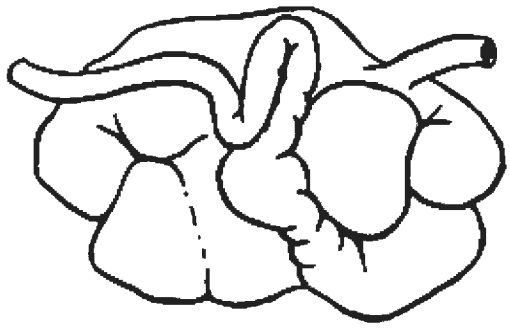
Abstracts

Conference on Rumen Function

Volume 23, 1995



44 Years of Interaction

1951-1995

23rd Biennial Conference on Rumen Function
Chicago, Illinois
November 14-16, 1995

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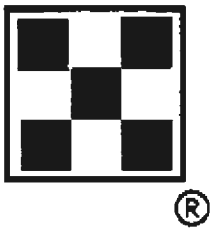


23rd BIENNIAL

CONFERENCE ON RUMEN FUNCTION

1951-1995

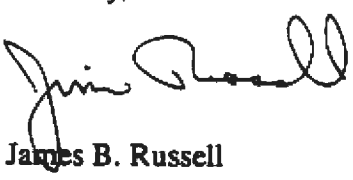
Welcome to the 23rd Biennial Conference on Rumen Function. The Rumen Function Conference has been meeting in the Congress Hotel since November 1951. The Conference originally focused on the problem of bloat, and this aspect of rumen function was a central theme until 1961. Since this time, the Conference 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, USDA

Agenda

November 14, 1995

8:00 - 11:30 pm Mixer in Great Hall. Posters may be presented at this time.

November 10, 1993

Nutrition-Agronomy Panel

9:00 Brief introduction by James B. Russell.

9:15 #8 MODEL OF HYDROGEN ION FLOW THROUGH THE RETICULORUMEN. M. S. Allen, Dept. of Animal Science, Michigan State University, East Lansing, MI 48824 (517-432-1386)

9:30 #10 THE USE OF NUMERICAL METHODS TO INTEGRATE A LOGISTIC MODEL OF SUBSTRATE DIGESTION WITH AN EXPONENTIAL MODEL OF PASSAGE. M.C. Barry, Department of Animal Science, Cornell University, Ithaca* NY 14853 (607-255-3973)

9:45 #4 INTAKE AND DIGESTION OF ORCHARDGRASS AND ALFALFA SILAGES TREATED WITH CELLULASE, FORMIC ACID, AND INOCULANT. E. M. G. Nadeau, D. R. Buxton, J. R. Russell, E. Lindgren, and P. Lingvall. Dept. Agronomy, USDA-ARS, Dept. Animal Science, Iowa State Univ., Ames, IA 50011; Dept. Animal Nutr. and Mgmt., Swedish Univ. Agric. Sci., Uppsala, Sweden (515-294-5076)

10:00 #13 CRYOGENICALLY PROTECTED AND FRESH RUMEN INOCULUM FOR DIGESTIBILITY STUDY . O.A. Ayangbile[1], J.C. Meier[1], and M.K. Vogel[1]; J. Robertson[2]; A.R. McElroy[3]; A.R. Komarek[4] [1]Analab, Division of Agri-King Inc., Fulton, IL 61252 (815-589-2525); [2]Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-4478); [3]Plant Research Center, Agriculture and Agri- food Canada, Ottawa, Ontario K1A OC6 (613-759-1305); [4]ANKOM, Fairport, NY 14450 (716-425-3940)

10:15 #1 EFFECT OF NITROGEN SUPPLEMENTATION ON IN VITRO DIGESTION KINETICS OF PERENNIAL GRASSES. D. J. R. Cherney and J. H. Cherney. Departments of Animal Science and Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY 14853 (607-255-0604)

10:30 Brief break

11:00 #7 EFFECT OF MATURITY ON DIGESTION KINETICS OF WATER-SOLUBLE AND WATER-INSOLUBLE FRACTIONS OF ALFALFA AND BROME. B. Stefanon, A. N. Pell*, and P. Schofield, Dipartimento di Scienze delle Produzioni Animali, Universita' degli Studi di Udine, Udine, Italy and Dept. of Animal Science, Cornell University, Ithaca, NY 14853. (607)-255-2876.

11:15 #5 EFFECTS OF MONENSIN AND ORGANIC ACID TREATMENT ON IN VITRO FERMENTATION OF CRACKED CORN BY MIXED RUMINAL MICRO-ORGANISMS. T. R. Callaway* and S. A. Martin[1,2], Departments of Animal and Dairy Science[1] and Microbiology[2], University of Georgia, Athens, GA 30602-2771 (706-542- 1065)

11:30 #2 THE EFFECT OF pH ON IN VITRO METHANE PRODUCTION FROM RUMINAL BACTERIA. J. S. Van Kessel[1*] and J. B. Russell[2]. Department of Animal Science[1] and Section of Microbiology[2], Cornell University and Agricultural Research Service, USDA[2], Ithaca, NY 14853 (607-255-4508)

11:45 #11 STUDIES ON RUMEN MICROBIAL SYNTHESIS FROM DIFFERENT NITROGEN SOURCES USING IN VITRO AND IN VIVO METHODS. Jia-qi Wang. Animal Science Institute, Chinese Academy of Agricultural Sciences Beijing t00094, China

12:00 Lunch

1:30 #9 POTASSIUM ACCUMULATION IN PERENNIAL GRASSES. J. H. Cherney, D. J. R. Cherney, D. R. Dewing and R. F. Lucey. Department of Soil, Crop, and Atmospheric Sciences and Department of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-0945)

1:45 #6 COMPARISON OF NEUTRAL DETERGENT-SOLUBLE FIBER FERMENTATIONS AMONG FEEDSTUFFS. M.B. Hall*, A.N. Pell and L.E. Chase. Dept. of Animal Science, Cornell University, Ithaca, NY 14853. (607)-255-4478.

2:00 #3 SOLUBLE CARBOHYDRATE CONCENTRATION OF BOVINE RUMINAL FLUID AS AFFECTED BY DIET. R. S. Pinder, M. A. Cecava and J. A. Patterson. Dept of Animal Sciences, Purdue University, West Lafayette, IN 47907 (317-494-4826).

2:15 #12 ALTERING RUMINAL FERMENTATION AND INHIBITING RUMINAL SULFIDE PRODUCTION WITH 9,10 ANTHRAQUINONE (AQ). A. O. Hession*, L. Kung, Jr., and C. A. Bessett. Department of Animal and Food Sciences, University of Delaware, Newark, DE 19717. (302-831-2522).

Microbiology-Physiopathology Panel

2:30 #15 GLUCOSE AND HYDROGEN UTILIZATION BY A RUMINAL ACETOGEN. R. S. Pinder and J. A. Patterson. Dept. of Animal Sciences, Purdue University, West Lafayette, IN 47907 (317)-494-4826.

2:45 #16 FACTORS AFFECTING MALATE UTILIZATION BY <u>SELENOMONAS RUMINANTIUM</u>. J. D. Evans and S. A. Martin. Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706-542-0886).

3:00 #14 GROWTH INHIBITION OF <u>RUMINOCOCCUS FLAVEFACIENS</u> BY <u>RUMINOCOCCUS ALBUS</u>. W. W. Chan and B. A. Dehority. Dept of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691-4096 (216-263-3908)

3:15 #30 FRUCTOSE 1,6-DIPHOSPHATE MAY SERVE AS AN ENERGY SIGNAL TO THE F_1F_0-ATPASE OF STREPTOCOCCUS BOVIS. D. R. Bond and J. B. Russell. Section of Microbiology, Cornell University and ARS/USDA, Ithaca, NY 14853 (607-255-4508).

3:30 Brief break

4:00 #24 LAG AND MAXIMUM GROWTH RATE STUDIES FOR RUMINOCOCCUS ALBUS ON FIBER SUBSTRATES. Peter Schofield* and Alice N. Pell. Dept. Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876).

4:15 #29 ISOLATION AND PARTIAL PURIFICATION OF THE PROTEOLYTIC ACTIVITY OF PREVOTELLA RUMINICOLA 118B. K. E. Griswold and R. I. Mackie. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-8809).

4:30 #25 EFFECTS OF SACCHAROMYCES CEREVISIAE CELLS ON RUMINAL MICROBIAL FUNCTIONS AS ASSESSED BY IN VITRO MEASUREMENTS. F. Chaucheyras [1,2], G. Fonty [1], G. Bertin [2], Ph.Gouet [1], [1]Laboratoire de Microbiologie, INRA, 63122 Saint-Genes Champanelle, France (Phone 19 33 73 62 40 00); [2]Santel-goupe Agritek, 85 rue Anatole France, 92300 Levallois-Perlet, France (Phone 19 33 1 41 34 00 90)

4:45 #28 INTERACTIONS OF TANNINS AND RUMINAL BACTERIA. K. E. Nelson*, A. N. Pell, and B. I. Giner-Chavez. Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607)-255-2876.

5:00 #17 COMPARISON BETWEEN MICROBIAL POPULATIONS IN THE RUMEN AND CONTINUOUS CULTURE FERMENTERS USING RIBOSOMAL RNA-TARGETED DNA PROBES. C. J. Ziemer[1], R. Sharp[2] M. D. Stern[1], M. A. Cotta[3], T. R. Whitehead[3] and D. A. Stahl[2]. [1]Department of Animal Science, University of Minnesota, St. Paul, MN 55108 (612) 624-6216), [2]Northwestern University, Evanston, IL and [3]ARS, USDA, Peoria.

5:15 #19 PHYLOGENETICALLY-BASED PROBES FOR THE STUDY OF RUMEN MICROBIAL POPULATIONS. R. Shalp[1], C.J. Ziemer[2], M.D. Stern[2], M.A. Cotta[3], T.R. Whitehead[3], and D.A. Stahl[1]. [1]Department of Civil Engineering, Northwestern University, 2145 Sheridan Road, Evanston, Il 60208. (708) 4671074, [2]Dept.of Animal Science, University of Minnesota, 130 Haecker Hall, 1364 Eckles Avenue, St Paul. MN 55108,and [3]FBRU, USDA/ARS/ NCAUR, 1815 N.University Street, Peoria, IL 61604.

5:30 Dinner at your discretion.

8:15 Introduction of Invited Speaker by James B. Russell.

DIGESTION KINETICS OF RUMINANT FEEDS AND PLANT ENZYME MEDIATED PROTEOLYSIS IN RUMINANTS

by Michael K. Theodorou

Institute of Grassland and Environmental Research, Plas Gogerddan,
Aberystwyth, Dyfed, SY23 3EB, United Kingdom
[Tel:44(0) 1970 828 255; FAX: 44 (0) 1970 828 357; E-mail: theodorou@bbsrc.ac.uk]

Introduction. This presentation will be made in two parts. In the first part, forage digestion procedures in ruminant nutrition will be considered together with an overview of how the most recent techniques in gas production can be used to (a) rank forages relative to their in vitro fermentability, (b) describe forage digestion kientics in ruminants and (c) evaluate the likely effects of anti-nutritional (anti-fermentative) factors on rumen function. In the second part of the presentation, accepted concepts concerning microbially mediated proteolysis in ruminants will be reviewed. I will then present a new hypothesis that considers the possibility of plant enzyme mediated proteolysis in the grazing ruminants.

Digestion kinetics of ruminant feeds. In vitro gas production procedures rely upon measuring gas accumulation above fermenting batch cultures inoculated with rumen microorganisms. The method in its various forms has been used over many years to measure rumen microbial activity, to observe seasonal variations in rumen microbial populations, to construct growth curves for mixed and axenic rumen microorganisms, to screen for bloat causing and bloat safe legumes in legumes breeding, and to rank the fermentability of forages in forage evaluation tests. By using pressure transducers and/or pressure sensors and recently derived mathematical descriptions, it is now relatively simple to measure and quantify in vitro gas production. However, although the new methods are simple, cost effective, and precise, the underlying mechanisms that give rise to gas and other fermentation end-products are complex and not entirely understood. Some problems associated with the use of in vitro gas production in forage evaluation will be highlighted together with comments on those areas of forage evaluation where the current techniques show promise.

Plant enzyme mediated proteolysis in ruminants. Evidence in favour of the new hypothesis that plant enzymes and not microbial enzymes are responsible for proteolysis in ruminants includes (a) comparison with the ensilage process where plant enzyme mediated proteolysis is considered the norm, making a significant contribution to protein breakdown in cut herbage, (b) considerations of plant proteolytic activities occurring naturally during leaf senescence and the likelihood that these processes may occur in ingested herbage where largely intact cells are incubated anaerobically for many hours prior to rumination and (c) my laboratory has shown that when incubated under rumen-like conditions, Rubisco (leaf fraction 1) protein in Lilium perenne, as visualized by SDS-PAGE electrophoresis, was broken down rapidly and at similar rates in both the presence and absence of rumen microorganisms. The fact that lack of rumen microorganisms in one treatment did not inhibit removal of Rubisco, but did lead to accumulation of lower molecular weight protein breakdown products, was of note in this experiment. The breakdown products did not accumulate in herbage incubated with rumen microorganisms, presumably because they were metabolised further by the rumen microorganisms.

If the plant enzyme mediated proteolysis hypothesis is correct, it will have a significant impact on the way we view proteolysis in ruminants and present us with alternative strategies to align protein and carbohydrate breakdown in the rumen. Research from my laboratory supporting the hypothesis was presented by Merry *et al.* at the Microbiology Satellite Meeting (16-17 September 1995) of the Fourth International Symposium on the Nutrition of Herbivores at Clermont Ferrand in France. The work is currently being considered for scientific publication.

9:15 Panel Discussion

November 16, 1993

Microbiology-Physiopathology Panel, Continued

9:00 #18 THE USE OF 16S RIBOSOMAL RNA DIRECTED OLIGONUCLEOTIDES TO STUDY THE RUMEN MICROBIAL POPULATION. J.M.Wood, K.P.Scott, G. Avgustin and H.J. Flint,Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB2 9SB, IJK (0224 715251).

9:15 #20 THE USE OF RIBOSOMAL RNA PROBES TO ACCESS THE CONTRIBUTION OF OBLIGATE AMINO ACID FERMENTING BACTERIA TO RUMINAL DEAMINATION. D. O. Krause* and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508).

9:30 #26 MUTAGENESIS OF PREVOTELLA RUMINICOLA B14 AND SELECTION OF MUTANTS DEFECTIVE IN PEPTIDE UTILIZATION. L. Peng and M. Morrison*, Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908 (402-472-6405)

9:45 #22 CHAPERONINS FROM RUMINOCOCCUS FLAVEFACIENS FD-1: CLONING OF dnaK ANI groEL BY A POLYMERASE CHAIN REACTION (PCR) BASED APPROACH. Bryan A. White[1], Isaac K. O. Cann[1], W. Michael Russell[1], and Philip E. Vercoe[2]. Department of Animal Sciences, University of Illinois at Urbana-Champaign[1], and Department of Animal Science, University of Western Australia, Nedlands, 6009, Western Australia[2]. (217-333-2091)

10:00 Brief break

10:30 #23 CONJUGAL TRANSFER OF TRANSPOSON Tn1545 INTO EUBACTERIUM CELLULOSOLVENS 5494. K.L. Anderson, J.A. Megehee, and V.H. Varel. Dept. Biol. Sci., Mississippi State University, Miss. St., MS 39762 (601-325-8825), and USDA/ARS, Clay Center, NE 68933 (402-762-4207).

10:45 #21 TRANSFORMATION OF THE RUMEN BACTERIUM BUTYRIVIBRIO FIBRISOLVENS WITH RECOMBINANT cDNAs ENCODING FIBER-DEGRADING ENZYMES. K.S. Gobius[1], C.S. McSweeney[2] and G.P. Xuel. ICSIRO DTCP, St. Lucia 4067, Australia (+61-7-3377-0261). 2CSIRO DTAP, Private Bag No 3 PO, Indooroopilly, QLD 4068, Australia.

11:00 #27 MARKED ELEVATIONS OF RUMEN H_2S GAS ASSOCIATED WITH DIET-INDUCED POLIOENCEPHALOMALACIA (PEM). D.H. Gould, B.A. Cummings, D.W. Hamar, Department of Pathology, Colorado State University, Fort Collins, CO 80523 (970 491-6144)

11:15 General meeting

ABSTRACTS

#1 EFFECT OF NITROGEN SUPPLEMENTATION ON IN VITRO DIGESTION KINETICS OF PERENNIAL GRASSES. D. J. R. Cherney and J. H. Cherney, Departments of Animal Science and Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY 14853 (607-255-0604)

Objectives were to assess the influence of N fertilization of reed canarygrass and timothy on fiber digestion kinetics. Rate and extent of digestion were higher for grass fertilized with 214 kg of N/ha (k=.06, extent=73%) than for unfertilized grass (k=.04, extent=67%) when no supplemental N was added to in vitro incubations. When urea (.5g/L of buffer) was added to the incubations, there was no difference in extent of fiber digestion between fertilized and unfertilized grass, indicating that that N limited fiber digestion of unfertilized grass in vitro (CP=9.5%). Rate of fiber digest of N/ha compared to unfertilized grass. In another study, trypticase (4g/L of buffer) added to in vitro incubations increased extent of digestion of grasses by 2% compared to no N supplementation, while added urea increased fiber digestion of unfertilized grass by 10%. After 3h, urea treatment resulted in ammonia concentrations 8-fold higher, and trypticase treatment ammonia concentrations 2-fold higher, than in untreated samples. It appears that trypticase did not meet N requirements of ruminal microorganisms for maximum fiber digestion during the first 24h of digestion. Podium.

#2 THE EFFECT OF pH ON IN VITRO METHANE PRODUCTION FROM RUMINAL BACTERIA. J. S. Van Kessel[1]* and J. B. Russell[2]. Department of Animal Science[1] and Section of Microbiology[2], Cornell University and Agricultural Research Service, USDA[2], Ithaca, NY 14853 (607-255-4508)

When ruminants are fed high concentrate diets, the ruminal acetate to propionate ratio decreases, and methane production declines. Concentrate diets also cause a decrease in ruminal pH, but the relationship between pH and methanogenesis had not been determined. When fistulated cows were fed forage or concentrate diets, the ruminal pH values were 6.80 and 5.42, respectively. Mixed ruminal bacteria from the forage-fed cow converted CO_2 and H_2 to methane, but no methane was detected with ruminal fluid from the concentrate-fed cow. When the pH was increased to 7.0, the ruminal fluid from the concentrate-fed cow produced methane, but the lag time was 4 hours longer. Based on the zero-time intercept, the concentrate-fed cow had approximately 10-fold fewer methanogens than the forage-fed cow. When the ruminal bacteria were incubated in a medium containing 100 mM acetate, the addition of HCl caused a dramatic decrease in methane production, and no methane was detected at pH values less than 6.0. When the acetate concentration of the medium was decreased, the pH-dependent inhibition of methane production could be completely reversed. Based on these results: 1) ruminal methanogens are very sensitive to low pH, 2) pH-dependent decreases in the ratio of acetate to propionate are probably caused by an inhibition of methanogenesis, and 3) the inhibition of methanogenesis is caused by the toxicity fermentation acids at low pH. Podium.

