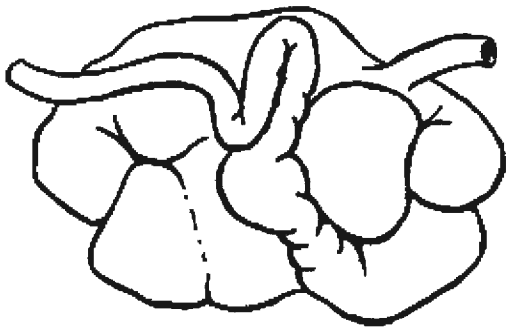
Abstracts

Conference on Rumen Function

Volume 24, 1997



46 Years of Interaction

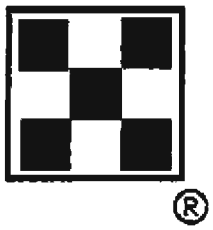
1951-1997

24th Biennial Conference on Rumen Function
Chicago, Illinois
November 11-13, 1997

This proceedings publication is a courtesy of

Purina Mills, Inc.

St. Louis, Missouri



24th BIENNIAL
CONFERENCE ON RUMEN FUNCTION
1951-1997

Welcome to the 24th Biennial Conference on Rumen Function. The Conference has been meeting in the Congress Hotel since November 1951. Initially, the Conference focused on the problem of bloat which continued as a central theme until 1961. The Conference has since 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 and J. B. Russell, ARS/USDA served as Chairman from 1991 to 1995.

Additional information regarding the Rumen Function Conference and future meetings can be accessed via the internet at http://pilot.msu.edu/user/rumen/. I hope that this current Conference will provide a stimulating and interesting forum.

Sincerely,

Michael S. Allen
Department of Animal Science
Michigan State University

Agenda

Tuesday, November 11, 1997

8:00 - 11:00 p.m. Poster session and mixer, Great Hall

Wednesday, November 12, 1997

Microbiology-Physiopathology Panel, Great Hall

9:00 a.m. Brief introduction by M. S. Allen

9:10 a.m.
#1 ADAPTATION BY *BUTYRIVIBRIO FIBRISOLVENS* TO RESIST PREDATION BY RUMINAL CILIATE PROTOZOA. C.J. Newbold, F.M. McIntosh, and R.J. Wallace, Rowett Research Institute, Bucksburn, Aberdeen, UK

9:30 a.m.
#2 COMPETITION AMONG THREE STRAINS OF RUMINAL CELLULOLYTIC BACTERIA IN CONTINUOUS CULTURE. J. Chen and P.J. Weimer, Dept. of Bacteriology, University of Wisconsin-Madison, and USDA-ARS, U.S. Dairy Forage Center, Madison, WI

9:50 a.m.
#3 FACTORS AFFECTING GLUCOSE AND MALTOSE PHOSPHORYLATION BY THE RUMINAL BACTERIUM *MEGASPHAERA ELSDENII*. S.A. Martin and E.L. Wani, Dept. of Animal and Dairy Science, University of Georgia, Athens

10:10 a.m.
#4 IDENTIFICATION OF A BIFUNCTIONAL XYLOSIDASE/ARABINOSIDASE IMPORTANT FOR XYLOOLIGOSACCHARIDE FERMENTATION BY THE RUMINAL ANAEROBE *SELENOMONAS RUMINANTIUM* GA192. T.R. Whitehead and M.A. Cotta. USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL

10:30 a.m.
#5 CELLULASE DIVERSITY AND DENSITY IN THE RUMEN ECOSYSTEM. THE USE OF DEGENERATE POLYMERASE CHAIN REACTION PRIMERS BASED ON DIFFERENT CELLULASE FAMILIES. S.A. Kocherginskaya and B.A. White, Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana

10:50 a.m. Brief break

11:10 a.m. Invited paper
#7 ADHERENCE TO CELLULOSE BY RUMINAL BACTERIA: WHERE COMPARISON TO THE CELLULOSOME PARADIGM ENDS AND COMPARATIVE PATHOBIOLOGY BEGINS? M. Morrison, Dept. of Animal Science and School of Biological Sciences, University of Nebraska, Lincoln

12:10 p.m. Lunch

1:30 p.m.
#6 IDENTIFICATION OF A SELENIUM-REDUCING BACTERIUM FROM THE SHEEP RUMEN. T. Wickman and M. Rasmussen, National Animal Disease Center, ARS, USDA, Ames, IA

1:50 p.m.
#8 METABOLISM OF ETHYLENE GLYCOL BY MIXED POPULATIONS OF RUMINAL MICROBES. M.J. Allison, H.M. Cook, and M.A. Rasmussen, National Animal Disease Center, ARS-USDA, Ames, IA

2:10 p.m.
#9 EXACERBATION OF LACTATE ACCUMULATION IN AN *IN VITRO* RUMINAL FERMENTATION MODEL BY MONILIFORMIN (A MYCOTOXIN). W. Wu[1], D.M. Schaeffer[1], D.K. Combs[2], and R.F. Vesonder[3], [1]Dept. of Animal Sciences, [2]Dept. of Dairy Science, University of Wisconsin, Madison, WI, [3]NCAUR, USDA/ARS, Peoria, IL

2:30 p.m.
#10 *IN VITRO* RUMEN FERMENTATION CHARACTERISTICS OF CONTINUOUS CULTURE RUMEN FERMENTERS FED A SUB-THERAPEUTIC LEVEL OF CHLORTETRACYCLINE AND TWO DIETARY PROTEIN LEVELS . T.E. Dawson, K.R. McLeod, and R.L. Baldwin, Nutrient Conservation and Metabolism Laboratory, USDA-ARS, Beltsville, MD

2:50 p.m.
#11 FIELD STUDIES OF POLIOENCEPHALOMALACIA (PEM) AND PATHOLOGIC RUMINAL HYDROGEN SULFIDE (H_2S) PRODUCTION IN CATTLE. D.H. Gould[1], G.H. Loneragan[1], D.W. Hamar[1], and J.J. Wagner[2], [1]Integrated Livestock Management Program and Diagnostic Lab, Colorado State University, Fort Collins, CO [2]Continental Beef Res, Lamar, CO

3:10 p.m.
#12 USE OF PLASMA GOSSYPOL TO ASSESS GOSSYPOL AVAILABILITY IN DAIRY COWS FED COTTON BY-PRODUCTS. M.C. Calhoun, B.C. Baldwin, Jr., and S.W. Kuhlmann, Texas Agricultural Experiment Station, Texas A&M University System, San Angelo, TX

3:30 p.m. Brief break

Nutrition-Agronomy Panel, Great Hall

4:00 p.m.
#13 AN EVALUATION OF AUTOMATED AND MANUAL *IN VITRO* METHODS FOR THE ESTIMATION OF NDF DIGESTION. M.A. Cohen, M.T. Maslanka, H.E. Swain, and L. Kung, Jr., Dept. of Animal and Food Sciences, University of Delaware, Newark

4:20 p.m.
#14 THE ROLE OF PH IN REGULATING RUMINAL METHANE PRODUCTION AND ACETATE TO PROPIONATE RATIO. R.P. Lana, J.B. Russell, and M. Van Amburgh. Section of Microbiology, and Dept. of Animal Science, Cornell University, Agricultural Research Service, USDA, Ithaca, New York

4:40 p.m.
#15 THE ROLE OF PH IN REGULATING RUMINAL DEAMINATION AND AMMONIA ACCUMULATION. R.P. Lana, J.B. Russell, and M. Van Amburgh. Section of Microbiology, and Dept. of Animal Science, Cornell University, Agricultural Research Service, USDA, Ithaca, New York

