled lactate from ^{13}C-1 and ^{13}C-2 glucose suggest the presence of other pathways leading to these products. Likewise, formation of labelled formate from ^{13}C-1 glucose was not expected. Thus, the metabolism of glucose by the anaerobic fungi appears to be more complex than initially assumed.

#68 NMR ANALYSIS OF GLYCOGEN STORAGE AND DEGRADATION IN *Fibrobacter succinogenes* G.Gaudet, E.Forano, and A.M.Delort [(1)], Lab. de Microbiologie, INRA, Centre de Recherche de Clermont-Ferrand-Theix, 63122 S[t] Genes-Champanelle, France; and (1) Lab. de Chimie Organique Biologique, Universite Blaise Pascal, 63170 Aubiere, France.

^{13}C and ^{1}H NMR spectroscopy was used "in situ" to follow glycogen synthesis and degradation in resting cells of *Fibrobacter succinogenes* S85. The cells were incubated at 37°C, in anaerobic conditions with ^{13}C glucose specifically labelled. ^{1}H NMR spectra were used to quantify the percent enrichment by ^{13}C of products of the metabolism. Glucose was utilized for energetic requirements of the bacteria, essentially via the Embden-Meyerhof pathway, leading to the synthesis of succinate and acetate, while glycogen was stored. From $^{13}C1$ glucose, labelling occured on the C2 positions of succinate and acetate, and both on the positions C1 and C6 of glycogen, the C1 position beeing predominant. The labelling of glycogen on C6 position may be explained by the scrambling of the glycolytic pathway. When the bacteria were first incubated with $^{13}C1$ glucose, washed and then incubated with $^{13}C2$ glucose, the pattern of ^{13}C labelling in the products of the metabolism, as shown by ^{13}C and ^{1}H NMR spectra, indicated that glycogen was degraded at the same time it was stored.

#69 MOLECULAR CLONING AND EXPRESSION OF AN ENDOGLUCANASE GENE FROM *Fibrobacter succinogenes* IN *Escherichia coli*. E. Forano, V. Broussolle, Y. Ribot, G. Gaudet and J.A. Bryant (1), Lab. de Microbiologie, INRA, Centre de Recherche de Clermont-Ferrand-Theix, 63122 St Genes-Champanelle, France; and (1) Dept of Biological Science, University of Exeter, Exeter - EX4 4QG, Great Britain.

A *F. succinogenes* genomic library was constructed in PUC18 and expressed in *E. coli*, and one clone particularly active against carboxymethylcellulose (CMC) was studied. The original inserted fragment was shortened to a 2,2 kb fragment (ED 11) retaining full CMCase activity. The CMCase activity was located in the cytoplasm of the E. coli cells. The enzyme was active against CMC, ß glucan and xylan, but could not hydrolyse laminarin, lichenan, arabinogalactan and glucomannan. Its specificity and the analysis of its activity against CMC (reduction of viscosity compared to soluble sugars released) suggested that the enzyme was a ß1-4 endoglucanase. The pH for optimal activity on CMC was 6.3. The temperatures for optimal activity were 25°C and 37°C, the activity decreasing rapidly at higher temperatures. Analysis of the protein profile on SDS PAGE of crude extracts of *E. coli* cells containing either pUC18 or pED11 showed that the ED11 fragment coded for a major protein of molecular weight 64,000. This result was confirmed by ^{35}S labelling of the proteins by using a minicell expression system. Sequencing of the ED11 fragment is now in progress.

#70 LIGNINASE (LIGNIN PEROXIDASE) GENES OF THE WOOD-DEGRADING WHITE-ROT FUNGUS, *Phanerochaete chrysosporium*. C. A. Reddy, Department of Microbiology, Michigan State University, East Lansing, MI 48824. Phone: (517)-355-6499.

Lignin constitutes an important barrier for the efficient digestion of lignocellulosic plant materials in the rumen. Biological delignification of lignocellulosics using ligninolytic fungi or their enzymes is a pretreatment alternative of considerable potential benefit to the livestock industry. With the eventual objective of generating genetically engineered microbes that overproduce lignin peroxidases (LIPs), we have cloned and characterized several major LIP genes of the lignin degrading white-rot fungus, *Phanerochaete chrysosporium*. Our results showed that each of the LIP genes encodes a mature protein that is 344 aa long and a 27 to 28 aa leader sequence that ends in a Lys-Arg cleavage site. The coding region in different LIP genes is interrupted by 8 to 9 small introns that range in size from 50 to 62 bp. Northern blotting analyses showed that the expression of these genes occurs only during secondary metabolism and that it is regulated at the transcriptional level. There is a high degree of homology (70-90%) among the different LIP genes. Further sequence analyses revealed that there are two distinct sub-families with in the LIP genes of *P. chrysosporium*. Research on heterologous expression of the LIP genes is in progress.

#71 STUDIES ON THE EXPRESSION OF CELLULASE BY *Ruminococcus flavefaciens* FD-1. B. A. White, R. I. Mackie, and K. C. Doerner. Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 (217-333-2091)

Ruminococcus flavefaciens has been hypothesized to produce cellulase constitutively. However, the work of Pettipher and Latham (J. Gen. Microbiol. 110:29-38,1979.) only addressed the production of carboxymethyl cellulase activity and did not address the regulation of true cellulase activity towards insoluble cellulose. We have studied the effect of carbon source, either cellobiose or cellulose, on the production of cellulase in batch cultures of *R. flavefaciens* FD-1, using carboxymethylcellulose, para-nitrophenyl-beta-D-cellobioside, and ^{14}C-cellulose as the substrates. Cells were grown to mid-log phase and then the cells were separated from the supernatant, and each of these preparations was used as a source of cellulase enzyme. Total carboxymethylcellulase and ^{14}C-cellulase activity was approximately 2-fold higher in cellobiose grown cells than in cellulose grown cells, whereas para-nitrophenyl-beta-D-cellobiosidase activity was not affected by culture conditions. There was also a marked difference in the distribution of enzyme activity between cells and culture supernatant. The ratio of cell associated to cell free carboxymethylcellulase increased slightly from cellobiose grown cells, whereas the ratio of cell associated to cell free ^{14}C-cellulase decreased by 1/2 with cellulose grown cultures. The rate of celluase production was also measured. Two cellobiose cultures, one with cellulose added in the log phase of growth, were monitored for para-nitrophenyl-beta-D-cellobiosidase and ^{14}C-cellulase activity. The addition of cellulose to cells growing on cellobiose did not alter the amount or rate of cellulase production. Although the adherence of cells and cellulase enzyme to insoluble cellulose can complicate interpretations of this data, the results indicate that cellulase synthesis by *R. flavefaciens* is constitutive.

AUTHOR INDEX BY ABSTRACT NUMBER

The Organizing Committee

James B. Russell, Chairman
M. T. Yokoyama and D. E. Akin, Micrbiology
F. N. Owens and R. S. Swingle, Nutrition
D. R. Buxton and R. Hatfield, Agronomy
R. H. Dunlop and E. C. Crinchlow, Physiopathology
J. R. Russell, Treasurer
S. F. Kotarski, Secretary

would like to thank

Alltech, Inc.

Lilly Research Laboratories

Monsanto

Moorman Manufacturing Company

The Upjohn Company

for providing the mixer, refreshments, poster boards and postage

and

James E. Wells

for optically scanning the abstracts.