#3 SOLUBLE CARBOHYDRATE CONCENTRATION OF BOVINE RUMINAL FLUID AS AFFECTED BY DIET. R. S. Pinder, M. A. Cecava and J. A. Patterson. Dept of Animal Sciences, Purdue University, West Lafayette, IN 47907 (317-494-4826).

The influence of dietary forage content (FC),ruminally undegradable intake protein (UIP) and time after feeding on concentrations of soluble carbohydrates in ruminal fluid of steers was determined. Four ruminally cannulated steers, in a 4 x 4 latin square design, were offered diets containing high (75% of DM) or low (25% of DM) DF and high (G% of DM) or low (5% of DM) UIP. Ruminal hexose concentration in steers fed low DF diets (145.1 μM) was higher (P = .079) than in steers fed high DF diets (124.5 μM). UIP did not affect (P = .538) ruminal hexose concentrations. Ruminal hexose concentration declined immediately after feeding and did not rise until 3 hr. after feeding (P < .0001). Cellobiose and glucose (~90% and ~10%, respectively) were the major soluble hexoses present in RF. Maltose was not detected. Ruminal glucose concentration (13.0 μM) was not modified by UIP (P = .403) nor DF (P = .GOG). However, a DF by post-prandial time interaction was detected (P = .017). Pentose concentrations were lower (P = .021) in rumen fluid of steers fed high DF (100.2μM) than steers fed low DF (177.0 μM). UIP did not influence (P = .346) ruminal pentose concentration. Soluble pentoses were not identified, but free xylose and arabinose were not detected. These data indicate that soluble calbohydrate concentrations in RF are close to, or below, the substrate affinities of many rumen bacteria and may not influence fiber digestion under these conditions. Podium.

#4 INTAKE AND DIGESTION OF ORCHARDGRASS AND ALFALFA SILAGES TREATED WITH CELLULASE, FORMIC ACID, AND INOCULANT. E. M. G. Nadeau, D. R. Buxton, J. R. Russell, E. Lindgren, and P. Lingvall. Dept. Agronomy, USDA-ARS, Dept. Animal Science, Iowa State Univ., Ames, IA 50011; Dept. Animal Nutr. and Mgmt., Swedish Univ. Agric. Sci., Uppsala, Sweden (515-294-5076).

Cellulase can decrease cell-wall concentration and subsequently increase the soluble and rapidly digestible portion of silage dry matter. This study determined the effects of cellulase (Multifect™ CL, Trichoderma longibrachiatum) alone or combined with a bacterial inoculant (Biomate® SI, Lactobacillus plantarum and Pediococcus cerevisiae) or formic acid on intake and digestion of orchardgrass and alfalfa silages. Intake and in vivo digestibilities were determined on lambs and rumen in situ digestion kinetics were determined on cows. Because of improved silage characteristics in terms of greater sugar and lower acetic acid and NH_3-N concentrations, cellulase combined with formic acid or inoculant increased dry-matter intake by 13 and 8% compared with control silage when averaged across plant species. As a result of an extensive cell-wall degradation by cellulase during ensiling, cellulase-treated orchardgrass and alfalfa silages had 34 and 15 % lower cellulose intakes than control silages. Total cell-wall disappearance during early hours of rumination was, however, greater for cellulase+formic acid treated silage than for the control, with a greater effect in orchardgrass than in alfalfa. Podium.

#5 EFFECTS OF MONENSIN AND ORGANIC ACID TREATMENT ON IN VITRO FERMENTATION OF CRACICED CORN BY MIXED RUMINAL MICRO- ORGANISMS. T. R. Callaway* and S. A. Martin[1] , [2], Departments of Animal and Dairy Science[1] and Microbiology[2], University of Georgia, Athens, GA 30602-2771 (706-542- 1 065)

Monensin has been included in cattle diets for 20 yr, while organic acids are commonly found in forages and grains fed to ruminants. The objective of this study was to determine the effects of monensin and organic acids on the in vitro fermentation of cracked corn by mixed ruminal microorganisms. Ruminal fluid was collected from a steer fed 36.3 kg of wheat silage and 4.5 kg of concentrate supplement once per day. Mixed ruminal microorganisms were incubated in anaerobic media (40 mL) that contained 20% (vol/vol) ruminal fluid and 0.4 g of cracked corn in batch culture for 24 h at 39°C. Organic acids (OA) (aspartate, fumarate, and malate) were added to serum bottles (n=4) to achieve final concentrations of 0, 4, 8, and 12 mM. Monensin, dissolved in ethanol, was included in the serum bottles to achieve a final concentration of 5 ppm. Addition of both monensin and OA decreased the acetate to propionate ratio ($P<0.05$). Organic acid plus monensin treatments increased propionate production ($P<0.05$), while OA addition increased total volatile fatty acid production ($P<0.05$). Organic acid addition increased the final pH of the media even in the presence of monensin ($P<0.05$). Total gas production was increased by OA addition ($P<0.05$) and it appeared that an increase in $C0_2$ production accounted for most of this increase. Methane production was decreased by monensin treatment ($P<0.05$). Monensin and OA treatment yielded similar results and in some cases additive effects were observed. Given concerns over increased antibiotic resistance in animal production systems, organic acids offer possible alternatives to feeding antimicrobial compounds to production ruminants. Podium.

#6 COMPARISON OF NEUTRAL DETERGENT-SOLUBLE FIBER FERMENTATIONS AMONG FEEDSTUFFS. M.B. Hall*, A.N. Pell and L.E. Chase. Dept. of Animal Science, Cornell University, Ithaca, NY 14853. (607)-255-4478.

Neutral detergent-soluble fiber (NDSF) is the portion of non-starch plant carbohydrates that is extracted by neutral detergent but which remains in 90% ethanol-insoluble residue (EIR). Dried citrus pulp (DCP), dried sugar beet pulp (DBP), mature alfalfa stems (MAS) and leaves (MAL), and immature alfalfa stems (IAS) and leaves (IAL) were analyzed for NDSF. For each feed, dry residues of NDF soaked overnight in 1 M ammonium acetate to remove detergent, and EIR were prepared. Whole feed, EIR and NDF were fermented for 24 h with mixed rumen microbes in vitro, gas production was measured and logistic rates of NDSF fermentation determined on EIR minus NDF gas data. NDSF percentages of feed dry matter were 34.5, 34.4, 11.9, 22.7, 21.4 and 23.9 for DCP, DBP, MAS, MAL, IAS and IAL. One-pool logistic rates with lag were .1 3 1 , .154, .130, .136, .1 1 and .125/h on the same feeds, respectively. Acetate to propionate ratios were consistently higher for EIR fermentations than for those of whole feed or NDF. The higher Ac:Pro ratios are consistent with those reported for fermentations of feeds high in NDSF components such as pectic substances. Podium

#7 EFFECT OF MATURITY ON DIGESTION KINETICS OF WATER-SOLUBLE AND WATER-INSOLUBLE FRACTIONS OF ALFALFA AND BROME. B. Stefanon, A.N. Pell*, and P. Schofield, Dipartimento di Scienze delle Produzioni Animali, Universita' degli Studi di Udine, Udine, Italy and Dept. of Animal Science, Cornell University, Ithaca, NY 14853. (607)-255-2876.

Alfalfa and bromegrass hays, each harvested at five stages of maturity, were separated into water-soluble and water-insoluble fractions. The NDF levels ranged from 19 to 43% for alfalfa, and from 42 to 58% for brome. The rates of digestion by mixed ruminal microbes of the whole forage, and of the water-insoluble and -soluble fractions were measured in vitro using pressure sensors to measure gas production. NDF disappearance was measured at the end of the digestion run. Both forages showed the expected decline in fiber digestibility with increasing maturity. A dual-pool logistic model gave gas pool sizes, specific rates, and a single lag time for both the faster- and slower-digesting fractions. The main difference between alfalfa and brome was in the soluble pool. This pool produced approximately 40% of the total gas in alfalfa and 25% in brome. The specific digestion rates of the brome soluble pool were approximately 50% higher than those for alfalfa. Net VFA production showed a somewhat higher acetate:propionate ratio for brome (3.2) compared with alfalfa (2.2), but there was little change with increasing maturity within a given forage. Podium.

#8 MODEL OF HYDROGEN ION FLOW THROUGH THE RETICULORUMEN. M. S. Allen, Dept. of Animal Science, Michigan State University, East Lansing, MI 48824 (517-432-1386)

A model of hydrogen ion flow through the reticulorumen was developed which predicted the production of hydrogen ions from ruminally degraded OM and removal by absorption across the rumen wall, flow through the reticuloomasal orifice, and dehydration of carbonic acid. Hydrogen ion production is calculated by fermentation balance from available hexose equivalents after correcting for cell yield. The relative importance of ditferent routes of removal change as the amount of VFA produced and saliva buffer flow changes. However, the primary route of removal is by absorption of VFA, accounting for about one-half of the tolal hydrogen ion removal under most conditions. Over one-fourth is incorporaled into water by the dehydration of carbonic acid, and nearly ten percent flows from the reticulorumen as dihydrogen phosphate. A minor traction flows from the reticulorumen a VFA, ammonium ions, and associated with particulate matter and there is negligible flow of free hydrogen ion~. Our understanding of several factors atfecting ruminal pH is inadequate; among them are amount and composition of saliva flow, surface area for VFA absorption in the reticulorumen, and variation in microbial cell yield. Podium.

#9 POTASSIUM ACCUMULATION IN PERENNIAL GRASSES. J. H. Cherney, D. J. R. Cherney, D. R. Dewing and R. F. Lucey, Department of Soil, Crop, and Atmospheric Sciences and Department of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-0945)

Intensively-managed perennial grass can achieve a level of milk production similar to high quality alfalfa forage. While rumen bacteria require high concentrations of K for protein synthesis, excessive K in grass forage can lead to severe dietary disorders when fed to dry cows in late pregnancy. Our objectives were to assess the effects of grass species, variety, time of season and N fertilization on K concentration. Patterns of K uptake throughout the spring were monitored under 0, 56, and 112 kg N fertilizer/ha. Unlike forage N content, K concentration in grass did not decline during spring growth throughout May. Nitrogen fertilization of grasses increased forage K concentration one percentage unit at spring harvest, with a much smaller effect on regrowth forage. Of the five species examined, orchardgrass consistently had the highest K concentration (exceeding 4.5% at first harvest), with smooth bromegrass having the lowest K concentration (24% lower than orchardgrass) at similar maturity stages. No varietal differences within species in K content were found. Reed canarygrass, timothy and tall fescue were intermediate in K concentration. Grass K concentration in dry cow forage can be controlled through grass species selection, N and K fertilization, and harvest management. Podium.

#10 THE USE OF NUMERICAL METHODS TO INTEGRATE A LOGISTIC MODEL OF SUBSTRATE DIGESTION WITH AN EXPONENTIAL MODEL OF PASSAGE. M.C. Barry, Department of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-3973).

Estimation of extent of digestion is critical in modeling the digestive process of ruminants. Several current models currently assume a bi-exponential decay of substrate due to passage and digestion with no lag. Published data suggest that this assumption is not appropriate, particularly in the case of substrates associated with the fibrous portion of feedstuffs. The bi-exponential model is convenient for practical application because passage and digestion can be integrated by relatively simple methods, and because only three parameters are needed to specify the model. A dual pool logistic model has been used to characterize gas production resulting from the digestion of feedstuffs. Fourth- order Runge-Kutta numerical integration was used to integrate the rates of passage and digestion over time, using exponential and logistic equations for respective processes This integration allows estimation of extent of ruminal digestion of substrate for various feedstuffs. For comparison, equivalent exponential rates that yield the same extent of digestion as the logistic model at given passage rates were estimated. The sensitivity to variations in passage rate in these exponential rates was found to be relatively low (+/- 0.01 /hfor passage rates 0.05 /h +/- 0.03 /h). This method enables us to combine the effects of multiple physical processes with differing kinetic behavior. Podium.

#11 STUDIES ON RUMEN MICROBIAL SYNTHESIS FROM DIFFERENT NITROGCN SOURCES USING IN VITRO AND IN VIVO METHODS. Jia-qi Wang, Animal Science Institute, Chinese Academy of Agricultural Sciences Beijing t00094, China

Traditional measurement of microbial activity and syntllesis using in vivo method is both time consuming and laborious. A rumen simulation technique (RST) was introduced in this paper to study the effect of different nitrogen sources on rumen microbial fermentation. The in vivo method was used at the same time for comparison. The RST experiment: the RST had six anaerobic fermentation vessels; buffer and strained rumen fluid were mixed with the proportion of 1:2 and was injected into each fermenters at 1.36ml/min by a peristaltic pump. The three diets with different nitrogen sources for RST were hay supplemented: I) soybean meal(H+SBM); 2) urea(H+U); 3) starch-urea complex (H+SUC). A factorial design was used. The measurement and calculation included degradabilities of organic matter(OM), neutral detergent fibre(NDF) and acid detergent fibre(ADF). the energy efficiency for microbial synthesis expressed as microbial nitrogen(MN) g/kg fermented OM(FOM) and the nitrogen efficiency for microbial synthesis expressed as MN g/g rumen degradable nitrogen(RDN) in the diets(Czerkawski, 1986). The in vivo experiment: three cattle with rumen and abomasum cannulae were used in a 3x3 Latin square design, the diet treatment and fermentation parameters measurcd were the same as in RST experiment. Cr_2O_3 was used as an indicator for determining the digestion of OM and nitrogen in the rumen. The results showed that different nitrogen sources had no signifcant effect on OM degradability for the three diets($p>.05$). Nitrogen degradability was significantly different($p<.05$). RDN utilizationfrom the three nitrogen sources was different($p<.05$). The RDN of SBM had the highest efficiency for rumen microbial synthesis compared with the other two nitrofen sources. Starch-urea complex was made from heating the starch-urea mixture at 130°C, both RST and in vivo results showed that this processing increased RDN utilization by rumen microbe($p<.05$). The three different nitrogen sources had no significant influence on every efficiency for rumen microbial synthesis($p>.05$). In conclusion. both RST and in vi~o experiments on microbial metabolism showed consistent results. Rumen microbial fermentation and synthesis from different nitrogen sources:

Methods	RST				IN VIVO			
Nitrogen sources	urea	SUC	SBM	SEM	urea	SUC	SBM	SEM
Degradability, %								
--OM	52.2	51.7	51.0	0.84	51.6	50.2	50.9	0.79
-- Nitrogen	60.8	53.7	47.4	3.67	59.9	53.6	47.7	2.61
Fermentation efficiency,								
--MN/FOM, g/kg	19.1	20.6	19.7	0.73	19.9	18.5	17.0	0.81
--MN/RDN, g/g	0.66	0.85	0.91	0.01	0.83	0.88	0.94	0.01

#12 ALTERING RUMINAL FERMENTATION AND INHIBITING RUMINAL SULFIDE PRODUCTION WITH 9,10 ANTHRAQUINONE (AQ). A. O. Hession*, L. Kung, Jr., and C. A. Bessett. Department of Animal and Food Sciences, University of Delaware, Newark, DE 19717. (302-831-2522)

Excess production of sulfide in the rumen may be detrimental to the host animal. We studied the effects of various sulfur compounds and 9,10 AQ on ruminal fermentation and sulfide production in in vitro ruminal fermentations. Treatments included a pelleted commercial diet for lambs (CTRL) with (+) and without (-) 10 ppm (of the fluid) AQ. One percent additional sulfur was also added to

the control diet from sodium thiosulfate (TS), elemental sulfur (ES), calcium sulfate (CS) or sodium sulfate (SS) and supplemented with (+) or without (-) 10 ppm AQ. Additional sulfur had minimal effects on VFA concentrations with the exception that the cultures treated with CS(-) were lower in acetate when compared to other treatments. Sulfide production was greater in the fermentation gas phase (.054 mM) than in the liquid (.011 mM). In general, added sulfur increased total sulfide production ($P < .05$) by more than 60% (CTRL sulfide = .055 mM; average sulfide in sulfur supplemented groups (-) = .090 mM). Addition of AQ decreased ($P < .05$) total VFA by approximately 9% regardless of supplemental sulfur. The majority of this decrease was due to a depression in acetate. However, AQ increased ($P < .05$) the concentrations of propionate (6%) and butyrate (21%) across treatments. AQ inhibited sulfide production from all added sulfur sources except from TS. Total sulfide concentrations (mM) are shown below. In summary, high levels of dietary sulfur increased ruminal sulfide production but addition of AQ caused a beneficial increase in propionate and butyrate while suppressing sulfide production. Podium.

Supplement	CTRL	TS	ES	CS	SS
AQ (-)	.055c	.102a	.081b	.088ab	.088ab
AQ (+)	.029e	.098ab	.047cd	.033de	.027e

Means with unlike superscript differ ($P < .05$). SE = .004.

#13 CRYOGENICALLY PROTECTED AND FRESH RUMEN INOCULUM FOR DIGESTIBILITY STUDY. O.A. Ayangbile[1], J.C. Meier[1], and M.K. Vogel[1]; J. Robertson[2]; A.R. McElroy[3]; A.R. Komarek[4] [1]Analab, Division of Agri-King Inc., Fulton, IL 61252 (815-589-2525); [2]Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-4478); [3]Plant Research Center, Agriculture and Agri- food Canada, Ottawa, Ontario K1A OC6 (613-759-1305); [4]ANKOM, Fairport, NY 14450 (716-425-3940)

A study was designed to investigate the viability of a cryogenically protected inoculum from a standardized artificial fermenter, to inoculum obtained from the rumen of cattle; and to compare the conventional in vitro tube technique to the ANKOM batch type (Daisy 11) method. Eight forage samples were obtained from outside sources. Each sample run in triplicate was placed in both conventional containers and F56 ANKOM filter bags. Two sources of inoculum were used in this study. Strained rumen fluid from an artificial fermenter was cryogenically preserved. The frozen inoculum was thawed rapidly at 39°C. Fresh inoculum was obtained from cattle and kept at 39°C. The fluid was strained through four layers of cheese cloth. Buffer solution was placed in each container containing the sample, and in the Daisy 113.8 L bottles with the bags. The content was allowed to equilibrate to 39°C, then was inoculated with respective amounts of either preserved or fresh rumen fluid. The content was incubated for 48h followed by NDF assay procedure to obtain the true dry matter disappearance. The true dry matter disappearance (IVTDMD) for cryogenically preserved inoculum was similar ($p > .2$) to the values from the fresh rumen fluid. There was no difference ($p > .3$) between Daisy 11 and conventional technique. However, the standard deviation was lower for samples with cryogenically preserved inoculum than those of fresh rumen fluid. This study indicates that IVTDMD obtained from standardized cryogenically preserved inoculum is more repeatable and accurate. Podium.