5:00 p.m. Invited Paper
#16 IMPACT OF TISSUE MATURATION ON CELL WALL DEGRADABILITY OF ALFALFA STEMS. H.G. Jung and F. M. Engels, USDA ARS, St. Paul, MN and Wageningen Agricultural University, The Netherlands

6:00 p.m. Dinner at your discretion

Thursday, November 13, 1997

Nutrition-Agronomy Panel (continued), Great Hall

8:30 a.m.
#17 INHIBITION OF SULFIDE PRODUCTION IN *IN VITRO* RUMINAL FERMENTATIONS. J.P. Bracht and L. Kung, Jr, Dept. of Animal and Food Sciences, University of Delaware, Newark, DE

8:50 a.m.
#18 EFFECT OF INCREASED RUMINAL LEVELS OF POLYSACCHARIDE-DEGRADING ENZYMES ON RUMEN FERMENTATION, ENZYME BY-PASS TO THE DUODENUM AND *IN VIVO* DIGESTIBILITY. A.N. Hristov, T.A. McAllister, and K.-J. Cheng, Agriculture and Agri-Food Canada Research Centre, Lethbridge

9:10 a.m.
#19 EVALUATION OF A FIBROLYTIC ENZYME ADDITIVE IN DAIRY COW DIETS. W.Z. Yang, K.A. Beauchemin, and L.M. Rode, Agriculture and Agri-Food Canada, Lethbridge

9:30 a.m.
#20 FEEDING VALUE FOR LAMBS OF CANOLA MEAL ARISING FROM BIODIESEL PRODUCTION. T.A. McAllister[1], K. Stanford[2], G.L. Wallins[1], M.T.J. Reaney[3], and K.-J. Cheng[1], Agriculture and Agri-Food Canada Research Centres, [1]Lethbridge, AB and [3]Saskatoon, SK; and [2]Alberta Agriculture, Food and Rural Development, Agriculture Centre, Lethbridge

9:50 a.m.
#21 FEEDING DIFFERENT LEVELS OF PHOSPHORUS AND NITROGEN TO STEERS. J.P. Fontenot, N.B. Frank, and R.K. Shanklin, Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA

10:10 a.m. Brief break

10:30 a.m.

#22 THE ROLE OF RUMINAL FERMENTATION IN REGULATION OF DMI OF HIGH PRODUCING DAIRY COWS. M. Oba and M.S. Allen, Dept. of Animal Science, Michigan State University, East Lansing

10:50 a.m.

#23 EFFECTS OF FORAGE SOURCE IN HIGH GRAIN DIETS ON TIME SPENT RUMINATING, RUMINAL PH AND FIBER DIGESTION. [2]M. Krause, [1]K.A. Beauchemin, [1]L.M. Rode, [1]B.I. Farr, and [2]P. Nørgaard, [1]Agriculture and Agri-Food Canada, Research Center, Lethbridge, AB, and [2]The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

11:10 a.m.

#24 FEEDING BEHAVIOUR OF FEEDLOT CATTLE MONITORED WITH RADIO FREQUENCY TECHNOLOGY. D. Gibb[1], R. Wiedmeier[2] and T.A. McAllister[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, and [2]Utah State University, Logan

11:30 a.m. Business meeting

12:00 noon Adjourn

Posters

#25 BIOTRANSFORMATION OF ANTI-NUTRITIONAL FACTORS BY RUMEN MICROBIOTA. A.H. Smith[1, 2], A.A. Odenyo[3], P.O. Osuji[3], and R.I. Mackie[1], [1]Dept. of Animal Sciences, University of Illinois, Urbana, IL 61801, [2]Agricultural Research Council, Irene, South Africa; [3]International Livestock Research Institute, Addis Ababa, Ethiopia

#26 EFFECT OF STEROIDAL SAPONIN FROM *YUCCA SCHIDIGERA* EXTRACT ON THE GROWTH OF RUMINAL BACTERIA. Y. Wang[1], T.A. McAllister[1], L.J. Yanke[1], P.R. Cheeke[2], and K.-J. Cheng[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, and [2]Desert King International, Chula Vista CA

#27 THE ROLE OF MICROORGANISMS IN LOCOWEED TOXICITY. T. May, C.M. Liddell, J.L. Riggs, T. Cook, K. Brown, and E.S. Woodruff, Depts. of Animal and Range Sciences and Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces

#28 THE POTENTIAL FOR SWAINSONINE BIOTRANSFORMATION OR UTILIZATION BY RUMINAL MICROORGANISMS. M. Morgan and T. May, Dept. of Animal and Range Sciences, New Mexico State University, Las Cruces

#29 TANNIN-TOLERANT BACTERIA FROM THE GASTROINTESTINAL TRACTS OF BIRDS, RUMINANTS, AND PRIMATES. T.K. Woolston, A.N. Pell, and K. De Leeuw, Dept. of Animal Science, Cornell University, Ithaca, NY

#30 THE EFFECTS OF INOCULATION WITH TANNIN-TOLERANT BACTERIA ON FIBER AND NITROGEN DIGESTIBILITY OF LAMBS FED HIGH TANNIN DIETS. D.O. Molina, A.N. Pell, and D.E. Hogue, Dept. of Animal Science, Cornell University, Ithaca, NY

#31 TOTAL RNA EXTRACTION FROM RUMEN BACTERIA EXPOSED TO TANNINS. P.B. Arcuri, A.N. Pell, and M.L. Thonney, Dept. of Animal Science, Cornell University, Ithaca, NY

#32 PCR DETECTION OF PYRIDINEDIOL-DEGRADING RUMINAL BACTERIUM, *SYNERGISTES JONESII*, IN THE RUMEN FLUID OF CATTLE. J. Yang[1], N. Du[2], J.R. Carpenter[2], and D. Borthakur[3], Depts. of Biochemistry[1], Animal Sciences[2] and Plant Molecular Physiology[3], University of Hawaii at Manoa, Honolulu, HI

#33 SMALL SUBUNIT RIBOSOMAL RNA SEQUENCE ANALYSIS OF ANAEROBIC RUMINAL FUNGI ISOLATED IN NEW ZEALAND. L.A. Thill[1], S.A. Kocherginskaya[1], G.E. Naylor[2], K.N. Joblin[2], and B.A. White[1], [1]Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL and [2]AgResearch, Grasslands Research Centre, Private Bag , Palmerston North, New Zealand

#34 CHARACTERIZATION OF PIROMYCES SP. (B3) ISOLATED FROM RUMEN OF A REVERINE BUFFALO. A.K. Samanta, T.K. Walli, V.K. Batish, S. Grover, and A.K. Montany, National Dairy Research Institute, Karnal , India

#35 USE OF THE AN-IDENT SYSTEM IN IDENTIFICATION OF RUMINAL BACTERIA. L.J. Yanke and K.-J. Cheng, Agriculture and Agri-food Canada Research Centre, Lethbridge, Alberta, Canada

#36 QUALITATIVE AND QUANTITATIVE STUDY OF THE EQUINE CAECAL CELLULOLYTIC BACTERIAL POPULATION. V. Julliand[1], A. de Vaux[1], L. Millet[2] and G. Fonty[2], [1]Laboratoire associé de Recherches Zootechniques INRA-ENESAD, Dijon Cédex, [2]Laboratoire de Microbiologie, INRA, CR de Clermont-Ferrand-Theix, Saint-Genès-Champanelle, France

#37 CULTIVATION OF CELLULOLYTIC COCCI FROM BUFFALO RUMEN. S. Iqbal, R. Singh, and S.P.S. Sodhi, Dept. of Veterinary Physiology, Punjab Agricultural University, Ludhiana, India

#38 ECOLOGICAL ROLE OF A SUCCINATE-DEGRADING BACTERIUM FROM THE RUMEN. T.L. Weddington, D.M. Hopkins, H.J. Strobel, and K.A. Dawson, Dept. of Animal Sciences, University of Kentucky, Lexington