#14 GROWTH INHIBITION OF RUMINOCOCCUS FLAVEFACIENS BY RUMINOCOCCUS ALBUS. W. W. Chan and B. A. Dehority, Dept of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691-4096 (216-263-3908)

While trying to develop a most probable number (MPN) selective medium for enumeration of Ruminococcus albus 7 and R. flavefaciens B1a in coculture, it was observed that when the two cultures were mixed, growth of R. flavefaciens was inhibited. Subsequent studies indicated that R. albus 7 produced an inhibitory substance that was present in cell-free culture filtrates. Level of inhibition increased with quantity of R. albus filtrate and reached almost 100% at a ratio of 3 ml R. albus filtrate to 1 ml of R. flavefaciens inoculum R. albus 7 plus two additional strains of R. albus M02a and M03g were tested for production of inhibitory activity against R. flavefaciens B1a, B34b, R13e2 and C1a,F. succinogenes S-85, B. fibrosolvens H17c and P. ruminicola H2b R. flavefaciens B1a and C1a were found to be inhibited by all strains of R. albus and strain B34b was inhibited by R. albus M02a and M03g R. flavefaciens R13e2, F. succinogens, B. fibriosolvens, and P. ruminicola were not inhibited by any R. albus strains. No inhibition was found between the three strains of R. albus. The inhibitory substance(s) produced by R. albus 7 M02a and M03g was heat-labile and degraded by the proteolytic enzyme, protease. This would suggest that the inhibitory substance(s) is proteinaceous in nature and may be a bacteriocin. It is of interest that various strains within the same species respond quite differently when grown in coculture with other bacterial species. Podium.

#15 GLUCOSE AND HYDROGEN UTILIZATION BY A RUMINAL ACETOGEN. R. S. Pinder and J. A. Patterson. Dept of Animal Sciences, Purdue University, West Lafayette, IN 47907 (317)-494-4826.

Isolate A10, an acetogen isolated from rumen contents, was able to grow on either glucose or H_2:$C0_2$ (80:20). Growth on glucose occurred in 6h, whereas detectable growth on H_2:$C0_2$ occurred after approximately 18 h. Acetate, formate and H_2 were detected during growth on glucose, whereas only acetate was detected during growth on H_2:$C0_2$. Regardless of the atmosphere (N_2:$C0_2$ or H_2:$C0_2$), generation times during growth on glucose were $0.47h^{-1}$ while growth on H_2:$C0_2$ was slower ($0\ 12h^{-1}$). When grown under a H_2 atmosphere with glucose and $NaH^{13}CO_3$ as the sole organic and inorganic carbon sources, respectively, unlabeled acetate and formate were detected during the early stages of growth. After glucose was exhausted (but during formate consumption), $CH_3{}^{13}COOH$ was detected in the culture supernatant. Following formate depletion, $^{13}CH_3{}^{13}COOH$ was detected as well. These findings suggest that formate is utilized as a carbon source for the methyl group of acetate. Hydrogenase activity (37 U/mg protein) was lower in cells growing on glucose than in cells growing on H_2:$C0_2$ (26O U/mg protein). Intracellular[NAD+] was high during growth on glucose (14 μM/g bacterial DM) and low during growth on H_2:$C0_2$ (4 μM/g bacterial DM). Intracellular [NADH] was low during growth on glucose (4 μM/g bacterial DM) but higher (15 μM/g bacterial DM) during growth on H_2:$C0_2$. We conclude that isolate A10 is a true acetogen t is not capable of mixotrophic growth on glucose and H_2:$C0_2$. Podium

#16 FACTORS AFFECTING MALATE UTILIZATION BY SELENOMONAS RUMINANTIUM. J. D. Evans and S. A. Martin, Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706-542-0886)

Dicarboxylic acids have been shown to stimulate lactate uptake by Selenomonas ruminatium HD4, while the ruminal selenomonad strain H18 has a strict requirement for dicarboxylic acids when grown on lactate. The objective of this study was to examine factors affecting malate utilization by strains HD4 and H18. Both strains were grown in batch culture (550 ml) on DL-lactate (25 mM) or D-glucose (17 mM) with and without added L-malate (7 to 17 mM). Samples were taken from each fermentation vessel over time and growth was monitored by measuring optical density at 600 nm. Glucose in supernatant samples was quantitated by a coupled enzyme assay, while lactate, malate, and succinate were measured using HPLC. Acetate and propionate were determined by gas chromatography. When strain H18 was grown in medium that contained glucose and malate, lactate accumulated with some production of acetate and propionate. However, malate utilization did not occur until all of the glucose had been fermented. Incubation of both strains in lactate plus malate medium resulted in co-utilization of both carbon sources and succinate accumulated to a concentration of 8 mM during the first 24 hr. Once the succinate concentration reached 8 mM, lactate and malate utilization stopped and no more succinate was produced. When compared to cells grown only on lactate, strain HD4 produced less propionate in the presence of malate. These results suggest that succinate plays a role in regulating the fermentation of lactate and malate by both strains HD4 and H18. Podium

#17 COMPARISON BETWEEN MICROBIAL POPULATIONS IN THE RUMEN AND CONTINUOUS CULTURE FERMENTERS USING RIBOSOMAL RNA-TARGETED DNA PROBES. C. J. Ziemer[1], R. Sharp[2] M. D. Stern[1], M. A. Cotta[3], T. R. Whitehead[3] and D. A. Stahl[2]. [1]Department of Animal Science, University of Minnesota, St. Paul, MN 55108 (612) 624-6216), [2]Northwestern University, Evanston, IL and [3]ARS, USDA, Peoria.

Small subunit (SSU) rRNA was extracted from samples taken from the rumen (daily for 4 d) and directly from fermenters (daily, from 96 to 168 h of operation). Samples were hybridized to radiolabeled SSU rRNA-targeted DNA oligonucleotide probes which targeted: total SSU rRNA, Bacteria, Eucarya, Archaea, Fibrobacter genus, F. succinogenes, F. intestinalis, and 2 F. succinogenes subgroups. Data were analyzed using General Linear Models procedure of SAS. Single degree of freedom contrasts compared ruminal and fermenter samples. Expressing population data as a percentage of total SSU rRNA, ruminal samples had higher Eucarya (52.23% vs 1.83%), lower Bacteria (48.00% vs 84.47%) and Archaea (1.32% vs 2.11%) than fermenters. While there were no differences between ruminal and fermenter samples for Fibrobacter (2 . 60%), F. succinogenes was lower (.47% vs . 92%) and F. intestinalis was higher (.05% vs 01%) in ruminal samples compared with fermenter samples. F. succinogenes subgroup 1 was higher (.53% vs .18%) and subgroup 3 was lower (.81% vs 1.41%) in the rumen compared with fermenters. With the exception of protozoa, continuous culture fermenters supported a diversity of key ruminal populations comparable to that found in the rumen environment. Podium.

#18 THE USE OF 16S RIBOSOMAL RNA DIRECTED OLIGONUCLEOTIDES TO STUDY THE RUMEN MICROBIAL POPULATION. J.M.Wood, K.P.Scott, G. Avgustin and H.J. Flint, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB2 9SB, UK (0224 715251).

Conventional culture techniques for assessing the rumen microbial population are time consuming and provide little phylogenetic information. For this reason there has been increasing interest in the use of oligonucleotide probes correspondillg to regions of the rRNA genes. This allows the design of specific oligonucleotides for a particular species, genera or phyla. Here we report on the use of such oligonucleotides to investigate the rumen microbial population and in particular the prevalence of the rumen bacterium Prevotella ruminicola (Avgustin G. et al (1994) Int. J. Syst.Bacteriol. 44, 246-255). In this study two approaches are being investigated. The first involves the direct analysis of RNA extracted from rumen material whereas the second method involves extracting DNA which is then amplified using universal primers. Once the DNA and RNA have been transferred to a membrane, generally by slot blotting, they are hybridised with the ^{32}P labelled oligonucleotides. The bound probe is then detected either by autoradiogaphy or by using a Packard Instant Imager. After these methods have been optimised it is hoped they will allow quantitative analysis of P. ruminicola in various rumen samples. We are also sequencing part of the 16S rRNA gene from Ruminococcus flavefaciens and R. albus strains in the hope of designing oligonucleotides which will allow the study of these bacteria in the rumen environment. Podium.

#19 PHYLOGENETICALLY-BASED PROBES FOR THE STUDY OF RUMEN MICROBIAL POPULATIONS. R. Shalp[1], C.J. Ziemer[2], M.D. Stern[2], M.A. Cotta[3], T.R. Whitehead[3], and D.A. Stahl[1]. [1]Department of Civil Engineeling, Northwestern University, 2145 Sheridan Road, Evanston, Il 60208. (708) 4671074. [2]Dept.of Animal Science, University of Minnesota, 130 Haecker Hall, 1364 Eckles Avenue, St Paul. MN 551()8, [3]FBRU, USDA/ARS/ NCAUR, 1815 N.University Street, Peoria, IL 61604.

The microhial community structure of model rumen systems (Hoover/Stern) was evaluated using phylogenetically-based probes. The model systems were opelated on two separate occasions for 240 hours, and sampled periodically, for nucleic acid extraction and hybridization with radiolabelled oligonucleotide probes complementary to small subunit (SSU) ribosomal rRNA. Bacterial, Eucaryotic and Archaeal rRNA accounted for 32.6%,, +/- 6.3, 39.5%, +/- 6.3, 3.2% +/- 0.9 respectively of total in the inoculum, and 80.8% +/- 3.3%, 6.3 %, +/- 1.7 and 5.2% +/- 1.1 respectively after 240h of fermenter operation. Within the Archaea, Methanobacteriales accounted for the greatest proportion of the total, 43.5%, +/- 1.5 in lhe inoculum and 28.7% +/- 4.1 after 240 h of operation. We hypothesize that this reduction in Methanobacteriales is a consequence of the loss of protozoa from the systems, as indicated by the reduction in Eucaryotic signal. Additional probes were developed for R. albus, R. flavefaciens and R. bromii, based upon new and existing gene sequences. R. albus and R. flaveflaciens accounted for 1.5% +/- 0.2 and 1.9% +/- 0.2% respectively in the inoculum, and 1.2% +/- 0.3 and 1.9 +/- 0.4 after 168 h of fermenter operation. Cloning and sequencing SSU rRNA genes from total rumen DNA revealed novel Ruminococcus like isolates. These results clearly demonstrate that phylogenetically based probes offer a powerful means of studying both specific microbial populations and the overall communily structure of rumen microorganisms. The. The fermenters appeared to maintain the same bacterial diversity as the rumen derived inoculum. Podium.

#20 THE USE OF RIBOSOMAL RNA PROBES TO ACCESS THE CONTRIBUTION OF OBLIGATE AMINO ACID FERMENTING BACTERIA TO RUMINAL DEAMINATION. D. O. Krause* and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508)

Some carbohydrate-fermenting ruminal bacterium can deaminate amino acids, but recently isolated monensin-sensitive, obligate amino acid-fermenting bacteria had 20 fold greater rates of deamination. Predominant ruminal bacteria (PRB) that were isolated from 108 dilution of ruminal fluid, could be maintained as a diverse population for several months, but did not utilize peptides as a sole source of energy, and had rRNA that did not cross-react with the three obligate amino acid-fermenting bacteria, (Peptostreptococcus anaerobius (C), Clostridium sticklandii (SR), and Clostridium aminophilum F). When F, C, and SR were co-cultured with PRB in continuous culture (3 g/liter mixed carbohydrates, 15 g/liter Trypticase, 0.075 h^{-1}), the steady state concentration of ammonia was more than twice as high than PRB alone. At lower concentrations of Trypticase, the difference in ammonia was less dramatic, and the rRNA probes indicated that F, C, and SR accounted for a smaller portion of the total population. When 5 μM monensin was added to the continuous cultures, ammonia production decreased and 16S rRNA analyses indicated that C and SR was washed out. F was more resistant to monensin, and its numbers increased when monensin was added to the continuous cultures. The continuous culture studies provided a realistic model of ruminal fermentation. When cows were fed monensin, ruminal ammonia accumulation decreased, the amount of rRNA that would hybridize with C and SR fell to undetectable levels, and F persisted. Podium.

#21 TRANSFORMATION OF THE RUMEN BACTERIUM BUTYRIVIBRIO FIBRISOLVENS WITH RECOMBINANT cDNAs ENCODING FIBER-DEGRADING ENZYMES. K.S. Gobius[1], C.S. McSweeney[2] and G.P. Xuel. ICSIRO DTCP, St. Lucia 4067, Australia (+61-7-3377-0261). 2CSIRO DTAP, Private Bag No 3 PO, Indooroopilly, QLD 4068, Australia.

Ruminant animals are unable to synthesize enzymes necessary for plant cell wall digestion but rely upon a symbiotic association with rumen microorganisms that partially degrade lignocellulosic fiber. Improved microbial fiber digestion holds the potential to increase productivity in the Australian beef and sheep industries. The introduction of novel genes for improved fiber-degradation to rumen bacteria has been impeded by the lack of suitable genetic transformation systems. Using the Staphylococcus aureus plasmid pUB110 and electroporation, we have genetically transformed several strains of B. fibrisolvens. Expression cassettes containing cellulase or xylanase cDNAs from the anaerobic rumen fungus Neocallimastix patriciarum were constructed and cloned into pUBl 10. Recombinant strains of B. fibrisolvens were isolated after transformation with pUB110-cellulase or -xylanase derivatives. Independent strains of B. fibrisolvens expressed and secreted the recombinant fungal fiber- degrading enzymes with varying efficiencies. Furthermore, two distinct B. fibrisolvens strains expressing the same xylanase cDNA increased plant fiber degradation with different efficiencies. The expression and secretion of recombinant fiber-degrading enzymes in the rumen bacterium B. fibrisolvens may require optimization with strain-specific regulatory elements. Genetic transformation and the expression of recombinant fiber-degrading enzymes in B. fibrisolvens strains will allow testing of the hypothesis that modified rumen bacteria may contribute to the improvement of fiber utilization in livestock. Podium.

#22 CHAPERONINS FROM RUMINOCOCCUS FLAVEFACIENS FD-1: CLONING OF dnaK ANI groEL BY A POLYMERASE CHAIN REACTION (PCR) BASED APPROACH. Bryan A. White[1], Isaac K. O. Cann[1], W. Michael Russell[1], and Philip E. Vercoe[2]. Department of Animal Sciences, University of Illinois at Urbana-Champaign[1], and Department of Animal Science, University of Western Australia, Nedlands, 6009, Western Australia[2]. (217-333-2091)

We report on experiments directed towards identifying molecular chaperonins in Ruminococcus flavefaciens FD-1. Chaperonins interact with other proteins to mediate ATP-dependent protein folding, refolding, assembly, and disassembly of proteins. We believe it is reasonable to expect that these chaperonin proteins are present in Ruminococcus and may be involved in both the phosphorylation transducing pathway for signaling of cellulose-dependent gene expression, as well as in assembly of the cellulase complex. Degenerate oligonucleotide primers designed on the basis of amino acid sequences conserved in these chaperonins were used to PCR amplify internal regions of dnaK and groEL. A 533 bp fragment of DNA containing a portion of the dnaK gene of R. flavefaciens FD-1 was cloned and the DNA sequence determined. The deduced amino acid sequence of this portion of the dnaK gene showed the highest homology with the amino acid sequence from positions 150 to 299 of the Lactococcus lactis DnaK protein (84%) and the Clostridium acetobutylicum DnaK protein (83%). A 1,406 bp fragment of DNA containing a portion of the groEL gene of R. flavefaciens FD-1 was cloned and the DNA sequence determined. The deduced amino acid sequence of this portion of the groEL gene showed the highest homology with the amino acid sequence from positions 31 to 494 to the C. acetobutylicum GroEL protein (72%). and the Bacillus stearothermophilus GroEL protein (71%). These cloned PCR fragments are being used to probe genomic libraries of R. flavefaciens FD-1 in order to clone the entire dnaK and groEL genes. Podium

#23 CONJUGAL TRANSFER OF TRANSPOSON Tn1545 INTO EUBACTERIUM CELLULOSOLVENS 5494. K.L. Anderson, J.A. Megehee, and V.H. Varel. Dept. Biol. Sci., Mississippi State University, Miss. St., MS 39762 (601-325-8825), and USDA/ARS, Clay Center, NE 68933 (402-762-4207).

Eubacterium cellulosolvens is a gram-positive, ruminal bacterium that actively degrades crystalline cellulose. However, elucidation of the molecular mechanisms underlying its cellulolytic activity has been severely limited by the lack of systems for genetic manipulation. The transposon Tnl 545 is one of several self- transmissible conjugative elements that are ubiquitous in the streptococci. This 25.3 kbp transposon carries resistance genes to tetracycline (tetM), erythromycin (ermAM), and kanamycin (aphA-3). Under anaerobic conditions, conjugal transfer of TnlS45 from Clostridium beijerinckii AA202 to E. cellulosolvens 5494 was found to occur at frequencies greater than 10^{-8}. Southern blot hybridization confirmed the presence of at least one copy of TnlS45 per transconjugate; resulting in conferred resistance to both tetracycline (20 ,µg/ml) and erythromycin (10 µg/ml). This demonstrates that E. cellulosolvens forms conjugal mating pairs with Clostridium, and readily expresses both tetM and ermAM genes. These results mark the first step in the genetic manipulation of E. cellulosolvens. Podium.

#24 LAG AND MAXIMUM GROWTH RATE STUDIES FOR RUMINOCOCCUS ALBUS ON FIBER SUBSTRATES. Peter Schofield* and Alice N. Pell, Dept. Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876).

The rate of fiber digestion in the rumen depends on microbial population size and composition, on the cellulolytic characteristics of this population, on fiber structure, and on the rumen environment, particularly the pH. We investigated the role of fiber structure in this process by measuring digestion rates and lag times for growth of Ruminococcus albus 8 (Ra8) on fiber substrates in a rumen fluid medium. Substrates studied included amorphous cellulose (prepared by regeneration from a -30 degrees Celsius solution in HCl), crystalline alpha cellulose, and NDF preparations from alfalfa and brome. Digestion was followed in batch culture using computer linked pressure sensors to monitor gas. The inoculum was a culture of Ra8 grown to substrate exhaustion on amorphous cellulose or grown to the beginning of stationary phase on cellobiose. Lag times depended on the inoculum size, and approached zero as this increased. Cellobiose grown cells behaved similarly to cellulose grown cells in this respect. The highest maximum rates of fiber digestion exceeded 30%/h (for amorphous cellulose) and varied widely with fiber source. Attempts to improve the efficiency of fiber digestion in ruminants by genetic manipulation of the cellulolytic apparatus in rumen microorganisms may be frustrated by this controlling role of fiber structure. Given the appropriate structure, or lack of it, the existing enzymatic apparatus of these microorganisms seems adequate to support high digestion rates. Podium.

#25 EFFECTS OF SACCHAROMYCES CEREVISIAE CELLS ON RUMINAL MICROBIAL FUNCTIONS AS ASSESSED BY IN VITRO MEASUREMENTS. F. Chaucheyras [1,2], G. Fonty [1], G. Bertin [2], Ph.Gouet [1], [1]Laboratoire de Microbiologie, INRA, 63122 Saint-Genes Champanelle, France (Phone 19 33 73 62 40 00); [2]Santel-goupe Agritek, 85 rue Anatole France, 92300 Levallois-Perlet, France (Phone 19 33 1 41 34 00 90)

The effects of two strains of Saccharomyces cerevi.siae, used as a microbial additive for ruminants, were investigated in vitro on the activity of anaerobic rumen microorganisms. The addition of live S. cereuisiae to a Neocallinnastix frontalis MCH3 culture stimulated zoospore germination, cellulose degraclation in a vitamin-deficient medium, and the rate of cellulolysis in a non deficient medium. This effect was due to the release of thiamine and the consumption of traces of oxygen by S. cerevisiae. Moreover, yeasts stimulated lactate utilization by Megasphaera elsdenii. The S. cerevisiae action seemed to be due to a supply of amino acids and vitamins. The lactate production from glucose by Streptococcus bovis was reduced in presence of live S. cerevisiae. Yeasts also influenced the results of the interaction S. bovis-M. elsdenii with glucose, maltose or starch as energy sources. S. cerevisiae, added in a culture of an acetogenic strain Ser 8 grown under H_2-$C0_2$, increased H_2 utilization and acetate production by the ruminal bacterial strain. Live yeast cells shifted the bacterial activity towards H_2-utilization, with glucose ancl H_2-$C0_2$ as energy sources. The improvement of H_2 consumption and acetogenesis by Ser 8 was also observed in a coculture associating Ser 8 and a strain of methanogenic archaea. Thus S. cerevisiae which has abilities to stimulate ruminal microbial species, can be used as ruminant feed additives to optimize ruminal fermentations. Podium.

#26 MUTAGENESIS OF PREVOTELLA RUMINICOLA B14 AND SELECTION OF MUTANTS DEFECTIVE IN PEPTIDE UTILIZATION. L. Peng and M. Morrison*, Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908 (402-472-6405)

In order to study the genetics and molecular biology of peptide utilization in Prevotella ruminicola B14, we developed a method using ethane methane sultonate (EMS) treatment plus ampicillin enrichment, for isolating mutants that grow poorly with peptides as the sole nitrogen source. Cells were exposed to EMS for 0, 5, 15, 30 and 45 minutes, washed, then allowed to recover in defined medium overnight. The mutation rate of Prevotella ruminicola B14 (as measured by the number of rifampicin resistant mutants) reached 1.57xl0-5 after EMS treatment for 45 minutes. Mutagenized cells were then added to a minimal medium containing trypticase as sole nitrogen source (having been treated to get rid ot most free ammonia) with ampicillin added. Because ampicillin inhibits actively growing cells of P. ruminicola, mutants with either limited or no ability to grow using peptides should be enriched for in the mutagenized cultures. Using this protocol, we were able to select five independent mutants on the basis of slow growth on defined agar medium containing peptides. The doubling times of the five mutants with 0.5% (w/v) trypticase is longer than that observed for the wild type parent strain, but similar for all strains when 10 mM ammonium chloride was used as sole nitrogen source. The dipeptidyl peptidase type I and glutamate dehydrogenase activities of three of these mutants has been measured, and the specific activities are also similar to that measured in the wild type parent strain. Further studies are needed to identify the genetic and phenotypic traits of these mutants, and one logical study will be to investigate rates of peptide transport/uptake for the mutant and parent strains. Podium.

#27 MARKED ELEVATIONS OF RUMEN H_2S GAS ASSOCIATED WITH DIET-INDUCED POLIOENCEPHALOMALACIA (PEM). D.H. Gould, B.A. Cummings, D.W. Hamar, Department of Pathology, Colorado State University, Fort Collins, CO 80523 (970 491-6144)

Concentrations of both rumen H_2S gas and rumen fluid sulfide were determined in 3 pairs of 120-140 kg steers fed a concentrate diet, either with or without added 1.8% sodium sulfate, by sulfide detector tube or sulfide selective electrode, respectively. Two of the 3 steers fed the high sulfate diet developed PEM. One steer had episodic ataxia and a blunted or absent menace reaction. The other 2 were asymptomatic. At necropsy the brain lesions of the 2 affected steers were characteristic of PEM. Increased ruminal H2S gas concentrations occurred in all 3 steers consuming the diet with added sulfate. Increases were more than 40- 60X control values. The onset of clinical signs coincided with the onset of elevated H_2S concentrations. In contrast, increases in rumen fluid sulfide concentrations usually began several days later and rose to 4X control values by the time of necropsy. The steers fed an identical diet, but without added sulfate, exhibited no signs or lesions of PEM, nor elevations of sulfide in rumen gas or fluid. These data indicate that large increases in rumen gas cap H_2S concentration may be a consistent indicator of the risk of H_2S neurotoxicity in cattle (supported in part by the Colorado Agriculture Experiment Station).Podium.

#28 INTERACTIONS OF TANNINS AND RUMINAL BACTERIA. K. E. Nelson*, A. N. Pell, and B. I. Giner-Chavez Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607)-255-2876

The interactions between purified tannins from three plant sources (quebracho (Schinopsis balanasae spp.), desmodium (Desmodium ovalifolium), and myrtle (Mirtus communis)) and five strains of rumen bacteria (Streptococcus bovis, Ruminococcus albus, Fibrobacter succinogens, Butyrivibrio fibrisolvens, and a recently isolated tannin tolerant bacterium) were investigated. Bacterial affinity for each tannin was evaluated by adding known amounts of purified tannins to cultures for fixed time periods, followed by two treatments of centrifugation, washing and resuspension. Bound tannins then were measured on the lyophilized pellet. Tannin binding was rapid with minimal binding occurring more than 5 min after tannin exposure. For each bacterial species, a saturation point was reached after which no additional tannins were bound. There was variation among bacteria in the amount of tannins bound and in the amount of tannin required to inhibit growth. Myrtle and desmodium had the highest binding and most microbial inhibition The level of total phenolics was highest for myrtle, and lowest in quebracho. Desmodium had the lowest degree of polymerization and the highest concentration of condensed tannins as measured by the vanillin and butanol-HCl assays Tannin-microbe interactions are strongly affected by tannin chemistry including degree of polymerization, total phenolics and concentration of condensed and hydrolyzable tannins. Podium.

#29 ISOLATION AND PARTIAL PURIFICATION OF THE PROTEOLYTIC ACTIVITY OF PREVOTELLA RUMINICOLA 118B. K. E. Griswold and R. I. Mackie. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-8809).

The extracellular proteolytic activities of P. ruminicola B14, D31d, M384, 118B, and GA33 were examined using a combination of the gelatin co-polymerized SDS-PAGE and protease inhibitor techniques. Only P. ruminicola 118B retained the same high molecular weight proteolytic activity (two distinct clearing zones at 110 -120 kD) when grown with either peptides or clarified rumen fluid as the sole N source. This proteolytic activity was inhibited by PMSF which indicated the presence of a serine protease(s). Purification of this activity was attempted with affinity chromatography. First, P. ruminicola 118B was grown to log phase in a 1 L batch culture. The culture was harvested, and the supernatant was concentrated, ultracentrifuged, and reconcentrated to 200x the original volume. This sample was applied to an affinity chromatography column, which consisted of Bacitracin (Sigma) ligated to Sepharose 4B (Pharmacia) packed in a 10 cm x 0.7 cm glass column. Fractions were eluted with 0.1 M KP04, 1 M NaCl, 25% isopropanol, pH 6.0, and tested against the artificial substrates, LPNA,BAPNA, and NSAAPPPNA for aminopeptidase M, trypsin, and chymotrypsin activities, respectively. The three activities were eluted separately. Active fractions were pooled, concentrated and tested for activity using the gelatin co-polymerized SDS-PAGE technique. The chymotrypsin-like activity peak separated into two distinct bands (65 and 100 kD) while the trypsin-like and aminopeptidase M activities did not create clearing zones. Further purification and characterization of this chymotrypsin-like, serine protease activity is in progress. Podium

#30 FRUCTOSE 1,6-DIPHOSPHATE MAY SERVE AS AN ENERGY SIGNAL TO THE F_1F_0-ATPASE OF STREPTOCOCCUS BOVIS. D. R. Bond and J. B. Russell. Section of Microbiology, Cornell University and ARS/USDA, Ithaca, NY 14853 (607-255-4508)

Bacterial energy tranduction has generally been partitioned into growth and maintenance, but some organisms also "spill" energy in futile reactions. In the absence of a nitrogen source, Streptococcus bovis can dissipate ATP 10 times faster than its maintenance rate. Because this non-growth energy consumption was inhibited by the F1FO ATPase inhibitor, DCCD and enhanced by the protonophore, TCS, it appeared that the energy spilling of S. bovis was being caused by the F1FO ATPase and a cycle of protons though the cell membrane. When S. bovis was grown in glucose-limited continuous cultures, energy spilling was proportional to the glucose consumption rate but not intracellular ATP. Because energy spilling cells produced large amounts of lactic acid, and the lactate dehydrogenase of S. bovis is activated by fructose 1,6 diphosphate (FDP), it appeared that FDP might be a regulator of energy spilling. When FDP was added to permeablized S. bovis cells, the rate of ATP hydrolysis increased 2-fold. The ATPase activity of cell-free extracts was only mildly stimulated by FDP, but membrane fractions had a higher affinity for ATP when FDP was present. The role of FDP in energy transduction was supported by the observation that cells using ammonia as a nitrogen source had an inherently higher rate of lactate production and energy spilling than cells provided with amino nitrogen.

POSTERS

#31 THE USE OF INTRACELLULAR POTASSIUM TO ASSESS THE ADAPTATION OF RUMINAL MICROORGANISMS TO MONENSIN. R.P. Lana and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508)

Monensin is often described as a sodium/proton antiporter, but this ionophore also has the ability to facilitate an electroneutral exchange of potassium and protons. When monensin sensitive ruminal bacteria are treated with monensin, there is an influx of sodium and protons and an efflux of potassium. When mixed ruminal bacteria from unadapted cows were treated with 6.4 μM monensin in vitro, intracellular potassium decreased, but approximately 45% of the potassium pool was monensin-resistant. As the monensin concentration was decreased, the amount of potassium lost declined, and the relationship between monensin concentration and potassium efflux could be described by a Michaelis-Menten function. The maximal efflux constant (Emax) for the unadapted cows was 55% and the half maximal efflux constant (K_e) was 0.3 μM. When cows were fed monensin, the K_e increased approximately 4 fold, but there was little change in E_{max}. Virtually all of this adaptation occurred within 4 days. Unadapted ruminal bacteria were more sensitive to lasalocid (K_e of 0.12 μM), but the E_{max} was only slightly higher (62%). Ruminal bacteria from fed-monensin cows adapted more slowly and never as completely to lasalocid, and the K_e was never greater than 0.3 μM. Based on these results: 1) a large pool of ruminal bacteria are naturally monensin resistant, 2) the adaptation to monensin in vivo occurs very quickly, and 3) lasalocid is a much more potent ionophore than monensin. Poster.

#32 DETECTION OF ESCHERHCHIA COLI 0157:H7 IN FEEDLOT CATTLE RECEIVING IONOPHORE TREATMENTS. B.N. Shipp*, M. L. Gaylean, and T. May, Dept. of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003. (505-646-2016)

Overgrowth of Escherichia coli 0157:H7 in cattle may be caused by stress. The role dietary ionophores play is not understood. One hundred twenty steers (341.7 kg; SE=6.9) were assigned to one of four treatments: Rumensin, Bovatec, Catalyst, or control. Fecal grab samples were taken from 2 steers in each pen (n=10 per treatment). Feces were screened for E. coli 0157:H7 by enrichment culturing. Samples were suspended and incubated in a modified trypticase soy broth (mTSB) medium containing bile salts, vancomycin, and cefixime. Following 24 hours of incubation, enriched cultures were diluted and plated on sorbitol MacConkey agar containing potassium tellurite and cefixime (TS-SMAC). Non-fermenting colonies from TS-SMAC were streaked onto lactose MacConkey agar. All lactose negative cultures were identified presumptively as E. coli 0157:H7. These colonies were submitted to a latex agglutination test to verify which colony belonged in serogroup 0157. Interestingly, seven fecal samples continually sustained positive signs of E. coli 0157:H7. Once a latex agglutination test was implemented all seven were negative, thus indicating no E. coli 0157:H7 was present in this population of cattle. Further sampling times are being tested throughout the finishing phase. Dietary and (or) management practices may be important for controlling overgrowth of this organism. Poster.

#33 A NON-INDUCIBLE ENDOGLUCANASE FROM FIBROBACTER SUCCINOGENES WITH A BASIC TERMINAL DOMAIN. A.H. Iyo and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Ontario, NlG 2Wl,Canada. (519)824-4120 x3433.

The celG gene from Fibrobacter succinogenes S85 has an open reading frame of 1557 bps, encoding a protein of approximately 55 kDa. It consists of an N-terminal catalytic domain separated from a largely hydrophilic C-terminal stretch by a tetrapeptide repeat of serine residues. A 45 kDa proteolytic degradation product which precludes this domain is as active indicating that this domain is not required for catalytic activity. The enzyme has a molecular weight of 54.9 kDa and a pH and temperature optima 5.5 and 25°C, respectively. It had a specific activity of 16.5 μmol/min/mg on barley β-glucan. CelG produces cellotriose as a major product from the hydrolysis of acid swollen cellulose and cellooligosaccharides. Antiserum raised against the purified form of CelG from E. coli failed to react with proteins from the native organism when grown on a variety of cellulose substrates. This may indicate that the enzyme in the native host is either non- inducible or that it's induction may involve a novel type of regulation. Poster.

#34 BREAKDOWN OF RUMEN BACTERIA BY PEPTIDOGLYCAN HYDROLASES AND BY RUMEN CILIATE PROTOZOA. H.C. Martin, C.J. Newbold, F.M. McIntosh and R.J. Wallace. Rowett Research Institute, Aberdeen, AB2 9SB,UK (+44 1224 712751)

The turnover of bacterial protein by protozoa in whole rumen fluid ranged from 11% h^{-1} with Butyrivibrio fibrisolvens to 3.5 % h^{-1} with Streptococcus bovis. It has been suggested that the breakdown of peptidoglycan limits the degradation of bacteria by protozoa. When a range of bacteria were incubated in solutions of lysozyme, mutanolysin or N-acetylglucosaminidase, B. fibrisolvens was degraded at 10, 18.7 and 0.98% h^{-1} respectively and S. bovis at 5.5, 0.03 and 0.93% h^{-1}. There was a poor correlation between the breakdown of bacteria in rumen fluid and their sensitivity to N-acetylglucosaminidase (R = 0.09), but a better correlation was observed with the two N-acetylmuramidases (R^2 = 0.37 for lysozyme and 0.63 for mutanolysin). When added to whole rumen fluid, Selenomonas ruminantium was degraded at 5.8 % h^{-1}. Breakdown was inhibited by 30% in the presence of chitotriose (an inhibitor of N- acetylmuramidase) but not by N-acetylmuramic acid (an inhibitor of N-acelylmuramyl-L-alanine amidase) or 2-acetamido-2-dcoxygluconolactone (an inhibitor of N-acetylglucosaminidase). Attempts were made to visualize the cell wall degrading activities in an extract prepared from the rumen protozoan Entodinium caudatum by SDS-PAGE with gels contailling 0.4% Micrococcus luteus. Whcn renatured in the presence of Triton X-100, bands of activity could be seen at 14kDa, 17.5kDa and 25kda. However, similar bands were also obtained from protozoa-free rumen fluid, indicating that there were similar activities of bacterial origin. It seems likely that thc major bacteriolytic enzyme of the rumen protozoa is of the N-acetylmuramidase group, similar in action to lysozymc. Poster.

#35 EFFECT OF CALCIUM AND SODIUM HYDROXIDE COMBINATIONS ON WHEAT STRAW DIGESTIBILITY AND RUMEN FUNCTION. S. G. Haddad* and R.J. Grant, Department of Animal Science, University of Nebraska, Lincoln 68583-0908 (402-472-6442).

Sodium hydroxide is commonly used to improve the ruminal digestibility of low quality forages. Calcium hydroxide could be used as an alternative, but is less effective. The objectives of these studies were to determine an optimum sodium and calcium hydroxide combination in vitro at pH 6.8 or 5.5, and to measure the effect of this treatment on kinetics of wheat straw fiber measured in vitro and rumen function in lactating dairy cows. In Experiment 1, wheat straw was treated with 0, 1, 2, 3, 4, or 5% NaOH, $Ca(OH)_2$, or both in a 6 x 6 factorial arrangement. In Experiment 2, four ruminally fistulated cows in midlactation were assigned to diets that contained 40, 30, 20 and 0% of 3% Na(OH) + 3% $Ca(OH)_2$-treated wheat straw. All diets contained 40% concentrate mix, with the remainder alfalfa silage. In Experiment 1, pH increased the lag and decreased the extent of digestion, with little effect on rate. Treatments that contained $Ca(OH)_2$ seemed to be affected less severely at low pH. Electron microscopy was used to visually evaluate the effect of $Ca(OH)_2$ and pH on microbial attachment to forage particles. In Experiment 2, alkali-treated wheat straw did not affect ruminal pH (6.4), osmolarity (322 mOsmol/kg), or in situ NDF digestive kinetics (lag, 5h; rate, 4.8%/h; extent, 86.1%). The inclusion of 20% (DM basis) alkali-treated wheat straw did not affect milk production or milk composition. A combination of 3% NaOH + 3% $Ca(OH)_2$ resulted in relatively high ruminal NDF digestibility, even at low pH, and normal ruminal function in dairy cows. Poster.

#36 GENERIC DISTRIBUTION OF RUMEN CILIATE PROTOZOA IN GRAZING RUMINANTS IN THE R. ARGENTINA. C.Arakaki, F.Rigalt[1], C.Peruchena[2], G.Berra, J.B.Viera and G.Rebuffi[3]. Instituto Patobiologia,CICV-INTA, CC77, (1708) Moron,Argentina [1] EEA-INTA, Catamarca. [2] EEA-INTA, Reconquista, 3EEA-INTA Abra- Pamapa.