#39 EFFECT OF RUMEN CELLULOLYTIC BACTERIAL CONCENTRATIONS ON *IN SITU* DIGESTION OF FORAGE CELLULOSE. B. A. Dehority and P. A. Tirabasso, Dept. of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster

#40 A COMPARATIVE STUDY OF THE *FIBROBACTER* POPULATION IN THE RUMEN OF DIFFERENT CATTLE BREEDS FED A LOW-QUALITY ROUGHAGE. B.G. Greyling, A.H. Smith, and E.H. Osler, Agricultural Research Council, ANPI, Irene, South Africa

#41 EFFECT OF DIET ON RUMEN MICROFLORA AND MICROFAUNA IN BUFFALO CALVES. S. Singh and R. Singh, Dept. of Veterinary Physiology, College of Veterinary Science, Punjab Agricultural University, Ludhiana (Punjab) India

#42 VARIATION OF SULFATE REDUCING BACTERIA POPULATIONS IN BOVINE RUMEN. M.G.Delgado[1] and R.L. Sager[2], [1]Private practitioner, [2]Instituto Nacional de Tecnologia Agropecuaria, EEA San Luis, Villa Mercedes, San Luis, Argentina

#43 EFFECTS OF A MICROBIAL ADDITIVE, LEVUCELL® SC, ON GROWTH AND METABOLISM OF A RUMINAL ACETOGENIC BACTERIAL STRAIN *IN VITRO*. F. Durand-Chaucheyras[1,2], G. Fonty[1], and G. Bertin[2], [1]Laboratoire de Microbiologie, INRA, CR Clermont-Ferrand Theix, 63122 Saint-Genès Champanelle , [2]SANTEL-groupe Agritek, Levallois-Perret, France

#44 EFFECT OF NONIONIC SURFACTANT ON FERMENTATION CHARACTERISTICS AND ENZYME ACTIVITY DURING *IN VITRO* INCUBATION OF BARLEY GRAIN. D.R. ZoBell[1]*, T.A. McAllister[2], J. A. Shelford[3], M. Goto[4] R. Wiedmeier[5] and K.-J. Cheng[2]. [1]Alberta Agriculture, Food and Rural Development, Lethbridge, AB, [2]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB; [3]Dept. of Animal Science, University of British Columbia, Vancouver, BC ; [4]Faculty of Bioresources, Mie University, Tsu, Japan, [5]Utah State University, Logan UT

#45 BACTERIA FROM CATTLE FED CONCENTRATES BECOME RESISTANT TO MONENSIN BUT NOT NISIN. T.R. Callaway, R.P. Lana, and J.B. Russell, Section of Microbiology, Cornell University, and Agricultural Research Service, USDA, Ithaca, New York

#46 THE ADVERSE EFFECT OF NITROGEN LIMITATION AND EXCESS CELLOBIOSE ON *FIBROBACTER SUCCINOGENES* S85. G. Maglione and J.B. Russell, Section of Microbiology and Cornell University and Agricultural Research Service, USDA, Ithaca, New York

#47 COMPLEMENTATION OF AN *ESCHERICHIA COLI* ADENYLATE CYCLASE MUTANT WITH A GENE FROM *PREVOTELLA RUMINICOLA* D31D. T.R. Whitehead[1], M.A. Cotta[1], and M.B. Wheeler[2], [1]USDA/ARS, National Center Agricultural Utilization Research, Peoria, IL, and [2]Dept. of Animal Sciences, University of Illinois, Urbana

#48 ACID TOLERANCE OF *ESCHERICHIA COLI* O157:H7 UNDER AEROBIC CONDITION. E.S. Woodruff and T. May, Dept. of Animal and Range Sciences, New Mexico State University, Las Cruces

#49 COMPETITIVE COLONIZATION OF SHEEP BY *E. COLI* O157:H7. N.A. Cornick, T.A. Casey and H.W. Moon, Veterinary Medical Research Institute, Iowa State University and National Animal Disease Center, Ames, IA

#50 REGULATION OF GLYCOGEN FORMATION IN CONTINUOUS CULTURE BY THE RUMINAL BACTERIUM *PREVOTELLA BRYANTII* $B_1 4$. H.J. Strobel and C.R. Jones, Dept. of Animal Sciences, University of Kentucky, Lexington

#51 INITIAL CHARACTERIZATIONS OF PROTEOLYTIC BACTERIA TOLERANT TO CONDENSED TANNINS. C.S. McSweeney, B. Palmer, and D. O. Krause, CSIRO Tropical Agriculture, Indooroopilly, Australia

#52 ISOLATION AND CHARACTERISATION OF MUTANTS OF *PREVOTELLA ALBENSIS* (FORMERLY *PREVOTELLA RUMINICOLA*) M384 WITH DECREASED PEPTIDASE ACTIVITY. N.D. Walker and R.J. Wallace, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK.

#53 THE GINGIPAIN-LIKE (PrtA) ACTIVITY OF *PREVOTELLA BRYANTII* STRAIN $B_1$4. H.M.F. Madeira and M. Morrison, Dept. of Animal Science, University of Nebraska-Lincoln, Lincoln

#54 SEQUENCING AND CLONING OF A FAMILY I GLUTAMATE DEHYDROGENASE FROM *RUMINOCOCCUS FLAVEFACIENS* FD-1. D.A. Antonopoulos, R. Aminov, B.A. White, and R.I. Mackie, Dept. of Animal Sciences, University of Illinois at Urbana-Champaign

#55 THE GLUTAMINE SYNTHETASE (GlnN) OF *PREVOTELLA BRYANTII* $B_1$4. Z. Wen and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln

#56 PARTIAL PURIFICATION OF ENDOGLUCANASE OF *PIROMYCES* SP. (B3) ISOLATED FROM RUMEN OF A REVERINE BUFFALO. T.K. Walli, A.K. Samanta, Y.S. Rajput, V.K. Batish, S. Grover, and A.K. Mohanty, National Dairy Research Institute, Karnal, India

#57 A CELLULOSE-BINDING PROTEIN FROM THE GRAM-POSITIVE BACTERIUM *RUMINOCOCCUS ALBUS* BELONGS TO THE PIL-FAMILY OF PROTEINS AND IS INVOLVED WITH SURFACE ASSOCIATED PROTEIN COMPLEXES. M.A. Larson, R.S. Pegden, R.J. Grant, and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln

#58 ADHERENCE TO CELLULOSE BY *RUMINOCOCCUS ALBUS* STRAIN 8. S.K. Reddy and M. Morrison, Dept. of Animal Sciences, University of Nebraska, Lincoln

#59 CELLULOSE-BINDING PROTEINS (GLYCOPROTEINS) INVOLVED IN ADHERENCE OF *FIBROBACTER SUCCINOGENES* S85 TO CELLULOSE. E.E. Egbosimba, J. Miron, and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Guelph, Ontario

#60 CELLULAR LOCALIZATION OF NUCLEASE ACTIVITY IN *FIBROBACTER SUCCINOGENES* S85. S.R. MacLellan and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Guelph, Ontario

#61 TEMPERATURE AND PH PROFILES OF RUMINAL ENZYME FRACTIONS FROM ANIMALS FED INCREASING PROPORTIONS OF CORN IN A CORN/CORN SILAGE DIET. V.L. Nsereko, L.M. Rode, K.A. Beauchemin, A.F. Furtado, Z. Mir, and P.S. Mir, Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

#62 RUMINAL POLYSACCHARIDASE ACTIVITIES IN ANIMALS FED INCREASING PROPORTIONS OF CORN IN A CORN/CORN SILAGE DIET V.L. Nsereko, L.M. Rode, K.A. Beauchemin, A.F. Furtado, Z. Mir, and P.S. Mir., Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada

#63 DIGESTION KINETICS OF CORN GRAIN. Y.-K. Chen, P. Schofield, A.N. Pell, and L.E. Chase, Dept. of Animal Science, Cornell University, Ithaca, NY

#64 *IN VITRO* DIGESTION KINETICS OF TROPICAL FORAGES. F.I. Juarez, P. Schofield, and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY

#65 SOYHULL DIET SUPPRESSION OF *IN SITU* DIGESTION KINETICS. R. Uctuk, D.E. Johnson, and G. Bernal, Dept. of Animal Science, Colorado State University, Fort Collins

#66 EFFECT OF BM3 MUTATION ON RATE AND EXTENT OF NEUTRAL DETERGENT FIBER DIGESTION OF CORN SILAGE GROWN IN MULTIPLE ENVIRONMENTS. Y. Ying and M.S. Allen, Dept. of Animal Science, Michigan State University, East Lansing

#67 EFFECTS OF CORN GRAIN PARTICLE SIZE AND MATURITY AT HARVEST ON FEEDING BEHAVIOR AND RUMINAL PH OF HOLSTEIN HEIFERS BEFORE AND AFTER CALVING. Y. Ying, M.S. Allen and M.J. VandeHaar, Michigan State University, East Lansing

#68 EFFECT OF FATTY ACIDS AND THEIR Ca-SALTS ON RUMINAL FERMENTATION, DIGESTION, AND MICROBIAL GROWTH DURING INCUBATION OF GRASS HAY *IN VITRO*. U.M. Yang and H. Fujita, Dept. of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro-shi 080, JAPAN

#69 PREDICTION OF VFA PROPORTIONS FROM NUTRIENT INTAKE AND AVAILABILITY [1]K. A. Beauchemin, [2]M.R. Weisbjerg, [2]T. Hvelplund, [2]B.M. Bibby, and [3]P. Nørgaard, [1]Agriculture and Agri-Food Canada, Research Center, Lethbridge, AB; [2]Danish Institute of Animal Science, Tjele, Denmark; and [3]The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

#70 TRACE ELEMENTS STATUS IN THE DIFFERENT FRACTIONS OF RUMEN LIQUOR UNDER DIFFERENT DIETARY REGIMS. S. Singh and R. Singh, Dept. of Veterinary Physiology, College of Veterinary Science, Punjab Agricultural University, Ludhiana (Punjab), India

#71 ALTERED DIETARY ENERGY UTILIZATION BY LACTATING DAIRY COWS FED HIGH LEVELS OF WHOLE COTTONSEED. J.R. Carpenter, H.A. Rachuonyo, and R.Y. Niino-DuPonte, Dept. of Animal Sciences, CTAHR, University of Hawaii at Manoa, Honolulu

#72 EFFECT OF *YUCCA SCHIDIGERA* EXTRACT ON RUMINAL FERMENTATION *IN VITRO*. Y. Wang[1], T.A. McAllister[1], L.J. Yanke[1], P.R. Cheeke[2], and K.-J. Cheng[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB and [2]Desert King International, Chula Vista CA

#73 EFFECT OF SESBANIA SPECIES ON RUMINAL PROTOZOA IN ETHIOPIAN AND SCOTTISH SHEEP. B. Teferedegne[1,2], P.O. Osuji[2], A. Odenyo[2], R.J. Wallace[1], and C.J. Newbold[1], [1]Rowett Research Institute, Aberdeen, UK and [2]International Livestock Research Institute (ILRI) , Addis Ababa, Ethiopia

#74 EFFECT OF 9,10-ANTHRAQUINONE ON MICROBIAL METHANE PRODUCTION, RUMINAL FERMENTATION, AND NUTRIENT DIGESTION IN SHEEP. K.M. Koenig[1], L.M. Rode[1], L. Kung, Jr.[2], and L. Neill[1], [1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta and [2]Dept. of Animal Science and Agricultural Biochemistry, University of Delaware, Newark

#75 EFFECT OF FREEZING AND EXTRACTION METHOD ON POLYSACCHARIDE-DEGRADING ACTIVITIES OF RUMEN CONTENT. A.N. Hristov, T.A. McAllister, and K.-J. Cheng, Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB

#76 TANNIN-PROTEIN INTERACTIONS IN SOLUTION STUDIED BY FLUORESCENCE QUENCHING. P. Schofield and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY

Abstracts -Podium

#1 ADAPTATION BY *BUTYRIVIBRIO FIBRISOLVENS* TO RESIST PREDATION BY RUMINAL CILIATE PROTOZOA.

C.J. Newbold, F.M. McIntosh, and R.J. Wallace, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK (44-1224-712751)

Butyrivibrio fibrisolvens SH13 was labelled by growing in the presence of [^{14}C]leucine and was then incubated in ruminal fluid *in vitro*. Ciliate protozoa caused a rapid rate of breakdown (34.7%/h) of labelled bacteria. This appeared to be incompatible with the survival of *B. fibrisolvens* in the rumen, where the liquid outflow rate is usually less than 10%/h. When filter-sterilized extract prepared from sonicated washed protozoa was added to the original growth medium, the rate of breakdown of *B. fibrisolvens* grown in this medium fell to 8.0%/h due to an increased production of extracellular polysaccharide. Autoclaving the extract abolished its effect. In contrast, growth of *Selenomonas ruminantium* Z108 in the presence of the protozoal extract had no effect on its susceptibility to protozoal lysis. Fewer bacteria were retained within protozoa when *B. fibrisolvens* was grown in the presence of protozoal extract and the rate of breakdown decreased with whole but not sonicated protozoa, indicating that adaptation of *B. fibrisolvens* caused decreased engulfment rather than increased resistance to bacteriolytic enzymes.

#2 COMPETITION AMONG THREE STRAINS OF RUMINAL CELLULOLYTIC BACTERIA IN CONTINUOUS CULTURE.

J. Chen and P.J. Weimer, Dept. of Bacteriology, University of Wisconsin-Madison, and USDA-ARS, U.S. Dairy Forage Center, Madison, WI (608-264-5408)

Strains of 3 predominant species of ruminal cellulolytic bacteria -- *Fibrobacter succinogenes* S85, *Ruminococcus flavefaciens* FD-1, and *Ruminococcus albus* 7 -- were grown in triculture in cellulose-limited chemostats at dilution rates of 0.016, 0.026, and 0.046/h. Oligonucleotide probes to species-specific 16S rRNAs were used to estimate both the cellulose-adherent and nonadherent (planktonic) populations. *F. succinogenes* S85 represented 52-74% of the adherent population and 41-68% of the planktonic population. *R. albus* 7 accounted for 22-45% of the adherent population and 27-45% of the planktonic population. *R. flavefaciens* FD-1 was present in both adherent and planktonic populations, but usually at <5% of each population. The data indicate that all 3 strains can coexist under steady-state conditions. The similar distribution of the 3 strains in the adherent and planktonic populations suggest that these strains are competing within the same niche rather than interacting as specialist populations preferring cellulolytic or cellodextrin-fermenting (non-cellulolytic) growth modes.

#3 FACTORS AFFECTING GLUCOSE AND MALTOSE PHOSPHORYLATION BY THE RUMINAL BACTERIUM *MEGASPHAERA ELSDENII*.

S.A. Martin and E.L. Wani, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA 30602 (706-542-1065)

Previous research demonstrated that *Megasphaera elsdenii* phosphorylates hexoses (glucose, fructose, and 2-deoxyglucose) using the phosphoenolpyruvate phosphotransferase system (PEP- PTS). However, little information is available detailing factors that affect hexose phosphorylation or maltose phosphorylation by this important ruminal anaerobe. Therefore, the objectives of this study were to examine the effects of growth substrate and extracellular pH on PEP-dependent glucose phosphorylation as well as examine how maltose is phosphorylated by *M. elsdenii* B159. Phosphoenolpyruvate-dependent glucose phosphorylation by toluene-treated cells was constitutive, and extracellular pH values of 6.5 and 5.0 reduced glucose phosphorylation by 7 and 54%, respectively. When toluene-treated cells were incubated in histidine buffer, little maltose phosphorylation occurred in the absence of inorganic phosphate. However, the addition of increasing concentrations of either potassium or sodium phosphate increased maltose phosphorylation. Maximal phosphorylation activity was observed between 25 and 50 mM with either inorganic phosphate source. Compared to the control incubations, maltose phosphorylation was increased over 3-fold with 25 mM of either potassium or sodium phosphate. Phosphoglucomutase activity was detected in cell extracts of *M. elsdenii* and this enzyme had a Km of 3.2 mM for glucose 1-P and a Vmax of 1,836 nmol of NADP+ reduced/mg of protein per min. To our knowledge, this is the first report of a maltose phosphorylase in *M. elsdenii*.

#4 IDENTIFICATION OF A BIFUNCTIONAL XYLOSIDASE / ARABINOSIDASE IMPORTANT FOR XYLOOLIGOSACCHARIDE FERMENTATION BY THE RUMINAL ANAEROBE *SELENOMONAS RUMINANTIUM* GA192.

T.R. Whitehead* and M.A. Cotta. USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604 (309-681-6272)

Xylan is an abundant polysaccharide in plant cell walls and, as such, comprises a significant portion of the diets of ruminant livestock. The enzymatic hydrolysis of xylan is accompanied by the formation of xylose, arabinose, and methyl-glucuronic acid containing oligosaccharides. These oligosaccharides can be utilized by several species of xylanolytic ruminal bacteria and *Selenomonas ruminantium*, a non-xylanolytic species. The objective of this study was to examine the fermentation of xylooligo-saccharides by strains of *S. ruminantium* and determine the enzymes and genes that may be important in the utilization of xylooligosaccharides by this organism. Strains of *S. ruminantium* varied in their capacity to ferment xylooligosaccharides. The ability of *S. ruminantium* strains to utilize xylooligo-saccharides was correlated with the presence of xylosidase and arabinosidase activities. A genetic locus from *S. ruminantium* GA192 was isolated and sequenced that produced both xylosidase and arabinosidase activities in *Escherichia coli*. Analyses indicated that a single protein was responsible for both activities in *S. ruminantium* GA192 and the *E. coli* clone. The enzyme expressed in *E. coli* was capable of degrading xylooligosaccharides. Attempts are underway to introduce the cloned gene into *S. ruminantium* strains.

#5 CELLULASE DIVERSITY AND DENSITY IN THE RUMEN ECOSYSTEM. THE USE OF DEGENERATE POLYMERASE CHAIN REACTION PRIMERS BASED ON DIFFERENT CELLULASE FAMILIES. S.A. Kocherginskaya and B.A. White, Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801 (217-333-2091)

Cellulase-specific polymerase chain reaction (PCR) primer pairs based on conserved cellulase family-specific sequences for families B, C, F, and G were used to generate cellulase gene sequence diversity profiles from samples from the rumen. First, the primer sets were used to analyze various cellulolytic *Ruminococci*. These data show that PCR products of different sizes are generated from different strains. When these primer sets were used with DNA from *Streptococcus bovis,* no PCR products were detected suggesting that these primers are specific for cellulolytic microorganisms. These cellulase specific primers were then used with rumen samples from a hay fed steer and grain fed steer. Different rumen samples give different patterns of genes and all of the primer pairs tested showed a PCR product. In addition, the overall complexity of the PCR product profiles appears to be much simpler than those derived from pure cultures. Furthermore, the PCR patterns derived from the microbial populations from the hay fed steer are much simpler than the patterns derived from the grain fed steer. This suggests that the hay diet selects for a highly specialized cellulolytic population, whereas the grain diet seems to select for a more diverse non-specific cellulolytic population. In order to confirm that the PCR products detected using the degenerate cellulase primers represent cellulase genes, we cloned and sequenced the predominant cellulase F family product derived from the PCR of a rumen sample from a hay fed steer. The amino acid sequence derived from the DNA sequence of this PCR product shows similarity to family F cellulases in the GenBank database. We believe that this method can be used to measure cellulase gene diversity and density and in correlation with rumen community structure data can provide an accurate picture of both community diversity and functionality.

#6 IDENTIFICATION OF A SELENIUM-REDUCING BACTERIUM FROM THE SHEEP RUMEN. T. Wickman and M. Rasmussen, National Animal Disease Center, ARS, USDA, Ames, IA 50010 (515-239-8200)

The capacity to reduce selenate to insoluble, elemental selenium has been described for several groups of microorganisms ranging from facultative anaerobes such as *E. coli* to common rumen species like *Selenomonas ruminantium.* This process may serve as either a means of respiration or detoxification. Reduction of selenium by rumen microorganisms may play a role in the dietary availability of selenium because reduced selenium is not nutritionally useful to the animal. However, a positive repercussion of microbial metabolism is that the animal is able to tolerate increased levels of this potentially toxic element. A strictly anaerobic, gram-negative, selenium-reducing bacterium was isolated from the rumen of a sheep. 16S ribosomal RNA analysis revealed a 98% similarity to *Wolinella succinogenes*, yet this organism failed to grow on a common *Wolinella* media containing formate and fumarate. The organism appears to use selenate specifically as an electron acceptor. Growth occurred in the presence of H_2 and selenate but not with H_2 and sulfate or nitrate. Growth was poor when lactate or acetate were substituted for H_2 as electron donors. Oligonucleotide probes have been designed for several selenium-reducing bacteria and will be used to examine changes in rumen populations in animals which are fed different levels of dietary selenium.

#7 **INVITED PAPER**

ADHERENCE TO CELLULOSE BY RUMINAL BACTERIA: WHERE COMPARISON TO THE CELLULOSOME PARADIGM ENDS AND COMPARATIVE PATHOBIOLOGY BEGINS? **M. Morrison, Dept. of Animal Science and School of Biological Sciences, University of Nebraska, Lincoln, NE 68583-0908 (402-472-9382)**

The cellulosome paradigm, developed largely from the study of *Clostridium* spp., is the most firmly established example of a stable, multienzyme complex specialized in attachment to and degradation of crystalline cellulose. However, mechanism(s) involved with rumen bacterial and enzyme binding to cellulose are still poorly understood, impeding our ability to manipulate a rate-limiting step in ruminal fiber digestion kinetics. Although large molecular mass cellulase complexes have been identified in a variety of anaerobic bacteria, including *Bacteroides cellulosolvens*, *Fibrobacter succinogenes*, and *Ruminococcus albus*, it is still unclear to what extent the Clostridial paradigm can be applied to these other bacteria. With specific reference to *Ruminococcus* spp., recent analysis of the EndA gene from *R. flavefaciens* revealed that a distant relationship exists between regions of an 80 amino acid sequence in EndA, and the duplicated 23 amino acid dockerin sequences found in *C. thermocellum* cellulosomal enzymes. Even though such findings are consistent with the hypothesis that cellulosome-like structures are produced by at least some *Ruminococcus* spp., virtually all of the endoglucanase genes cloned to date lack other features characteristic of cellulosomal enzymes. Recent research has shown that *Ruminococcus albus* strain 8 produces a cellulose-binding protein (*cbpC*) containing the amino terminal motif characteristic of the Pil-family of proteins. The Pil-protein family is involved with synthesis and assembly of surface-associated protein complexes, including Type 4 fimbrial proteins, proteins of the general secretory pathway (gsp), and DNA transfer systems. The *R. albus* strain 8 *cbpC* protein possesses a striking degree of structural homology with Type 4 fimbrial proteins, which are the best characterized of the Pil-proteins, and until now, were only known to be produced by gram-negative pathogenic bacteria. In addition to the Type 4 fimbriae, a number of plant pathogens synthesize and secrete via the gsp cellulases and other plant cell wall hydrolases, once colonization of the cell surface has occurred and(or), in response to other exogenous stimuli. Interestingly, micromolar concentrations of phenylacetic (PAA) and phenylpropionic (PPA) acids stimulate cellulase enzyme production by *R. albus*, as well as the number of cells adherent to cellulose, and cellulose digestion kinetics. The cell morphology of *R. albus* is also altered in response to micromolar concentrations of PAA and PPA, both vesicular and fimbrial structures are produced, and cellulases remain associated with the bacterial capsule as part of large molecular mass protein complexes. Southern blot analyses has revealed that a number of *R. albus* strains possess multiple genes encoding Pil-protein homologs, and Australian researchers have found the genome of at least one strain of *B. fibrisolvens* also encodes a gsp protein homolog(s). Therefore, this system of surface-complex assembly in fiber-degrading ruminal bacteria may be relatively common. Findings such as these suggest that rumen microbiologists and nutritionists may learn more about factors affecting fiber degradation from comparative pathobiology, in addition to comparisons with the cellulosome paradigm. Our findings also raise interesting questions relevant to the origin and evolution of cellulose-degrading complexes in ruminal bacteria: do some genes originate from plant pathogens, and if so, how were they transferred to ruminal bacteria?