The study was made using animals kept in their native environment in different geographical regions and climate conditions, i.e: sheep and goat (semi-arid regions) , cattle (temperate vs. sub-tropical regions) and llama (Andes mountain vs. temperate regions). Between the domestic ruminants protozoa concentration ranged from 6 to 20 xlO(4)/ml. Entodinium was the dominant protozoa, the concentration ranged from 69% to 88.5%. Holotrichs represented the second most abundant population. A wide distribution of large protozoa was detected. Epidinium sp. a high cellulolytic protozoa was observed in cattle, sheep and goat. Elytroplastron, Enoploplastron and Ophryoscolex were detected in sheep and goat but not in cattle. Llama from Andes mountain showed a mono-fauna type composition, (only Entodinium). Within the llamas from temperate regions the number of protozoa was higher (l xlO(5)/ml) than those observed in the mountain area (2 xlO (4)/ml) and only three kind of protozoa could be observed: Entodinium, Diplodinium and Eudiplodinium. The poor colonization of protozoa in the llamas forestomach might indicate possible effects of the host physiological factors and kind of diet in the establishment of protozoa. The wide distribution of high cellulolytic protozoa among the grazing ruminants, may indicate a positive role of protozoa on the digestion of fastidious plant material . Poster

#37 THE EXPRESSION OF A FIBROBACTER SUCCINOGENES S85 XYLANASE GENE IN MAMMALIAN CELLS. J.X. Zhang, P.J. Krell, J.P. Phillips, and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Guelph, Ontario, Canada NlG 2Wl (519) 824-4120 x4478

The generation of transgenic domestic animals able to secrete their own fibre digesting enzymes could provide a substantial benefit to the animal industry. With this objective in mind, we have been studying the expression of the truncated XynC domain B xylanase gene in Chinese hamster ovary (CHO) cells and mouse pancreas cells (266-6). The fusion gene consisting of the mouse Amy-2.2 signal peptide sequence and XynC-B, transcribed from the SV40 early enhancer/promoter, was stably transfected into CHO cells. The level of expression was up to 1.2U/mg cell protein in a 72h culture after amplifying by methotrexate, and more than 90% of the enzyme was secreted into the medium. Tissue-specific expression of this truncated enzyme was also achieved by stably transfecting the fusion gene, transcribed from Amy-2.2 enhancer/promoter, into pancreas cells 266-6. Our current studies are aimed at enchanting expression of the gene. Poster.

#38 STIMULATORY ACTIVITIES FROM LOW-MOLECULAR WEIGHT FRACTIONS DERIVED FROM SACCHAROMYCES CEREVISIAE STRAIN 1026.1. D. Girard and K. A. Dawson, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606 257-7552)

A series of studies examined the stimulatory activities of fractions derived from cultures of S. cerevisiae strain 1026 on the growth of representative strains of ruminal bacteria in batch cultures. The addition of small amounts of a yeast cell culture (providing 2.8 x 104 yeast cells/ml) grown on Tryptic Soy Broth (TSB) decreased the time required to initiate growth of Prevotella ruminicola strain GA-33, Butyrivibrio fibrisolvens strain D-1, Ruminicoccus albus strain 7, R. flavefaciens strain FD-1 and Megasphaera elsdenii strain T-81 by 20 to 40%. Cell yield and growth rate were increased by 160 and 77%, respectively, when cultures of Fibrobacter succinogenes strain S-85 were treated with the yeast supplement. Similar stimulatory activities were not associated with commercial yeast extract. Two low-molecular weight fractions (less than 10,000 Da) containing heat-stable (A) and heat labile (B) stimulatory components were derived from the supernatant of the yeast preparation and the cytosol of yeast cells, respectively. The addition of fractions A or B (same relative concentration as 2.8 x 105 yeast cells/ml) decreased the lag time of P. ruminicola, B. fibnsolvens, R. albus, R. flavefaciens and M. elsdenii by 35 to 50% and by 15 to 50%, respectively. Growth of B. fibrisolvens was not stimulated by fraction B. Growth characteristics of F. succinogenes were not affected by either fraction. These data suggest that yeast cells grown on TSB may stimulate the growth of some ruminal bacteria by providing heat-stable and heat-labile low-molecular weight components. Poster.

#39 NEW FAMILY, GENUS AND FIVE NEW SPECIES OF ENTODINIOMORPH PROTOZOA FROM THE FORESTOMACH OF MARSUPIALS. B. A. Dehority, Dept. of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691- 4096 (216-263-3908).

A unique group of entodiniomorph protozoa was found in forestomach contents from the quokka (Setonlix brachyurus), western grey kangaroo (Macropus fuliginous), red kangaroo (M. rufus) and euro (M. robustus erubescens). A new genus, Macropodinium n.g., containing five new species, is described. Three new species were present in forestomach contents of the quokka: M. baldi n. sp., M. moiri n. sp. and M. setonixum n. sp. A single species, M. ennuin sp., was found in the red kangaroo and euro. The last species, M. yalanbee n. sp.,occurred in forestomach contents from the western grey kangaroo. These five new species of ciliate protozoa, all classified into the genus Macropodium n.g., differ markedly from all other families of Entodiniomorphida. The features which set this genus apart are the two lateral convex ribbed channels, one along the right and one along the left sides of the cell, which extend from the vestibular opening to the cytoproct and divide the cell into upper and lower halves, a narrow band of somatic cilia which completely encircles the lower half of the cell longitudinally at a level just below the ribbed channels, and a diamond-shaped pattern formed by narrow grooves on the pellicle. Based on these marked structural differences with the eight established families of Entodiniomorphida, creation of a new family, Marsupialidae, is proposed. Poster.

#40 THE USE OF 16S-rRNA TARGETED PROBES TO INVESTIGATE THE INTERACTIONS BETWEEN Fibrobacter succinogenes S85, Methanobrevibacter ruminantium, Ruminococcus albus 8, AND Ruminococcus flavefaciens FD-1. J. L. Rychlik* and T. May. Molecular Biology Program and Animal & Range Sciences Department, New Mexico State University, Las Cruces, New Mexico 80003 (505)-646- 20 1 6.

Information concerning the interactions between different species of ruminal bacterial is necessary for further understanding of the activities in the ruminal environment itself, feed degradation, and the energy supplied to the animal. Methanogens are important in promoting the growth of other bacteria by converting excess H_2 to CH_4 with the aid of $C0_2$. Specific oligonucleotide probes targeted to sites on the 16S rRNA molecule of F. succinogenes S85, M. ruminantium, R. albus 8, and R. flavefaciens FD-1, and a EUB 338 probe (complimentary to sequences within Eubacterial species) will be used to investigate bacterial interactions during the fermentation on soluble and insoluble substrates in defined mixed cultures. To date, tricultures of F. succinogenes S85, R. albus 8, and R. flavefaciens FD- 1, were grown on cellobiose and samples were taken each hour over a 24 hour period. Phenol extraction was used to isolate RNA, samples were immobilized on a nylon membrane using a slot blot, and hybridized with a 32P-labeled EUB 338 probe. Densitometry will be utilized to detect differences in probe intensity, which can be interpreted as variations in species and population abundance. By introducing a methanogenic strain, which is an important microbial component of the rumen, we hope to gain a better understanding of the ruminal microbial populations and its functions. Poster.

#41 A COMPARISON OF IN SITU DRY MATTER DISAPPEARANCE AND IN VITRO GAS PRODUCTION FROM MICRONIZED AND UNTREATED FULL FAT CANOLA. D. R. ZoBell1*, T. A. McAllister[2], H. D. Bae[2], Z. Mir[2], K.-J. Cheng[2] and T. Entz[2]. [1]Alberta Agriculture, Food and Rural Development, Lethbridge, AB TlJ 4C7; [2]Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B 1. (403-381-5237)

In situ dry matter disappearance (ISDMD) and gas production from in vitro digestion were determined and compared for untreated (C, control) and micronized (M) full-fat canola. The extent of ISDMD was determined from 3 g samples of whole canola seeds incubated in quadruplicate for 0, 2, 4, 8, 12, 24 48, 72 and 96 h in the rumen of a Holstein cow being fed a 50:50 alfalfa hay:barley concentrate diet. Additional bags were incubated concurrently to provide samples for examination by scanning electron microscopy (SEM). In vitro gas production was determined using the Menke's gas apparatus. The Menke's syringes contained 10 mL ruminal fluid, 20 mL of 0.1 mM phosphate buffer (pH 7.0) and 250 mg of canola. Gas production was measured (quadruplicate values) at various time points throughout a 72 h incubation at 38"C. Data from both experiments were analyzed by analysis of covariance. Initial rupture of the seed coat of the micronized canola was clearly evident by SEM examination. Extensive microbial colonization of interior structures of the seeds had occurred within 24 h. The ISDMD values of C and M were similar ($P > 0.05$) for the first 24 h of incubation, but by 72 h, the ISDMD of M, (60.1%) was significantly higher than that of M (23.5%). The rate and extent of gas production differed between treatments. Total gas production at 72 h was lower ($P < 0.05$) from C than from M (0.061 mL g^{-1} and 0.133 mL g^{-1} DM canola, respectively). At all time points, the rate of gas production from C was lower ($P < 0.05$) than that from M. Micronization was shown to be effective in increasing the ISDMD and in vitro digestibility of whole canola, however, this digestibility may still be too low for effective utilization of whole canola seed by ruminants. Poster.

#42 SOME PROPERTIES OF A NOVEL 180 kDa CELLULOSE BINDING PROTEIN FROM FIBROBACTER SUCCINOGENES. J. Gong, E.E. Egbosimba, C.W. Forsberg, Dept. of Microbiology, University of Guelph, Ontario, N1G 2W1, Canada. (519) 824-4120 x3433.

Fibrobacter succinogenes possesses at least four cellulose binding proteins (CBPs) in the outer membrane. These include proteins of 260, 210, 180, and 120 kDa. The 120 kDa protein was identified as endoglucanase 2 and was mainly in the periplasmic fraction. The CBPs were released from cellulose by washing with water, ethylene glycol or cellobiose. Antibodies raised against the 180 kDa CBP cross-reacted strongly with numerous cell envelope proteins. Immunogold labelling of whole cells using antibodies against the 180 kDa CBP showed that either it or immunologically related protein(s) were uniformly distributed on the cell surface, but congregated at the points of contact of cells with cellulose during hydrolysis. Antibodies to the 180 kDa CBP caused 62% inhibition of binding of F. succinogenes S85 to crystalline cellulose whereas those directed against whole cells caused less than 40% inhibition of binding. These data suggest that the 180 kDa cellulose-binding protein and/or the additional cross-reactive surface proteins may have a role in adherence. Poster.

#43 FORAGE INTAKE OF BEEF COWS GRAZING SMOOTH BROMEGRASS-ORCHARDGRASS PASTURES WITH OR WITHOUT BIRDSFOOT TREFOIL BY DIFFERENT STOCKING SYSTEMS. J. R. Russell, Dept. of Animal Science, Iowa State University, Ames, IA 50011 (515-294-4631)

Eight 4.04 ha pastures containing smooth bromegrass (SB) and orchardgrass (O) with or without birdsfoot trefoil (BfT) were grazed by continuous or rotational stocking at 1.48, 1.98 (year 1); 1.24, 1.73 (year 2); 1.24, 1.73 (year 3); and 1.23, 1.23 (year 4) cow-calf pairs/ha in 4 years. Hay was harvested from 25% of each pasture after 47 days of grazing in year 4. Forage intake (FI) was estimated after 58, 50, 45, and 57 days of grazing in years 1 through 4 from the passage kinetics of Cr-mordanted fiber (1.2% Cr) and in vitro digestible dry matter (IVDDM) concentration of forage selected during grazing by ruminally fistulated steers. Available pasture forage was measured for sward height, hand-clipped and analyzed for yield, botanical composition, and IVDDM. Mean FI (%BW) and selected forage IVDDM concentrations (%DM) of SB-O pastures with or without Bfr were 2.9, 55.8 and 2.8, 55.7 when continuously stocked and 2.8, 56.9 and 3.0, 56.8 when rotationally stocked. In quadratic regressions, FI (% BW) was correlated to sward height (r^2=.57), live forage allowance (r^2=.39), live forage yield (r^2=.30) and selected forage NDF (r^2=.22). In stepwise multiple regressions, sward height, total yield, live forage allowance and available forage IVDDM concentration were selected to predict FI (r^2=.66). Poster.

#44 CHARACTERIZATION AND ACCUMULATION OF GLYCOGEN IN THE RUMINAL BACTERIUM PREVOTELLA RUMINICOLA B14. J. Lou, K. A. Dawson and H. J. Strobel, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606-323-4762)

Prevotella ruminicola is one of the most common bacteria in the rumen and it utilizes a variety of carbohydrates. Polysaccharide accounted for 60% of the cell dry weight in maltose-grown cells and smaller amounts were also found in glucose-, cellobiose-, and xylose-grown cultures. This material was isolated using a KOH-ethanol extraction method and was found to be a glucose-polymer with little reducing capacity. Enzymatic characterization of the polymer indicated that the glucose units were linked via alpha 1,4 and 1,6 linkages. The molecular weight was greater than 2 million and the average linear chain length from each branch point was 8 glucose units. These results suggested that the polysaccharide accumulated by P. ruminicola was glycogen. In maltose-grown cultures, glycogen was synthesized during cell growth, but its rate of synthesis was greatly increased in late logarithmic growth and as the culture entered stationary phase. As much as 40% of maltose was converted into glycogen. The amount of glycogen decreased after cells reached stationary phase and there was a concomitant increase in acetate and succinate. Similar results were obtained using glucose-grown cells but the ratio of polysaccharide to cell protein was 3-fold lower than maltose-grown cultures. Since energy sources are often transiently available in the rumen, the ability to accumulate glycogen may be important for bacterial survival. Poster.

#45 EFFECT OF MULTIPURPOSE TREE (MPT) SUPPLEMENTS ON RUMINAL CILIATE PROTOZOA. A.A. Odenyo[1], P.O. Osuji[1] and R.I. Mackie[2] [1]International Livestock Research Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia (Tel. (251-1) 33 82 90); [2]Department of Animal Sciences, University of Illinois, Urbana 61801 (Tel. 217-244-2526)

Effect of 5 multipurpose trees (MPTs), Acacia angustissima 15132, Acacia cynophylla, Chamaecytisus palmensis, Leucaena pallida 14189 and Sesbania sesban 10865 used as supplements on ciliate protozoa was investigated in rumen cannulated Ethiopian highland sheep. MPTs contain secondary compounds which may affect protozoa. Both entodiniomorphs and holotrichs were counted. The S. sesban supplemented diet significantly ($p<0.04$) increased the numbers of ciliated protozoa. Maize stover alone and maize stover supplemented with C. palmensis or L. pallida, did not have any significant ($p > 0.05$) effect on the numbers of ciliate protozoa. Acacia cynophylla supplemented diet decreased the numbers of protozoa especially during the first 21 days (1.52 x 105 to 0.62 x 105 cells/mL rumen fluid). Differences in ciliate numbers among MPT supplements barely failed to reach significance ($p < 0.06$). Entodiniomorphs dominated (93.3%) the protozoa population in all diets, entodinia were the most predominant (80.7%). None of the MPTs tested eliminated protozoa. Also relationship between protozoal numbers and in sacco fiber degradation and neutral detergent fiber (NDF) digestibility was examined. Decreases in protozoal numbers did not result in increase in fiber degradation. There were no significant differences ($p>0.05$) in NDF digestibility among the diets except for the A. cynophylla supplemented diet which was significantly ($p<0.01$) lower than others. No significant relationship was observed between ruminal pH and protozoal numbers with all diets. Only A. cynophylla supplemented diets had a ruminal pH above 7.0. The animals on A. angustissima died after 21 days of the experiment. Poster.

#46 CELLULASE ACTIVITY AND POPULATION OF ANAEROBIC FUNGI IN RUMEN OF BUFFALOES FED THREE COMBINATIONS OF ROUGHAGE AND CONCENTRATE. A.K.Samanta, T.K.Walli, Sunita Grover and V.K.Batish, National Dairy Research Institute, Karnal132001, India.

Three adult fistulated male buffaloes were fed 3 types of dietary combination; Type I (roughage:concentrate:60:40, with wheat straw as the only roughage), type II (rough:concentrate: :60:40, with wheat straw and green maize fed in equal proportions) and type III (roughage:concentrate:: 70:30, with wheat straw and green maize in equal proportions). The results of the 5 trials showed that the average fungal population ranged from $0.50x10^2$ to $1.25x10^2$ in type I, from $0.63x10^2$ to $1.65x10^2$ in type II and from $0.55x10^2$ to $1.38x10^2$ in type III buffalo. Microscopic examination showed oval, rounded, elognated and globular sporangia. The CMCas activity (ug of glucose/mg of protein/minute) ranged from 0.200 to 0.337 in type I, from 0.506 to 0.722 in type II and from 0.463 to 0.586 in type III. The highest specific activity of the enzyme W2S seen on diet II i.e. 60:40 roughage:concentrate, with green and straw given in equal proportion. Poster

#47 FLOW RATE OF DIETARY AND MICROBIAL NAN AT ABOMASUM IN CROSSBRED CALVES FED GRADED LEVELS OF UNDERGRADED DIETARY PROTEIN. O.H.CHATURVEDI AND T.K.WALLI, DAIRY CATTLE NUTRITION DIVISION, NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL-132001

Four crossbred calves each fitted with a rumen as well as abomasal fistula were fed 4 types of concentrates, varying in CP content and RDP:UDP ratio viz. 23.37% and 79:21 for T_1, 20.96% and 68:32 for T_2, 18.37% and 59:41 for T_3 and 15.92% and 48:52 for T_4 group, alongwith some wheat straw and green maize, in a 4x4 latin sq switch over design. Group Tl received 20% higher CP, whereas group T_2, T_3 and T_4 received normal, 10% and 20% lower CP of the NRC (1989) recommendations. Chromium mordanted GN cake was used as digesta marker and RNA was used as the microbial marker. As the N intake decreased from 84.28+3.34 to 58.80+1.60 g/d and the UDP increased, the flow rate of NAN and alpha AAN (as g/d) at abomasum were similar on all 4 diets. Fractionation of total NAN showed a significant (P/0.05) increase in the dletary plus endogenous NAN flow (g/d) from 15.05 ± 0.41 g in T_1 to 40.69 ± 1.07g in T_4 and a corresponding decrease in the microbial NAN flow (P/0.01) from 51.85 ± 2.30g in T_1 to 25.63 ± 0.77g. As % N intake. the alpha AAN showed an increasing trend (P/0.01) from high CP low UDP diet (T_1) to low CP high UDP diet (T_4). Poster.

#48 EFFECT OF QUEBRACHO CONDENSED TANNINS ON FERMENTATION IN THE ARTIFICIAL RUMEN (RUSITEC). H. D. Bae[1]*, S. M. Moustafa[2], T. A. McAllister[1], L. J. Yanke[1], C. G. D'Silva[1] and K.-J. Cheng[1]. [1]Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1 (403-327-4561) and [2]El-Minia University, Minia, Egypt.