#8 METABOLISM OF ETHYLENE GLYCOL BY MIXED POPULATIONS OF RUMINAL MICROBES.

M.J. Allison, H.M. Cook, and M.A.Rasmussen, National Animal Disease Center, ARS-USDA, Ames, IA 50010 (515-239-8373)

Ethylene glycol is oxidized to oxalate by animal tissue enzymes (alcohol dehydrogenases) and the main pathologic feature of ethylene glycol poisoning in man and animals is a renal necrosis caused by calcium oxalate crystals. It is possible that ethylene glycol has a different fate in ruminants as a result of microbial metabolism in the rumen. We incubated mixed populations of ruminal microbes from sheep and/or cattle with ethylene glycol to determine the effects of added ethylene glycol on various fermentation parameters and to determine the metabolic fate of the ethylene glycol. No change was found in the amounts of acetate, propionate, butyrate, or methane produced during 2 h fermentations *in vitro* with 1% or 5% ethylene glycol. However, with 10 or 20% ethylene glycol, acetate, butyrate and methane productions were inhibited. When (UL) 14C-ethylene glycol was incubated with coarsely strained rumen fluid from a cow, the rate of loss of ethylene glycol from an initial concentration of 20 mM was 3.8 umol/ml/h. This rate was only slightly increased when a carbohydrate mixture (0.4% w/v) was added. The labelled products detected were mainly acetate and ethanol, and measurements obtained suggest that the ethanol formed was subsequently converted to acetate. We suggest that the most probable reaction sequence (for ethylene glycol) is dehydration to acetaldehyde followed by dismutation to acetate and ethanol.

#9 EXACERBATION OF LACTATE ACCUMULATION IN AN *IN VITRO* RUMINAL FERMENTATION MODEL BY MONILIFORMIN (A MYCOTOXIN).

W. Wu[1], D.M. Schaeffer[1], D.K. Combs[2], and R.F. Vesonder[3], [1]Dept. of Animal Sciences, [2]Dept. of Dairy Science, University of Wisconsin, Madison, WI 53706, [3]NCAUR, USDA/ARS, Peoria, IL 61604 (608-262-6004)

In vitro batch fermentation consisting of equal volume of ruminal fluid (as inoculum taken from a fistulated cow fed a diet with 66.6% alfalfa hay and 33.4% concentrate) and McDougall's buffer (pH adjusted to 6.0 with HCl) and a substrate (60 mM of glucose or 250 mg of alfalfa hay powder) was carried out at 39° C for 24 h to observe the effects of moniliformin (MN) on concentration changes of lactate, glucose, and volatile fatty acids (VFA). Only glucose formed an acidotic model. Compared with control, significantly 30% more lactate accumulated in the fermentation containing 25 uM (micromoles/liter) of MN by 3 h of incubation. Same effect was observed by 1.5 h with 50 uM or more of MN. More lactate (> 30 to 120% of control) was observed towards to 24 h of incubation. Transitional higher lactate (> 30% of control) was observed with 4.5 and 6 h samples from fermentation containing 5 uM of MN. MN did not affect glucose and VFA metabolism. Addition of 100 uM of thiamin did not negate the effect of 50 uM of MN on lactate accumulation, suggesting that the mode of action did not relate to thiamin. MN (100 uM) did not affect the alfalfa fermentation model. Since MN-producing molds have been recovered from high moisture corn associated with chronic acidosis, we postulated that MN can exacerbate grain overfed acidosis.

#10 *IN VITRO* RUMEN FERMENTATION CHARACTERISTICS OF CONTINUOUS CULTURE RUMEN FERMENTERS FED A SUB-THERAPEUTIC LEVEL OF CHLORTETRACYCLINE AND TWO DIETARY PROTEIN LEVELS . T.E. Dawson, K.R. McLeod, and R.L. Baldwin, Nutrient Conservation and Metabolism Laboratory, USDA-ARS, Beltsville, MD 20705 (301-504-8355)

An *in vitro* ruminal fermentation study was used to investigate the influence of feeding sub-therapeutic levels of chlortetracycline (CTC) at 2 levels of dietary protein (PRO) on ruminal fermentation characteristics. Rumen fluid was obtained at slaughter from steers fed 1 of 4 treatments (- or + CTC and 10% or 13% PRO) and used to initiate continuous culture artificial rumens. CTC and 13% PRO resulted in lower (P<.001) ammonia. However, CTC and 10% PRO had in similar (P>.05) concentrations of ammonia. CTC with 13% PRO resulted in higher (P<.001) pH and acetate, as well as, lower (P<.001) acetate, iso-butyrate, iso-valerate, n-valerate, and total VFA. Fermentors receiving 10% PRO and CTC had similar (P>.05) pH, and total VFA. When CTC was added to fermentors receiving 10% PRO, acetate, iso-butyrate, iso-valerate and the acetate to propionate ratio were lower (P<.001). Propionate was higher (P<.001) when CTC was fed with 10% PRO, but was similar (P>.05) when fed at 13% PRO. Acetate to propionate ratio was decreased (P<.001) by addition of CTC at both PRO levels, with the 10% PRO being lower (P<.001) than 13% PRO. Under these circumstances, CTC maintained similar ammonia concentrations at both 10% and 13% PRO, indicating a sparing of dietary protein.

#11 FIELD STUDIES OF POLIOENCEPHALOMALACIA (PEM) AND PATHOLOGIC RUMINAL HYDROGEN SULFIDE (H_2S) PRODUCTION IN CATTLE. D.H. Gould[1], G.H. Loneragan[1], D.W. Hamar[1], and J.J. Wagner[2], [1]Integrated Livestock Management Program (GHL) and Diagnostic Lab (DHG, DWH), Colorado State University, Fort Collins, CO 80523 (970-491-6144), [2]Continental Beef Res, Lamar, CO 81052

Using a field technique to estimate ruminal H_2S concentrations, we have investigated PEM under different circumstances of sulfur intake and shown that elevated ruminal H_2S concentrations coincide with the onset of PEM. In the first investigation, 3 groups of 9 feedlot steers (324 kg) were maintained on water with varying sulfate content (125, 500, 2000 ppm). Ruminal gas cap H_2S concentrations were measured during the feeding period. It was found that water sulfate levels were directly related to ruminal H_2S levels. The time period with the highest ruminal H_2S coincided with the portion of the feeding period when cases of PEM were most numerous historically. In the second investigation, 150, 8 month old, beef calves were weaned and fed grass hay containing Canada thistle. Between the 5th and 7th d post-weaning, 21 calves had signs of PEM and the brain of 1 necropsied calf had characteristic lesions of PEM. On the 7th d post-weaning, 10 clinically normal pen-mates at the ranch were examined. Rumen gas cap H_2S concentrations ranged from 2000-11,750 ppm H_2S, with a median of 6000 ppm H_2S. Excessive sulfur intake was demonstrated. Water sulfate concentration was 1990 ppm and the hay had 0.53% total sulfur on a dry matter basis. (Supported in part by the Colorado State Experiment Station and the Meat Research Corp, Australia).