The effects of an extract of quebracho tannins (QTE) on the ruminal environment were evaluated in the artificial rumen (Rusitec). Inoculum for the Rusitec was obtained from two steers fed a diet of 50% wheat, 35% alfalfa and 15% barley straw. The Rusitec was fed wheat and alfalfa (5 g d^{-1} each). Five levels of QTE were administered in the artificial saliva (0, 1.25, 2.5, 5.0 and 10.0 g L^{-1}). Microbial yield and total bacterial numbers were not affected ($P > 0.05$) by QTE (at any level), but cellulolytic bacterial populations decreased as QTE increased ($P < 0.01$). Xylanase and endoglucanase activities in fermenter liquid were inhibited to a greater extent by QTE than was protease activity. With no QTE, extensive microbial colonization and digestion of wheat were observed by SEM. However, with 10 g L^{-1} QTE, colonization was distinctly restricted to the protein matrix of the wheat. Starch granules were not colonized. At O g L^{-1} QTE, the predominant colonizers of filter paper placed in the fermenters resembled Fibrobacter sp. and Ruminococcus sp. At 2.5 g L^{-1} QTE, colonization did not occur. At 1.25 g L^{-1}, colonization was observed, but morphological changes and distinctive digestive patterns had been induced. These results clearly demonstrate the particular sensitivity of cellulolytic bacterial populations to QTE in the Rusitec, and offer an explanation for the tannin-induced reductions in ruminal fibre digestion observed in vivo. Poster.

#49 Withdrawn

#50 CLONING AND SEQUENCING OF NADP-DEPENDENT GLUTAMATE DEHYDROGENASE GENE, FROM THE CELLULOLYTIC RUMINAL BACTERIUM Ruminococcus flavefaciens FD-1. I. M. Ioannides*, P. E. Vercoe, B. A. White and R. I. Mackie, Departments of Animal Sciences, University of Illinois, Urbana, IL 61801, University of Western Australia, Nedlands, WA 6009 (217-333-8809)

Mutant complementation in the plasmid-free host strain E. coli PA340 (gdh-1, Δ(gltB-F)500) was used to clone and express the NADP-dependent glutamate dehydrogenase (GdhA) from Ruminococcus flavefaciens FD- 1. The specific activity of two different isolated clones (pIMI4, pIMIg) was 0.494 and 0.409 μmoles/min/mg protein respectively. The specific activity of clone pIMI4 was 4.4 times higher than the specific activity of the positive control (a plasmid containing gdhA from Bacteroides theraiotaomicron) and 67 times higher than the activity of the negative control (E. coli PA340). No enzymatic activity was observed when NADPH was replaced with NADH. Both gdh clones (1.1 kb) were sequenced and each encode the identical nucleotide sequence. This sequence contains a large (978 bp) open reading frame (ORF) capable of coding for the first 326 amino acids of a protein (MW 37,279). Several novel features emerged from alignments of the nucleotide and translated amino acid sequences performed using a Blast Search. Low similarity (<40%) was found with other published hexameric GDH's from either Gram-positive or Gram-negative bacteria. Southern hybridization was used to confirm that the gene, designated gdhA, was from R. flavefaciens FD-1. Further examination of the cloned 1.1 kb fragment was carried out using PCR and in each case the same 1.1 kb amplification product was obtained. This further confirmed that the gdhA gene cloned originated from R. flavefaciens FD-1. These interesting findings will be followed up by biochemical analysis of the protein encoded by the gdhA gene. Poster

#51 COMPARISON OF CELLULOLYTIC AND XYLANOLYTIC ACTIVITIES OF ANAEROBIC RUMEN FUNGI. L. J. Yanke, L. B. Selinger, H. D. Bae and K.-J. Cheng. Lethbridge Research Centre, Agriculture and Agri-Food Canada, P. O. Box 3000, Lethbridge, Alberta, Canada TlJ 4B1 (403-327-4561)

To compare cellulolytic and xylanolytic activities among genera of anaerobic fungi, strains of Piromyces communis, Orpinomyces joyonii and Neocallimastix sp. were grown on glucose, xylan and the cellulosic- substrates, filter paper and Avicel (microcrystalline cellulose). All the fungi had low constitutive extracellular fibrolytic activities which could be enhanced by growth on xylan or the cellulosic substrates. However, P. communis strain 22 and Neocallimastix patriciarum strain 27 exhibited substantially greater levels of fibrolytic activity than O. joyonii strain 19-2 or Neocallimastix frontalis strain RE1. Zymogram analysis suggested both structural and growth substrate-regulated differences amongst the enzyme systems of the fungi. Numerous and varied enzyme bands were evidenced for all the fungi, with substantial substrate influences on their xylanase activities. Most commonly, the smaller molecular weight bands, found exclusively extracellularly, appeared under the greatest regulatory control. Piromyces communis and O. joyonii demonstrated similar regulatory control of their endoglucanase activity, while the two Neocallimastix sp. strains did not appear to exert such control. These results suggest that while the enzymatic activities are functionally similar, there is likely significant variability in the enzyme systems of the anaerobic fungi and in their response to growth substrate. Poster

#52 ENZYMATIC CHARACTERIZATION OF A POLYCENTRIC RUMEN ANAEROBIC PHYCOMYCETE ORPINOMYCES sp. Zakia Sghir and Gerard Prensier, Laboratoire de Microbiologie, CNRS unite n° 138, Universite Blaise Pascal, Clermont-Ferrand France.

Carbohydrate degrading enzymes from Orpinomyces sp., a polycentric rumen anaerobic phycomycete isolated from rumen of sheep (Breton et al., 1989) were examined in batch culture incubations. The Orpinomyces sp. was maintained in Orpin medium and cultivated in 2 liter stirred fermentors (Lowe and Orpin medium) (pH 6.8, temperature 39 °C, stirring 100rpm, and C02 gassing). Best growth, as determined by dry weight, was obtained using Orpin medium containing glucose and cellobiose added freshly every 24 hours. The distribution of cellulolytic and hemicellulolytic enzymes between cells and supernatant was studied. Most of the enzyme activity (>70%) was located in the cell pellet.The biochemical characteristics of four polymer degrading enzymes (Amylase, Xylanase, CMCase, Avicelase) and three glycosidases (Xylosidase, Glucosidase and Fucosidase) were determined using SDS PAGE and IEF electrophoresis. Poster.

#53 PURIFICATION AND PUTATIVE CHARACTERIZATION OF THE YELLOW FLUORESCENT COMPOUND PRODUCED BY RUMINOCOCCUS FLAVEFACIENS FD-l DURING GROWTH ON CELLULOSE. I. M. Ioannides*, M. D. Berber-Jimenez, B. A. White and R. I. Mackie, Departments of Animal Sciences and Food Science, University of Illinois, Urbana, IL 61801(217-333-8809).

Ruminococcus flavefaciens and Ruminococcus albus are recognised as some of the most active bacteria involved in plant cell wall digestion in the rumen. R. flavefaciens FD-l was grown in a semi-defined medium containing cellulose filter paper discs. The crude yellow fluorescent compound (cYFC) adherent to cellulose discs was extracted with absolute ethanol and stored at -70°C. The cYFC is very soluble in ethanol and methanol. The UV/visible spectrum of the cYFC in ethanol exhibits two peaks at 385 and 405 nm. The same spectrum and maxima were observed after it had been stored at -70°C for one year. Two bands (YFCU and YFCL) were observed when the cYFC was chromatographed on gel H, developed in ethanol at 0-4°C, in darkness. Both bands were fluorescent with an Rf of 0.82 and 0.65 respectively. Chromatography using cellulose thin layer developed in ethanol has been unsuccessful due to adhesion of cYFC. Using 385 and 405 nm excitation wavelengths, the cYFC in ethanol exhibited the same broad fluorescence emission spectrum with maximum at 620 nm. Further purification of the cYFC, using reverse phase high performance liquid chromatography with a YMC-pack ODS-AM column was used. Low resolution negative ion FAB mass of the two yellow fluorescent bands indicated the following major peaks: 901.6 (YFCU with an R_f = 0.85) and 977.9 (675.4) (YFCL with an R_f = 0.72). Fourier transform infrared and X-ray photoelectron spectra revealed the following elemental composition for both TLC bands: carbon (C), hydrogen (H) and oxygen (0). NMR analysis of the cYFC demonstrated the presence of two chemical components: one highly aromatic and the other olefinic. Further investigations are being undertaken to fully characterize and identify the cYFC. Poster

#54 EFFECT OF STEARIC ACID, SPHINGOSINE AND FUMONISIN B_1 ON GROWTH OF PURE CULTURES OF RUMEN BACTERIA. *M W. Corley[1], M. Tumbelson[2], G.Meerdink[2], and R. I. Mackie[1] Departments of Animal Sciences[1] and Veterinary Biosciences[2] University of Illinois at Urbana Champaign, IL 61801 (217-244-2526)

Fumonisin B I (FB I) a mycotoxin prevalent in fungal contaminated cereal grains, is toxic to monogastrics in low concentrations, and targets the brain in horses, lungs in pigs and the esophagus in humans. Ruminants however are less susceptible to FB 1. The effect of FB 1, stearic acid (SA), and sphingosine (SO) on the growth rate of pure cultures of rumen bacteria, was therefore determined. SO and SA were used as analogues of FB 1 - Streptococcus bovis JB 1, Butyrivibrio fibrisolvens 49, Prevotella ruminicola D31D, and Selenomonas ruminantium D were grown in medium containing Trypticase and glucose as the carbon source. Ruminococcus albus 8 was grown in medium with cellobiose as the carbon source. The levels of inclusion of SA, SO, and FBI were 0.01mg/10 ml medium (LSA, LSO and LFB) and 0.05 mg/10 ml medium (HSA, HSO and HFB) respectively. The growth rate (μ) was determined for each bacteria. Growth of JBl, 8, D and 49 decreased by 29%, 63%, 27% and 28% respectively with LSA, whereas growth decreased by 38%, 65%, 42% and 91%, respectively with HSA. Growth of JB 1, D, and 49 decreased by 1.7%, 51% and 94%, espectively, with LSO. There was no difference in growth of D3 lD wilh LSO vs control. JBl, D, 49 and D31D showed no growth in HSO, whereas growth of D decreased by 91% in HSO. In contrast, growth of these pure cultures of rumen bacteria was not affected by addition of FB I to the medium. HPLC analysis is being carried out to determine degradation of this fungal mycotoxin. Poster

#55 THE USE OF CARBOHYDRASE ENZYMES AS FEED ADDITIVES FOR EARLY LACTATION COWS. M. R. Stokes and S. Zheng, Dept. of Animal, Veterinary and Aquatic Sciences, University of Maine, Orono, ME 04469-5763 (207-581-2737).

Effects of various combinations of cellulase, celiobiase and xylanase on digestion of silage DM were determined by measuring gas production during 24 h in vitro ruminal incubations of treated and control forage. Derived parameters of digestion kinetics did not differ between treatments but some enzymes increased rate of digestion 20-30% compared to control and increased gas production in the first 12 h of digestion. The most effective and economical combination of enzymes with FDA approval was evaluated over the first 16 wk of lactation of 16 Holstein cows. Dietary forage of the TMR (50% hay crop silage, 5% alfalfa hay, 45% barley-based concentrate, DM basis) was sprayed in a mixer wagon with water (control) before adding the concentrate or with 1.1 liter enzyme/ton forage DM diluted with 5 liters water per cow using a hose-end sprayer. Compared to control, enzyme treatment increased DMI (17.05, 19.10), yield of milk (28.48, 32.70) and FCM (28.56, 33.91; all kg/d, NSD). Treated cows maintained a higher BCS (2.64, 3.26, P<.076), milk yield peaked higher and 2 weeks earlier (wk 5, 29.20, 35.11; wk 7, 31.28, 33.35), and were confirmed pregnant earlier (85 vs 100 d, P<.04). Poster.

#56 INFLUENCE OF MONENSIN AND CARBOHYDRATE SOURCE ON THE GROWTH OF PURE CULTURES OF PREDOMINANT RUMEN BACTERIA. H. S. Hussein and R. I. Mackie. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-8809).

The effects of monensin addition and carbohydrate source (glucose, sucrose, or starch) on growth rate of three ruminal bacteria (Streptococcus bovis JB1, Butyrivibrio fibrisolvens 49, and Prevotella ruminicola B,4) and their fermentation products were investigated. These bacterial species differ in their membrane structure (Gram positive, thinned-walled Gram positive, and Gram negative, respectively) and, therefore, differ in their sensitivity to monensin. The medium concentrations of monensin were .5 mg/L for S. bovis and P. ruminicola and .1 mg/L for B.fibrisolvens (very sensitive). Fermentation was terminated when maximum OD and, therefore, cell yield was achieved. With glucose, sucrose, and starch, monensin addition reduced the growth rate of S. bovis (by 15, 9, and 27%, respectively) and B. fibrisolvens (by 33, 13, and 74%, respectively) but it did not affect the growth of P. ruminicola. Volatile fatty acid data were analyzed for each bacterial species as a completely randomized design with treatments being arranged as a 2 (with or without monensin) x 3 (carbohydrate sources) factorial. Monensin decreased ($P < .05$) concentrations of acetate, propionate, and butyrate by 33, 35, and 58%, respectively, when S. bovis was grown on sucrose. With B. fibrisolvens, monensin decreased ($P < .05$) the concentrations of acetate and butyrate by 93 and 50%, respectively, regardless of the carbohydrate source. With P. ruminicola, only propionate was decreased ($P < .05$) by 53 and 59%, respectively, when sucrose and starch were used. Responses of ruminal bacteria to monensin appear to be influenced by the carbohydrate source available for fermentation. Poster

#57 INFLUENCE OF MONENSIN AND INOCULUM SOURCE ON IN VITRO FERMENTATION OF DIFFERENT CARBOHYDRATE SOURCES. H. S. Hussein and R. I. Mackie. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-8809).

In order to determine the effects of monensin on in vitro fermentation, different carbohydrate sources were incubated for 24 h with inocula from different sources in a completely randomized design experiment. Treatments were arranged as a 2 x 3 x 3 factorial. These factors were monensin (with or without .5 mg/L), carbohydrate source (glucose, sucrose, or starch), and inoculum source (rumen fluid that was collected from 6 steers after being adapted to 3 diets for 60 d). Diets (2 steers/diet) differed in the forage (grass hay) to concentrate (corn) ratio and were forage (100:0), concentrate (90:10), and forage-concentrate (40:60). No monensin x carbohydrate source x inoculum source interactions were detected ($P > .05$) for any of the measurements evaluated. However, interactions ($P < .05$) were detected between monensin and the inoculum source and between monensin and the carbohydrate source. Monensin addition decreased ($P < .05$) methane production by 77% when the inoculum was from steers fed the concentrate diet. Monensin decreased ($P < .05$) total gas production by 16% and tended to decrease ($P = .07$) methane production by 55% when added to incubations containing sucrose. Concentration of acetate was decreased ($P < .05$) by 40% when monensin was added to incubations with inoculum from steers fed the forage-concentrate diet. Because concentration of propionate was not altered ($P > .05$) by this interaction, the acetate to propionate ratio was reduced ($P < .05$) by 36%. Results illustrate the importance of considering the proportion and the source of the carbohydrate fraction in the diet when adding monensin. In addition, the decrease in methane production when sucrose was used as a carabohydrate source suggest an energetic efficiency to adding monensin to diets containing molasses as a supplement. Poster.

#58 THE ANTI-CELLULOLYTIC FACTOR FROM CICER MILKVETCH IS AN ARABINOGALACTAN PROTEIN. T.C. Maleniak, R.D. Hatfield and P.J. Weimer, Dept. of Bacteriology, University of Wisconsin, and USDA/ARS Dairy Forage Research Center, Madison, WI 53706 (608-264-5408).

Leaves of the perennial legume Astragalus cicer (cicer milkvetch) are known to contain a factor that inhibits cellulose digestion by adherent ruminal cellulolyticbactelia. We havc isolated the active agent by ultrafiltration, alkaline treatment and isoelectric focusing of thc aqueous phase remaining after boiling and ether extraction of ground leaves. The purified product containcd 77% carbohydrate (primarily galactose and arabinose, and including 6% uronic acids), and 8% protein (particularly enrichcd in hydroxyproline and serine). Linkage analysis by GC/MS of methylated alditol acetates and by ^{13}C-NMR revealed a predominance Of beta-1,4-galactosyl and beta-1,3,6-galactosyl linkages, with arabinose, rhamnose, and glucose located primarily at chain termini. This polysaccharide structure suggests either a novel type of arabinogalactan (AG) or a mixture of Type I and Type II AGs. The agent was neutralized by the beta-glucosyl Yariv reagent (an arabinogalactan protein binding agent), but not by cellulose or by cells of Fibrobacter succinogenes or Ruminococcus flavefaciens. The data suggest that the agent inhibits cellulose digestion by interfering with the binding of cells to cellulose, without itself binding to either substrate. Poster.

#59 CLONING, EXPRESSION, AND SEQUENCING OF AN ARABINOFURANOSIDASE GENE FROM THE RUMINAL ANAEROBE BUTYRIVIBRIO FIBRISOLVENS H17C. T. R. Whitehead* and D.A. Lee, USDA/ARS, Natl. Cent. Agric. Utilizn. Res., 1815 N. University St., Peoria, IL 61604 (309-681-6272).

Improving digestion of xylan in the rumen through genetic manipulation of ruminal microorganisms has been suggested as a means to enhance ruminal utilization of this polysaccharide. Butyrivibrio fibrisolvens is one of the more important ruminal xylan-degrading microorganism, and would be a prime choice for this work as it expresses a full complement of xylanolytic enzymes (xylanase, xylosidase, arabinosidase, acetyl esterase, glucuronidase). As a part of this work we have cloned a genetic locus from B. fibrisolvens H17c that produces an arabinofuranosidase in Escherichia coli, as initially determined by screening on methylumbelliferyl-arabinofuranoside. The enzyme expressed in E. coli was capable of releasing arabinose from wheat arabinoxylan and oatspelt xylan. Sequencing of the locus indicated ihat the entire gene had not been cloned on the Sau3A fragment. A second EcoRI locus was cloned which contained the entire gene. DNA sequencing of the locus demonstrated the presence of an open reading frame corresponding to the arabinosidase activity which encodes tor a protein of 88,000 molecular weight. Analyses of the derived protein sequence did not result in any significant amino acid similarity with other reported arabinosidases. Attempts will be made to introduce the cloned arabinosidase gene back into B. fibrisolvens H17c using E. coli/Butyrivibrio fibrisolvens shuttle vectors and determine if overexpression of the gene occurs. The eflect of overexpression on the ability of this organism to degrade and utilize xylan will also be determined. Poster

#60 INTERACTIONS AMONG RUMINAL CELLULOLYTIC BACTERIA IN CELLOBIOSE-LIMITED CHEMOSTATS. Y. Shi and P.J. Weimer, Depts. of Dairy Science and Bacteriology, University of Wisconsin, and USDA-ARS Dairy Forage Research Center, Madison, WI 53706 (608-264-5420).