#12 USE OF PLASMA GOSSYPOL TO ASSESS GOSSYPOL AVAILABILITY IN DAIRY COWS FED COTTON BY-PRODUCTS.

M.C. Calhoun, B.C. Baldwin, Jr., and S.W. Kuhlmann, Texas Agricultural Experiment Station, Texas A&M University System, San Angelo, TX 76901 (915-653-4576)

A major step toward a better understanding of how animals use gossypol was the recent development of high performance liquid chromatography (HPLC) procedures for the simultaneous determination of total, and (+)- and (–)-gossypol in plasma, tissues and cotton by-products. The procedures involve separation by HPLC after precolumn derivitisation with (R)-(–)-2-amino-1-propanol. A 4 mm x 100 mm Inertsil C18 reverse- phase column operated isocratically with a mobile phase consisting of 80% acetonitrile, 20% 10 mM KH_2PO_4 adjusted to pH 3.0 with H_3PO_4 and a flow rate of 1.0 ml/min was used to separate (+)- and (–)-gossypol. Retention times were 2.3 and 3.8 min for the 2- amino propanol derivatives of (+)- and (–)-gossypol, respectively. These procedures are rapid, sensitive and specific and provide information on the availability of gossypol from different sources, as well as the effects of processing, dietary, environmental, and animal factors on its availability. Plasma gossypol reflects the level and availability of gossypol in the diet and the proportion of isomers being fed. It plateaus in 4 to 6 weeks and remains fairly constant until the diet is changed. Plasma and tissue levels are highly correlated. These characteristics make plasma gossypol an ideal tool for (1) understanding how gossypol from different sources is used, (2) assessing factors that alter gossypol availability, and (3) establishing levels of cottonseed and cottonseed meal that can be fed safely. Lactating dairy cattle fed whole, linted cottonseed at levels of 2.3 to 3.6 kg/day have 1.5 to 3.5 micrograms of total gossypol per ml of plasma. Gossypol poisoning has occurred in herds where plasma levels exceeded 10 micrograms of total gossypol per ml and 5.0 micrograms per ml is considered the safe upper limit for dairy cattle being fed cotton by-products for extended periods.

#13 AN EVALUATION OF AUTOMATED AND MANUAL *IN VITRO* METHODS FOR THE ESTIMATION OF NDF DIGESTION.

M.A. Cohen*, M.T. Maslanka, H.E. Swain, and L. Kung, Jr., Dept. of Animal and Food Sciences, University of Delaware, Newark, DE 19717 (302-831-2522)

Several procedures were tested for the estimation of *in vitro* NDF digestion (NDF-D) of corn silage (43.2% NDF). In Experiment 1, feed (0.5 g) was incubated in 100 ml tubes (Goering and Van Soest,1970). NDF-D after 12, 24, 48, and 72 h was 26.3, 43.9, 50.5, and 52.9% for tubes constantly shaken (orbital shaker) and 9.0, 23.5, 42.8, and 50.8% for tubes manually shaken 3 times per d. In Experiment 2, feed (0.5 g) was incubated in filter bags (F57, ANKOM Technology, Fairport, NY) in a revolving digestion incubator (Daisy II, ANKOM Technology) and compared to feed incubated in test tubes on an orbital shaker. Filter bags were tested as pre-washed (5 min acetone) and unwashed. NDF-D after 12, 16, and 24 h were 2.7, 4.9, and 9.8% for unwashed F57; 5.4, 10.1, and 20.6% for washed F57, and 22.4, 31.7, and 46.5% for test tube samples. Lower digestion in bags than from constantly shaken tubes may have been due to retention of gas and acid end products within the bags. In Experiment 3, NDF-D after 12, 24, 48, and 72 h was 7.5, 21.9, 46.7, and 55.4% from washed F57 (0.5 g feed); 22.3, 44.2, 56.2, and 57.1% from washed F57 (0.5 g) that were regularly compressed (every 1 min) in a prototype digestor; 20.0, 42.0, 52.8, and 57.6% from washed F57 (0.25 g); and 18.7, 41.6, 56.3, and 57.1% from tubes constantly shaken (0.5 g). This study indicates that constant shaking of test tubes improved NDF digestion values. With the Daisy II system, rinsing bags in acetone prior to filling and a smaller sample size (0.25 g) resulted in NDF-D values similar to that of shaking tubes. Using the Daisy II *in vitro* system dramatically reduces analytical time because NDF-D determinations can be processed in bulk.

#14 THE ROLE OF PH IN REGULATING RUMINAL METHANE PRODUCTION AND ACETATE TO PROPIONATE RATIO. R.P. Lana, J.B. Russell, and M. Van Amburgh. Section of Microbiology, and Dept. of Animal Science, Cornell University, Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Steers fed a 90% concentrate diet had a lower ruminal pH than steer fed forage (5.7 vs 6.5), and *in vitro* experiments indicated that acidic pH caused a decrease in methane production. Mixed ruminal bacteria from forage-fed steers produced methane at a rate of 48 nmol/ mg protein/ min at pH 6.5, but these bacteria produced only 7 nmol of methane/ mg protein/ min at pH 5.7. Mixed ruminal bacteria from concentrate-fed steers produced methane at a rate of 1.8 nmol/ mg protein/ min at pH 5.7, but these bacteria produced 14 nmol of methane/ mg protein/ min at pH 6.5. Based on the initial rates of methane production, steers fed forage had 3.6 times more methanogens than steers fed concentrate. When steers were fed increasing amounts of concentrate (0, 45, or 90% of DM), pH and the ratio of ruminal acetate to propionate declined ($P<.001$). The acetate to propionate ratio was highly correlated with the capacity of the bacteria to produce methane *in vitro* ($r^2 = .78$; $P<.001$). *In vivo* ruminal pH was highly correlated with the ratio of acetate to propionate ($r^2 = .82$, $P<.001$) and methane production in vitro ($r^2 = .50$, $P<.01$). Based on these results, reductions in ruminal pH can decrease ruminal methane production and increase ruminal acetate to propionate.

#15 THE ROLE OF PH IN REGULATING RUMINAL DEAMINATION AND AMMONIA ACCUMULATION. R.P. Lana, J.B. Russell, and M. Van Amburgh. Section of Microbiology, and Dept. of Animal Science, Cornell University, Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Steer fed a 90% concentrate diet had a lower ruminal pH than steer fed forage (5.7 versus 6.5). *In vitro* experiments indicated that acidic pH could cause a decrease in amino acid deamination, but mixed ruminal bacteria from forage and concentrate-fed steers responded differently. Bacteria from forage-fed steers produced ammonia at a rate of 28 nmol/ mg protein/ min at pH 6.5, but the deamination rate was 15 nmol of ammonia/ mg protein/ min at pH 5.7. Bacteria from concentrate-fed steers always produced ammonia at a rate <15 nmol/ mg protein/ min, and the lowest rate was 10 nmol of ammonia/ mg protein/ min at pH 5.7. When steers were fed increasing amounts of concentrate (0, 45, or 90% of DM), pH, total ruminal ammonia, and dissociated ammonia declined ($P<.05$). The total ruminal ammonia and dissociated ammonia concentrations were highly correlated with the capacity of the bacteria to deaminate amino acids *in vitro* ($r^2 = .59$, $P<.005$). Only 41% of the decrease in total ruminal ammonia could be explained by a decrease in ruminal pH, but the concentrations of dissociated ammonia and deamination rate *in vitro* were highly correlated with ruminal pH ($r^2 = .65$ and .63, respectively; $P<.005$). Based on these results, reductions in ruminal pH can decrease ruminal deamination and ammonia accumulation.