Interactions among Fibrobacter succinogenes S85, Ruminococcus flavefaciens FD-1 and R. albus 7 were examined in cellobiose-limited continuous cultures (pH 6.5-6.8) fed 1-4 g cellobiose/ L. Populations were assessed via characteristic fermentation products and via signature membrane fatty acids (MFAs). When FD-l was inoculated into a steady-state (D=0. 17 h^{-1}) culture of S85, or when the chemostat was simultaneously inoculatedwith both strains, FD-1 quickly predominated, as indicated by acetate/succinate ratios similar to FD-1 monocultures, and MFAs characteristic of FD-l, but not S85. The predominance of FD-1 is consistent with the measured Monod growth parameters (K_s and maximum growth rate) for these strains. Chemostats co-inoculated with 7 and either FD-1 (D=0.024 h^{-1}) or S85 (D=0.07 h^{-1}) produced abundant ethanol and acetate but no succinate, and contained MFAs characteristic of 7. Similar results were obtained when 7 was inoculated into steady-state cultures of FD-1 or S85 (D=0.07h^{-1}). Thepredominance of 7 was not expected based on the measured Monod growth parameters for these strains. Supernatants of effluents collected from chemostats dominated by 7 did not contain factors that inhibited growth of FD-l or S85 in batch culture. Poster

#61 EFFECT OF SILICA ON THE COLONIZATION OF RICE STRAW BY RUMINAL MICROORGANISMS. H. D. Bae[1], T. A. McAllister[1], E. G. Kokko[1], F. L. Leggett[1], J. K. Ha[2], H. T. Shin[3] and K.-J. Cheng[1]. [1]Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta (403-327- 4561); [2]Department of Animal Science and Technology, Seoul National University, [3]Department of Dairy Science and Technology, Sung Kyun Kwan University, Korea.

The relationship between microbial colonization and silica distribution in the cuticle and pith parenchyma of rice straw was examined using scanning electron microscopy and energy dispersive X-ray microanalysis (EDX). Rice straw stems cut into 1 cm lengths were either treated with 40 g NH_3 kg^{-1} DM (AMM) or left untreated (CON) . Microbial colonization was examined by growing Fibrobacter succinogenes S85, with rice straw as the sole source of carbohydrate. The extent of DM disappearance and colonization of AMM and CON rice straw by mixed ruminal microorganisms were examined using the nylon bag technique. EDX demonstrated that the surface of the cuticle was virtually covered by silica, with high levels of accumulation within distinctly arranged phytoliths (opaline bodies). Silica was distributed diffusely over most of the surface of the pith parenchyma which lacked phytoliths and contained regions void of silica. Ammonia treatment dramatically increased the DMD of rice straw, but did not alter the distribution of silica in the cuticle or pith parenchyma. Trichomes, the only cuticular structures void of silica, were major sites of invasion and colonization in the intact cuticle of rice straw. Damage or loss of the cuticle was more prevalent in AMM than CON rice straw and tissues underlying the cuticle were found to be void of silica and extensively colonized. The improved digestibility of AMM rice straw may be partially due to a weakening of the adhesion between the cuticle and underlying tissues. Detachment of the cuticle removes this silica-based barrier to digestion and exposes underlying silica-void tissues which are readily colonized. Poster.

#62 EFFECTS OF A SACCHAROMYCES CEREVISIAE CULTURE ON LACTATE UTILIZATION BY LACTATE UTILIZING RUMINAL BACTERIA E.S. Callaway and S.A. Martin, Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706)542-1065

The objective of this study was to determine the effects of a Saccharomyces cerevisiae culture (Diamond V Mills) on lactate utilization by the ruminal bacteria Selenomonas ruminantium (strains HD4 and H18) and Megasphaera elsdenii (strains B159 and T81). Because the yeast culture is bound to an insoluble carrier, a filter-sterilized filtrate of the yeast culture was used to evaluate effects of soluble components associated with the yeast culture on bacterial growth and end product formation. Bacterial growth was monitored by measuring optical density at 600 nm. A stock concentration of 1 g/50 ml deionized water of filter-sterilized filtrate was used. The filtrate was added to a basal medium that contained 5 g/L DL-lactate to achieve final concentrations of 1 or 5% (vol/vol). Medium without Trypticase or yeast extract was also used. The 1% filtrate concentration was not stimulatory and the 5% level was only slightly stimulatory for all four strains incubated in basal medium. Without Trypticase and yeast extract in the basal medium, both the 1 and 5% concentrations of filtrate increased growth of all strains. Growth of strains HD4 and H18 was stimulated 54% and 40%, respectively with 5% filtrate added to the minimal medium. In the presence of 5% filtrate, growth of strains B159 and T81 in minimal medium was stimulated 2.1-fold and 3.6-fold, respectively. Filtrates of 2.5 g/50 ml and 5 g/50 ml were also used and these filtrates increased growth of all strains and also increased VFA production. Analysis of the 1 g/50 ml filtrate by HPLC methods revealed that it contained 4 mM malate and .9 mM aspartate. These dicarboxylic acids stimulate growth of S. ruminantium on lactate. It is likely that the filtrate also contains B vitamins and other amino acids, which are required for growth on lactate by S. ruminantium and M. elsdenii. Poster.

#63 DETECTION OF BUTYRIVIBRIO FIBRISOLVENS STRAIN H17C pUB.xynA IN THE RUMEN BY PCR AMPLIFICATION. C.S. McSweeney[1], K.S. Gobius[2], G.P. Xue[2], P.M. Kennedy[1],B.P. Dalrymple[1]. [1]CSIRO DTAP, Private Bag No 3 PO, Indooroopilly, QLD 4068, Australia (+61-7-32142820). [2]CSIRO DTCP, 306 Carmody Rd, St. Lucia, QLD 4067, Australia.

The construction of a genetically-engineered rumen bacterium Butyrivibrio fibrisolvens H17c pUB.xynA that expresses a recombinant xylanase gene derived from the ruminal phycomycete Neocallimastix patriciarum has been a recent development in our laboratories. This report describes the detection of B. fibrisolvens H17c pUB.xynA in the rumen by PCR amplification of chromosomal and plasmid DNA. The primers for amplification of chromosomal DNA were selected from the nucleotide sequence of the gene encoding a type 111 glutamine synthetase from H17c. The primers for the plasmid pUB.xynA were derived from DNA sequences in the xylanase gene and transcription termination sequence. The establishment and persistence of B. fibrisolvens H17c pUB.xynA was examined in 4 cattle and 4 sheep, dosed with a pure culture of B. fibrisolvens H17c pUB.xynA which was equivalent to 1011 -1013 cells per animal, and fed ad libitum diets of high quality lucerne (3.2 %N) and low quality rhodes grass (0.7% N). B. fibrisolvens H17c pUB.xynA declined from 106 cells/gm digesta after dosing to <101 cells/g when detected by PCR with plasmid targeted primers . However, genomic primers indicated that the bacterium established in the rumen at 103-104 cells/gm digesta. These results indicate that strain H17c can establish in the rumen but the recombinant strain did not persist due to the instability of the plasmid. Poster

#64 THE POTENTIAL OF BACILLUS STEAROTHERMOPHILUS SPORES AS A MARKER FOR ESTIMATING DIGESTA PASSAGE RATE IN RUMINANTS, Z. Mir*, P.S. Mir, M. S. Zaman, L. B. Selinger,T. A. McAllister, L.J. Yanke and K-J. Cheng Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1 (403-327-4561).

Inorganic markers such as chromium-mordanted neutral detergent fiber (Cr-NDF) and cobalt-ethylenediamine tetraacetic acid (Co-EDTA) are widely used for measurement of solid and liquid passage rates in the gastrointestinal tract (GIT) of ruminants. Since these inorganic markers have limitations (Warner 1981, Nutr. Abstr. & Rev. [Series B] 51:789) there is a need to find alternate organic markers. The objective of the present study was to investigate the use of spores of the thermophilic bacterium Bacillus stearothermophilus (BSS; Marteau et al. 1990, Br. J. Nutr. 64:71), as a marker to study passage of digesta from the rumen. In the present study, solid and liquid passage rates from the rumen were determined by using BSS spores and these values were compared with rates determined with Cr-NDF and Co-EDTA. Chromium-mordanted hay (100 9), Co-EDTA (50 9) and spores BSS suspension (50 ml, containing 108 spores/ml) were administered through rumen fistulas to three mature steers and six mature heifers with an average weight of 659$\pm$6 kg. The animals were fed timothy grass hay (13.6% CP) at maintenance level of an average dry matter intake of 1.8% of body weight. Following administration of markers, rumen contents were collected up to 120 h. Aliquots of rumen digesta solids and liquids were analyzed for Cr and Co concentration and bacterial colonies of BSS were counted after incubation on nutrient agar plates at 65°C, overnight. Rates of passage were calculated as the slope of the regression of the natural logarithm of Cr, Co concentration and BSS colonies versus time. Data showed that solid rate of passage from the rumen estimated by Cr-NDF (3.46 %/h) was lower ($P<0.05$) than that measured by using BSS (4.38 %/h). The liquid rate of passage from the rumen estimated by Co-EDTA or BSS (4.30 vs 4.92 %/h) was not significantly different. The results of this experiment indicated that BSS may potentially be used as a marker for estimating both liquid and solid rate of passage in ruminants. Poster.

#65 CHARACTERIZATION OF A XYLANASE GENE FROM NEOCALLIMASTIX PATRICIARUM. L.B Selinger[1]., J.-H. Liu[2], Y.J. Hu[1], M.M. Moloney[2], C.W. Forsberg[3], and K.-J. Cheng[1]. [1]Agric. and Agri-Food Canada, Lethbridge, AB, (403) 327-4561 [2]Dept. Biol. Sci., Univ. of Calgary, Calgary, AB, (403) 220-6823 [3]Dept. of Microbiol., Univ. of Guelph, Guelph, ON, (519) 824-4120

Fibrolytic activity in the rumen arises primarily from the activities of three bacterial species: Fibrobacter succinogenes, Ruminococcus albus, and R. flavefaciens. Enzymes produced by other microorganisms, including fungi and protozoa, also contribute to fibre degradation. Ruminal fungi are noted for their production of potent fibrolytic enzymes and their ability to degrade the most recalcitrant of plant cell wall polymers. In order to understand and exploit the fibrolytic systems of ruminal fungi, researchers have begun to clone and characterize genes encoding fibrolytic enzymes. In the present study a xylanase gene, isolated from the anaerobic fungus Neocallimastix patriciarum, was characterized. Deletion and DNA sequence analysis of the genomic DNA clone identified a 1458 bp open reading frame (xynC). Translation of the ORF would result in the expression of a 486 amino acid polypeptide with a predicted molecular weight of 51 kDa. The first 22 amino acid residues are typical of a signal sequence encompassing a basic N-terminus and central hydrophobic core. Deletion analysis localized the catalytic domain to the N-terminus and a binding domain to the C-terminus of XynC. Sequence analysis indicated that the catalytic domain is followed by a highly reiterated proline rich linker region. A comparison of known protein sequences revealed that XynC has a family G catalytic domain and is most closely related to XynC from Fibrobacter succinogenes. This study presents a third xylanase gene cloned from N. patriciarum. Poster.

#66 GROWTH OF RUMINOCOCCUS ALBUS ON CELLOBIOSE AND CELLULOSE: AN ELECTRON MICROSCOPIC STUDY. Peter Schofield* and Alice N. Pell, Dept. Animal Science, Cornell University, Ithaca, NY 14853 (607- 255-2876).

Ruminococcus albus 8 can use cellobiose (CB) or cellulose (C) as primary carbon source. Cellulose requires a different enzyme system for its digestion. We have used transmission electron microscopy to look for structural changes when cells grown in CB were transferred to an amorphous form of C. Cells were examined unstained, negatively stained with phosphotungstate, or after embedding, sectioning, and staining with uranyl acetate and lead citrate. Interesting observations included: (1) the appearance of 'backpack' structures, translucent membrane-enclosed capsules filled with non-electron dense, non- staining material. These structures appeared in both CB- and C-grown cells under certain conditions; (2) a profusion of extracellular spherical and doughnut-shaped structures, some associated with, many remote from, C-grown cells; (3) late cultures grown on filter paper showed cells containing many egg yoke-shaped structures. These structures had a characteristic staining pattern in thin section and were also seen in negatively stained whole cells. These observations suggest that cellulolysis in Ruminococcus albus is accomplished by extracellular enzyme complexes, not all of which are cell bound. Poster.

#67 NITROPROPIONATE REDUCES CH_4 PRODUCTION BY RUMINAL MICROBES. R. C. Anderson. National Animal Disease Center, ARS, USDA, Ames, IA, 50010 (515-239-8200).

An attractive strategy to reduce ruminal CH_4 emissions, which result in estimated energy losses of 2 to 12% of GE intake and contribute to global warming, is to utilize alternative electron sinks. During incubations (20 h) with 24 mM formate and an H_2:$C0_2$ gas phase, mixed ruminal populations produced 19, 33, and 68% less CH_4 when 5, 10, or 20 mM nitropropionate (NPA) had been added. Populations incubated without NPA produced 15.6 µmol CH_4/ml. The total amounts of acetate, propionate, butyrate, and isobutyrate produced by incubations without NPA were 16.5 µmol/ml. The acetate:propionate ratio (A:P) was 5.2. While total VFA's produced by incubations with 5, 10, or 20 mM NPA were increased proportionally (R^2 = o.99), due to increased levels of acetate and propionate, the A:P ratios were decreased (R^2 = 0.88). In other experiments, inoculation of an NPA metabolizing bacterium, strain NPOH1, into mixed populations incubated with NPA did not change the effect of NPA on VFA and CH_4 production, although rates of NPA metabolism were increased. While these results clearly show NPA reduces CH_4 production, the mode of action remains to be determined. Poster.

#68 RUMEN FERMENTATION AND MICROBIAL GROWTH: INFLUENCE OF DILUTION RATE IN CONTINUOUS CIJLTIJRE . Q. Meng*, P. A. Ludden, and M. S. Kerley. Dept of Animal Sciences, University of Missouri-Columbia, Columbia, MO 65211 (314-882-0834)

Two continuous culture runs with 6 dilution rates (2.5, 5, 7.5, 10, 15, and 20 %/hr) were conducted to ascertain rumen fermentation and microbial growth responses to dilution rate (D). A pelleted diet consisting of ground corn, soybean hulls and isolated soybean protein (ISP), was formulatcd to provide CP content of 16.4% on DM basis and calculated non-structural carbohydrate to ruminal degradable protein ratio of 1:.2. Each of 12 fermenters was fed totally .31 g of dietary DM per day in 12 equal ponions. Digestibililies (apparent and corrected for microbes) of DM, OM, and CP were decreased (P <.001) with increased D. The maximum populations of bacteria and protozoa were maintained at the D of 10 and 5%/hr. respectively. although there was no signirlcant difference (P > .05) for bacterial population between D ranging from 5 to 15%,/hr. As D incrcased, ammonia concentration decreased (P<.001) and pH values increased (P < .001). Increasing D from 2.5 to 20%/hr increased total VFA production per day (quadratic; P<.001) and the molar proportions of acetate (quadratic; P < 00l) and propionate (linear; P < .0l). but decreased the proportions of butyrate (quadratic; P <.05) and valerate (lincar; P<.001). Increasing D also quadraticly (P < .001) increased rumen microbial efficiency (g of microbial N / kg of OM truly digested. R^2 =.97) and microbial N production per day (R^2 = .71). Using mathematical derivation, a theoretical maximum microbial efficiency, 59.6, was calculated at the D of 31.4%/hr. In the same way, it was obtained that the microbial N production plateaued at the D of 14.5%/hr. Based upon the results of the present study, we propose that it would be more reasonable to use a quadratic rather than linear model for estimating maximm microbial efficiency in the rumen. Poster.

#69 THE EFFECT OF PRESERVATION METHOD ON GAS PRODUCTION FROM THE NEUTRAL DETERGENT SOLUBLE FRACTION OF FORAGE. P. H. Doane* and A. N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876)

The fermentation of neutral detergent solubles (NDS) was assessed using a 3x3x3 factorial arrangement. Three forage species (alfalfa, bromegrass, and orchardgrass) were collected at three maturities and preserved by freeze drying, oven drying at 60 degrees Celsius, or by ensiling. Each feed sample and its isolated NDF were fermented in vitro and gas production monitored. Gas yield from NDS was determined as the difference between gas from the whole forage and its respective NDF. The forages ranged from 22% (immature alfalfa) to 68% NDF (mature orchardgrass). The silages were well fermented with a final pH < 4.5. There was little difference in 48-hour gas production between freeze-dried and oven-dried forage samples. Ensiling decreased the gas yield compared with the whole forage. When expressed as a percent of the total, gas yield from NDS decreased 4 to 17% when the silage was compared with the freeze-dried sample. The greatest decrease was seen in the immature orchardgrass. There were only small changes (< 3%) in NDF digestibility due to preservation method. Gas production may provide a method for making comparisons between dried and ensiled forages to account for discrepancies in energy values which are otherwise difficult to assess. Poster.

#70 CLONING, EXPRESSION, AND SEQUENCE OF THE L-LACTATE DEHYDROGENASE (LDH) GENE OF STREPTOCOCCUS BOVIS. H. A. Wyckoff, J. M. Chow, T. R. Whitehead, and M. A. Cotta. National Center for Agricultural Utilization Research, ARS/USDA. Peoria, IL 61604 (309-681-6271)

The ruminal bacterium, Streptococcus bovis is highly amylolytic and can convert crude starches completely to fermentation end products. During rapid, uncontrolled growth, S. bovis forms large amounts of lactic acid but under controlled growth it will produce acetate, ethanol and formate. The uncontrolled production of lactate in the rumen can lead to lactic acidosis. Cloning and characterization of the S. bovis JB1 ldh gene may allow modifications to the organism's metabolic pathways to decrease the amount of lactate produced for improved rumen function. A genomic library of S. bovis JB1 DNA was constructed in lambda ZAPII and screened using heterologous probes derived from a S. mutans ldh gene. Several clones were isolated that contained a common 2.9 kb fragment as determined by restriction analysis. DNA sequence analysis revealed a 987 bp open reading frame with extensive homology to S. thermophilus (88%) and S. mutans (82%) ldh nucleic acid sequences. Expression of S. bovis JB1 LDH activity in E. coli by the cloned gene was confirmed by using an in-gel activity assay. The cloned LDH activity of S. bovis JB1 was able to complement the LDH mutation of E. coli FMJ39, allowing anaerobic growth on glucose as a substrate. Attempts are being made to construct LDH mutants of S. bovis JB1 using insertional mutagenisis. Poster.

#71 EVALUATION OF A BLOAT RESISTANT CULTIVAR OF ALFALFA USING THE MENKE'S GAS TECHNIQUE. B. P. Berg[1]. T. A. McAllister[2], Z. Mir[2], R. M. Tait and K.- J.Cheng[2] [1]Alberta Agriculture and Rural (403-381-5836); [2]Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta; University of British Columbia.

Pasture bloat in cattle grazing alfalfa is caused by the formation of a persistent foam which prevents eructation and traps fermentation gases in the reticulo-rumen. Agriculture Canada has undertaken a breeding program to develop a bloat resistant alfalfa cultivar with a low initial rate of digestion (LIRD). The objective of this study was to determine if the rate of digestion, as measured by gas production, varied between LIRD and one of the original parental varieties (c.v. Beaver, BVR). Fresh leaves from BVR and LIRD were either left whole, or treated to enhance bacterial invasion. Treatments included sectioning in half, crushing with a mortar and pestle, or penetration of the cuticle with a needle eight times. Gas production was also compared between dried samples ground through a 1 mm screen. Samples (0.25 g DM) were incubated with rumen fluid collected form a Holstein steer fed alfalfa. All treatments increased ($P < 0.05$) the rate of gas production and reduced the lag time as compared to whole leaves. Lag time was longest for whole leaves followed by sectioning, penetration of the cuticle, crushing and grinding. Leaves from the LIRD cultivar had consistently longer lag times regardless of treatment. The fact that lag times differed between LIRD and BVR even after disruption of the cuticle suggests that other internal plant structures may also contribute to the differences in digestion rate between the two cultivars. Future experiments will determine if the differences in rate of gas production between these two cultivars is reflected in the rate of digestion of alfalfa in the rumen and the incidence of bloat in grazing cattle. Poster.

#72 ESTABLISHMENT OF <u>RUMINOCOCCUS FLAVEFACIENS</u> AND ACETOGENIC BACTERIA IN ABSENCE OF METHANOGENS IN THE RUMEN OF GNOTOBIOTICALLY-REARED LAMBS. B. Morvan, G. Fonty and Ph. Gouet. Laboratoire de Microbiologie, I.N.R.A. de Clermont-Theix, 63122 Saint-Genes-Champanelle, France (Phone: 33-73624000- Fax: 33-73624581).

The use of hydrogen by methanogens in the rumen greatly affects the activity and the product formation of fermentative microorganisms. Acetogenic bacteria which have also the potential to use H_2 are among the first species able to colonize the rumen immediately after birth. Their population which reach a quite high level two days after birth decreases rapidly while methanogens became established. Our aim was to study the establishment of <u>Ruminococcus flavefaciens</u>, an H_2-producing cellulolytic bacterium, in the rumen of gnotobiotic lambs in absence of acetogens and methanogens, and then the establishment of acetogenic bacteria. Four germ-free lambs received, at the age of 8 days a bacterial inoculum, free of acetogens and methanogens, constituted by the 10^{-7} dilution of rumen content taken from a sheep and previously incubated for 48 h in prescence of BESA. At the age of 1 month they were inoculated with <u>R. flavefaciens</u> (007 and FD1) and at the age of 3 months they received a culture of 2 acetogenic strains (SerS and Ser8). Lambs were fed cow milk and dehydratred alfalfa. Compared to the rumen contents of conventional lambs, total viable counts were similar in gnotobiotic lambs whereas <u>R. flavefaciens</u> became established at a slightly lower level(2.2×10^7 cells ml^{-1}). In contrast, acetogens reached higher numbers (1.5×10^6 cells ml^{-1}) than those observed in control lambs, probably due to the ahscnce of competition with methanogens. Poster.

#73 CHARACTERIZATION OF NEW ACETOGENIC SPORULATED COCCI, IMPLICATIONS OF THEIR PHYLOGENETIC PLACEMENT FOR REVISION OF THE GENUS CLOSTRIDIUM. F. Rieu-Lesme[1] and C. Dauga[2]. [1]Laboratoire de Microbiologie, I.N.R.A. Clermont-Theix, 63122 Saint Genes-Champanelle. [2]Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France.(Phone: 33-73624740).

Acetogenic bacteria are present in numerous anaerobic ecosystems where they use H_2+$C0_2$ as energy source catalyzing a total synthesis of acetate from this gas mixture.The current knowledge of the diversity of acetogenic rumen bacteria is still very poor. Such bacteria were isolated under $H_2/C0_2$ using the roll-tube technique. The present investigation revealed that the predominant acetogens in the rumen of lambs, llamas and bisons are cocci occuring in long chains forming endospores. Our isolates (thirteen) were the first Gram positive spore-forming recognized as acetogenic. All isolates show high phenotypic similarities. Electron microscopy observations show an original structure of the cell wall. All isolates are able to grow heterotrophically with numerous organic substrates. Under H_2 / CO_2 the amount of acetate formed reached 50 to 80 mM depending on the strains. The 16S rRNA sequence analysis of five isolates shows that they were new genomic species closely related to Ruminococcus hansenii, Ruminococcus productus and Clostridium coccoides. We have shown that these three species were also able to use H_2+$C0_2$. Our data are in full agreement with the necessity for a major revision of Clostridium and relatives assemblage. Poster.

#74 CHARACTERIZATION OF DIPEPTIDYL PEPTIDASE-I ACTIVITY OF PREVOTELLA RUMINICOLA $B_1$4. H. M. F. Madeira, and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln, NE. 68583-0908. (402-472-6405)

Despite its inability to use di- and tri-peptides for growth, bacteria currently classified as P. ruminicola are considered to be the primary source of dipeptidyl peptidase type I (DPP 1), the predominant peptidase activity measurable in rumen contents. Selective inhibition of this enzyme may be a useful means of slowing down ruminal protein degradation. The present study aimed to characterize the pH optimum, effect of inhibitors and nitrogen sources (peptides, ammonia, gelatin), and cellular location of the DPP I activity of P. ruminicola strain $B_1$4. Enzyme activity was measured using Gly-Arg-4-methoxy-2-napthylamide (Gly-Arg-MNA) as the substrate, and the specific activity of P. ruminicola whole cells harvested at mid-log phase of growth was 6.7 ($\pm$0.26) nmols MNA released min^{-1} mg^{-1} protein. Enzyme activity had a pH optimum of 7.5, and at pH 6.0, enzyme activity was significantly decreased. Oxygen, cysteine protease inhibitors (p-chloromercurobenzoate, iodoacetate), EDTA, and EGTA markedly inhibited DPP I activity. Addition of 5 mM calcium chloride reversed the inhibitory effects of EDTA and EGTA, but magnesium ions did not. Dithiothreitol (DTT) and phenylmethylsulfonyl fluoride (a serine protease inhibitor) had no effect on enzyme activity. Nitrogen sources also had little effect on enzyme activity. Isoelectric focusing gel electrophoresis of cell fractions identified two distinct proteins capable of hydrolyzing Gly-Arg- MNA. The membrane-associated protein has a pI of ~4.0, while the cyto-/periplasmic protein has a pI of ~7.0. Results indicate that the DPP I enzymes of P. ruminicola B,4 are calcium-dependant, cysteine-type peptidases, and activity may be affected by ruminal pH. Poster.

#75 CELLULOSE ADHERENCE FACTORS IN RUMINOCOCCUS ALBUS 8. R. Pegden, R.J. Grant, and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln, NE 68583 (402-472-6405)

Bacterial adherence is an important aspect affecting the kinetics of ruminal fiber digestion. A series of genetic and functional based screens have been employed in an attempt to identify macromolecules capable of binding to cellulose. Low stringency Southern hybridizations of R. albus 8 genomic DNA, with a probe specific for the cellulose binding domain of the xylA gene of Pseudomonas fluorescens subsp. cellulosa, identified a single DNA fragment with weak cross reactivity. Mid-log phase cultures of R. albus 8 grown on cellobiose were harvested, resuspended in phosphate buffer, and aliquots mixed in microtiter dishes with either lectins derived from jack bean, peanut, castor bean, winged pea, wheat germ, and lentil; or erythrocytes obtained from rabbit, ox, calf, guinea pig, horse, sheep, and goat. The cells showed no reaction with the lectins tested, but did agglutinate rabbit erythrocytes. I however, agglutination appeared to be affected by the age of the erythrocytes. Membrane proteins extracted lron cellobiose grown cultures of R. albus 8 were mixed with slurries of crystalline cellulose, then washed with various detergents. Four proteins (~22 kDa, ~30 kDa, ~65 kDa~ and ~116 kDa) have been identfied as putative cellulose binding proteins. Affinity of these proteins for cellulose is affected by the inclusion of dithiothreitol, calcium, and magnesium in the reaction mixture, and the proteins can also be "eluted" by different detergents. The 65 kDa protein reacts positively in Western blot assays for glycosylated proteins, as do protein(s) in excess of 100 kDa. Preliminary results indicate that degradation products of these protein(s) still possess a strong affinity for cellulose. Poster.

#76 CHARACTERIZATION OF THE GLUTAMATE DEHYDROGENASE ACTIVITY IN VARIOUS STRAINS OF PREVOTELLA RUMINICOLA. Z. Wen, and M. Morrison, Department of Animal Science, University of Nebraska, Lincoln, NE 68583-0908 (402-472-6405)

Little is known about ammonia assimilation in rumen bacteria such as Prevolella ruminicola, which is believed to play a central role in the nitrogen economy of the rumen. In whole cell assays under aerobic conditions, strains 23, D31d, and B,4 were found to produce no measurable NADH- or NADPH-dependent glutamate synthase activity, when grown with 10 mM ammonia as sole nitrogen source. Similar to previous findings with P. ruminicola $B_1$4, strains 23 and D31d possess glutamate dehydrogenase activity, both NADPH and NADH can catalyze glutamate biosynthesis in whole cell assays, and NADPH-dependent activity is stimulated by the addition of 0.2M KCI. The NADPH- dependent activity of strain $B_1$4 was decreased five-fold, when trypticase (1.5%, w/v) was used as nitrogen source. Northern blot analysis revealed that gdh gene expression was greater in ammonia grown cultures than in cultures grown on peptides, indicating that gene expression is modulated in response to nitrogen source, although the mechanism is unclear. The structural gene, gdh, contains an 1.3 kb ORF which encodes a 48.8 kDa polypeptide, and the deduced amino sequence possesses three regions similar to the highly conserved motifs typical of Family I GDHs. However, several unique amino acids were also evident within these motifs, and Southern blot analysis indicates that the gdh gene of strain $B_1$4 does not cross hybridize with genomic DNA isolated from strains 23 (subsp. ruminicola) or D31d (subsp. brevis). To evaluate whether GDH activity is the sole route of glutamate biosynthesis and primary mechanism of ammonia assimilation in P. ruminicola, future studies will involve construction of gdh mutants. Also, studies will be done to clarify the regulation of gdh expression and nitrogen assimilation. Poster.

#77 PHYLOGENY OF FOUR CILIATED ANAEROBIC RUMEN PROTOZOA INFERRED FROM SMALL SUBUNIT RIBOSOMAL RNA SEQUENCE COMPARISONS Abdelghani Sghir[1], Joel Dore[2], Liliane Millet[3], Danielle David[4] & Philippe Herve[5]. [1]Department of Animal Sciences, 1207 West Gregory Drive, University of Illinois at Urbana-Champaign 61801 IL USA. [2]INRA, Laboratoire de Nutrition et Securite Alimentaire - Domaine de vilvert - 78352 Jouy-en-Josas Cedex. [3]INRA, Laboratoire de Microbiologie, 63122 Saint-Genes-Champanelle Cedex. [4]CNRS, Laboratoire de Zoologie B.P. 26 - 63177- Aubiere Cedex. [5]CNRS, Laboratoire de Biologie Cellulaire 4 Universite Paris XI 91405 Orsay Cedex France.

Partial sequencing of 18S rRNA of four ruminal ciliate protozoa was carried out using total extracts from cell pellets purified from rumen contents of sheep inoculated with single protozoal species. The four species were Isotricha prostoma (Order vestibulifera), Eudiplodinium magii, Epidinium caudatum and Polyplastron multivesiculatum (Family Ophryoscolecidae). Dideoxynucleotide sequencing was performed using reverse transcriptase and six oligonucleotide primers complementary to evolutionarily conserved regions of the 18S-like rRNA. Sequences were aligned according to conserved features of primary and secondary structure. Sequence similarities were calculated using only positions of unambiguous alignment with a nucleotide in all sequences compared (769 positions). These were converted to evolutionary distances using "the Neighbor-joining method". There is a broad diversity within the rumen ciliates based on 18S-like rRNA sequence comparison. We confirm a monophyletic assemblage of this group of ciliates, but in contrast to the hypothesis of an evolutionary line (Dogiel 1947, Lubinsky 1957, Furness and Butler 1988), the entodiniomorphid ciliates appear to have followed a branched evolution. Isotricha, of the vestibulifera order, diverged early from the entodiniomorphida and within the latter, Epidinium diverged first from the branch Eudiplodinium-Polyplastron. Considering the difficulties encountered in culturing rumen protozoa in vitro, rRNA-based technologies based on hybridization probes specific for these microganismes should prove useful in the appreciation of the phylogenetic diversity and ecological role of ciliates in the ruminal microbial community. Poster.

#78 INTERCONVERSIONS OF C-18 FATTY ACIDS FROM CORN OIL INCUBATED IN VITRO. D.L. Palmquist, Eva Cenkvari, Ramon Casals and Donna Kinsey, Dept. of Animal Sciences, OARDC/The Ohio State University, Wooster, OH 44691 (216- 263-3795).

Lipolysis of glycerides and biohydrogenation (BH) of fatty acids is an active process in ruminal content. BH decreases the toxicity of fatty acids toward gram positive microbes and protozoa. Recently, interest has increased in cg, t,l, 18:2 ("CLA") and t 11 18:1, both intermediates in BH. CLA may enhance immunity to certain tumors, whereas t 18:1 decreases milk fat percentage and may be hyperlipidemic. The sequence of BH is: cg, c12 18:2 (isomerization) cg, t11 18:2 (BH) t11 18:1 (BH, slow) 18:0. Although little is known concerning accumulation of CLA, we have observed that 500 ml soy oil intraruminally increased CLA from 0.5% to 1.5% of milk fatty acids within 24 h. We incubated corn oil (high in cg, c12 18:2) with ruminal content and measured changes in proportions of the C-18 fatty acids. Significant effects on proportions of all fatty acids by dose of corn oil ~52, 82 and 111 mg/flask) and time of incubation (0, 3, 6, 9, 12 h) were observed. Only the proportion of t 18:1 was influenced by the interaction of dose x time of incubation, with higher doses causing higher accumulation of t 18:1. Linoleic acid (cg, c12 18:2) decreased rapidly from 33% to 7% of total fatty acids during 12 h, whereas t11 18:1 increased linearly from 2% to 23%. Increases in 18:0 followed the t11 18:1 changes. CLA was initially less than 0.5% and never exceeded 2% of total fatty acids. In order to cause larger increases in CLA content of ruminant fats, it will be necessary to identify metabolic inhibitors which block BH of cg, t11 18:2, without inhibiting the cg, c12 18:2 isomerase. Poster

#79 COMPARISON BETWEEN Yb AND Co-EDTA AND 15N AS MARKERS FOR ESTIMATION OF FLOW OF MICROBIAL PROTEIN IN COWS FITTED WITH RUMEN CANNULAE ONLY. A. N. Hristov[1], L. Rode[1] and G. A. Broderick[2], Research Center, Agriculture Canada, Lethbridge, AB T1J 4B1 (403-327-4561)1 and US Dairy Forage Research Center, Madison, WI 53706 (608-263-2030)2

A procedure for estimation of rumen microbial protein synthesis respectively feed protein degradation on animals with rumen cannulae only was proposed (Hristov and Broderick, 1994). In order to investigate the possibility of using one single marker instead of three as in the original method an experiment involving lactating dairy cows fed on a mixed forage-concentrate diets was designed. Ytterbium, Co-EDTA and $(15NH_4)_2SO_4$ solutions were infused continuously into the rumen and passage rates of Solid and Liquid phases were determined. Generally, the 15N-estimated dilution rates were lower by 25.1 to 42.7 % and by 28.6 to 54.8 % compared to the Co-EDTA- and Yb-rates. Respectively, the 15N-method underestimated the flow of microbial-N out of the rumen by 28.9 (33.2 g N/d) to 48.1 % (129.8 g N/d). Obviously, these differences represent the level of N recycling occurring in the rumen. The results were compared to those obtained through a routine sampling of the duodenal digesta. Poster.

#80 IN VITRO COMPARISON OF RUMEN FERMENTATION OF POTATO WASTES WITH BARLEY AND CORN. D. Grancher and S. Komisarczuk-Bony, Unit of Rumen Physiopathology. INRA-ENVL, B.P.83, F-69280 Marcy l'etoile, France (33- 78 87 25 29)

A short term incubation fermentation system was used to compare during 12 h the ruminal fermentation of raw potato offcuts (RPO), barley (B) and corn (C). Flasks were incubated anaerobically at 39°C with 200 ml of total rumen content, 200 ml of an artificial saliva (Mac Dougall), 230 mg of urea nitrogen, 20 mg of sulfur and 10 g DM of each substrate studied (1mm ground). pH and gas production kinetics revealed that RPO and B had similar rate and extent of degradation and were superior to C. Rate and extent of short chain fatty acids (SCFA) production an the amount of fermented organic matter (FOM) calculated according to Demeyer and Van Nevel (1978) were in the order RPO > O > C. The molar C2/C3 ratio decreased slightly along with the incubation time particularly with RPO.

Nitrogen incorporation in microbes measured as the decrease in $N\text{-}NH_3$ concentration in the flasks between the 3rd and the 6th hour was similar for all substrates.

According to these results, the ruminal fermentation of potato waste gives similar pattern compared with barley. There is no evidence for having any increased risk of ruminal acidosis in animals fed potato wastes. Poster.

#81 PHYLOGENETIC RELATIONSHIPS AMONGST BUTYRIVIBRIO-LIKE ISOLATES OF RUMEN BACTERIA AND THE DESIGN OF PROBES FOR DETERMINATIVE AND COMMUNITY STRUCTURE STUDIES IN THE RUMEN. R.J. Forster, J. Gong and R.M. Teather, Centre for Food and Animal Research, Agriculture Canada, Ottawa, ON, KIA 0C6 (613-759-1725).

The genus Butyrivibrio is thought to compose a large and important population in the rumen. At the present time the genus is composed of two validly described species, Butyrivibrio fibrisolvens and B. crossatus, of which the majority of isolates are B. fibrisolvens. The objective of this study was to determine the phylogenetic relationship amongst these organisms and to design probes that would be useful in evaluating these bacteria in the rumen. Phylogenetic relationships were determined by comparing the entire 16S rDNA sequence of 15 isolates, including the type strains. All isolates grouped within the Clostridial cluster XIVa of Collins et al. 1994, Int.J.Syst. Bacteriol. 44:812. However, the B. fibrisolvens type strain was shown to be genetically distinct from the majority of isolates. Further phylogenetic analysis was performed on 200 bp of variable region sequence from more than sixty Butyrivibrio-like isolates. Using these data a probe was designed which hybridized to the majority of Butyrivibrio-like strains, excluding the type strain. Additional probes were designed that hybridized to specific clusters within this diverse group of rumen bacterium. These new probes have proven useful in accessing the community profile and for use in determinative studies of Butyrivibrio in the rumen. POSTER

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