#16 INVITED PAPER

IMPACT OF TISSUE MATURATION ON CELL WALL DEGRADABILITY OF ALFALFA STEMS.

H.G. Jung and F. M. Engels, USDA ARS, St. Paul, MN 55108 and Wageningen Agricultural University, The Netherlands (612-625-8291)

Lignification of forages is generally regarded as the primary impediment to ruminal cell wall degradability. However, recently it has been suggested that physical cell anatomy and cell-wall substructure limit microbial accessibility to potentially degradable plant cell walls and may exert a greater impact on cell wall degradation than lignification. We examined, with light and scanning electron microscopy, the impact of tissue and cell-wall development of alfalfa stem internodes on the relative contributions of lignification and physical accessibility as limitations of cell wall degradability. The seventh internode of regrowth stems, counting from the base, were collected at five stages of development, from early elongation through mature seed set. The seventh internode was in the elongation phase of development at the first and second harvests. By the third harvest most internodes had just completed elongation whereas stem diameter increased continually due to cambial growth. The fourth harvest was taken after 31 d of regrowth and all stems were flowering. During internode elongation all tissues had thin, completely degradable primary walls except protoxylem which was already very well developed and lignified, as indicated by phloroglucinol staining, and remained undegraded after a 48 h *in vitro* fermentation of 100 micron thick sections. By the third harvest secondary xylem, xylem fibers, cortical fibers, and pith parenchyma began to deposit secondary cell walls and lignify. These tissues became undegradable. Lignification always began in the thin primary cell walls. At harvest four and five a number of xylem fiber cells deposited an additional, only marginally lignified, secondary wall layer which remained relatively degradable. In contrast, during cortical fiber development a thin, ring shaped part of the primary wall lignified prior to the deposition of a very thick secondary wall which never lignified and remained completely degradable. Chlorenchyma, secondary phloem, cambium, and protoxylem parenchyma remained thin walled, non-lignified, and degradable throughout maturation. Collenchyma and epidermis deposited thick primary and secondary wall layers, respectively, which remained non-lignified and degradable. When 1.5 to 2 cm long stem pieces from the fourth harvest were fermented *in vitro* for 24 h, degradability of tissues was altered compared to fermentation of thin sections. Those tissues which had non lignified primary walls were rapidly degraded and the degradation front passed through many cell layers to depths of 1 cm in stem pieces. In contrast, any tissue which had deposited lignin in the primary wall was only degraded in the cell layer actually exposed at the cut end. Even the non lignified, potentially completely degradable secondary wall of cortical fiber cells was not degraded in the cell layer immediately below the cut end of the stem. Pith parenchyma lignification proceeded from cells on the periphery of the pith into the center during development. Those internode pieces that still contained a non lignified pith parenchyma in the center of the stem showed degradation to depths of 0.7 cm, through multiple cell layers. We also noted that if the epidermal layer was undamaged, no access to potentially degradable tissues was available to rumen bacteria through the sides of stem pieces. Our observations of alfalfa stem tissues indicate that lignification is the factor which initiates reduced cell wall degradability. However, once the primary wall is lignified to the point of formation of a continuous barrier to degradation between adjacent cells, then lack of accessibility to potentially degradable cell-wall layers becomes the over riding limitation to extent of degradability. This suggests that further accumulation of lignin associated with secondary wall development is of little significance to cell wall degradability of large forage particles and may explain poor correlations observed between concentration of lignin and extent of cell-wall degradation in forages of similar maturity. Efforts to improve forage cell wall degradability should be targeted against the development of the initial lignin barrier in primary walls, a potentially very difficult task given the developmental pattern of plant cell walls.

#17 INHIBITION OF SULFIDE PRODUCTION IN *IN VITRO* RUMINAL FERMENTATIONS. J.P. Bracht and L. Kung, Jr, Dept. of Animal and Food Sciences, University of Delaware, Newark, DE 19717 (302-831-2522)

High levels of ruminal sulfide have been linked to polioencephalomalacia in ruminants. We evaluated the effect of several compounds on their ability to reduce the production of sulfide in *in vitro* ruminal fermentations when sulfur content of the diet was greater than 1% (DMB). Excess sulfur increased (P<0.05) ruminal sulfide production but had no effects on VFA production. The effect of 1, 10, and 25 ppm of 9,10 anthraquinone (AQ) and molybdate (MO) in high sulfur diets was also studied. AQ (10 ppm) and MO (25 ppm) reduced (P<0.05) hydrogen sulfide production by 71 and 77%, respectively. However, this level of MO approaches toxicity levels in cattle. AQ but, not MO decreased (P<0.05) the molar percentage of acetate and decreased methane production. AQ also increased (P<0.05) the molar proportions of propionate, butyrate, and valerate. Bacitracin (1.25 ppm), Oxytetracycline (1.25 ppm), Chlortetracycline (5 ppm), Lasalocid (5 ppm), Bambermycin (0.3 ppm), Monensin (5 ppm) and Avoparcin (5 ppm) were tested relative to AQ (10 ppm) for their ability to affect sulfide production. Only Chlortetracycline and Oxytetracycline reduced (P<0.05) sulfide production but they were less effective than AQ. Surprisingly, sulfide production was increased (P<0.05)by more than 50% when cultures were treated with Monensin. Interactions between methanogens and sulfate reducing organisms may be responsible for this finding. Several compounds have the ability to reduce ruminal sulfide production but require further evaluation before recommendations on their use for this specific application can be made.

#18 EFFECT OF INCREASED RUMINAL LEVELS OF POLYSACCHARIDE-DEGRADING ENZYMES ON RUMEN FERMENTATION, ENZYME BY-PASS TO THE DUODENUM AND *IN VIVO* DIGESTIBILITY. A.N. Hristov, T.A. McAllister, and K.-J. Cheng, Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB T1J 4B1 (403-327-4561)

An experiment on the effect of exogenous polysaccharide-degrading enzymes (PDE) on fermentation, enzyme by-pass and digestibility was conducted using eight fistulated heifers fed a 70% barley grain/30% barley silage diet. Prior to the morning feeding, animals were dosed intraruminally with 0, 100, 200, or 400 g/d of an enzyme preparation (GNC Bioferm Inc., Saskatoon, SK) described as a beta-glucanase and possessing high xylanase activity. All levels of PDE significantly increased carboxymethylcellulase (CMCase), xylanase and beta-glucanase activities in ruminal contents for up to 6 h after feeding. All levels of PDE increased in sacco DM disappearance of the mixed diet initially, but by 48 h, ISDMD was higher (P<0.05) only with the 400 g/d dose of PDE. Viscosities of ruminal fluid and duodenal digesta were reduced by PDE (P<0.05). The effects of PDE supplementation on rumen ammonia, total free amino acids and protozoal numbers were more variable. Large increases (P<0.05) in xylanase, CMCase, beta-glucanase and amylase activities in duodenal digesta were observed in heifers receiving 400 g/d of PDE, but this did not increase *in vivo* DM digestibility. Further investigations where 100 g/d of the product was infused directly into the duodenum also did not improve DM digestibility.

#19 EVALUATION OF A FIBROLYTIC ENZYME ADDITIVE IN DAIRY COW DIETS. W.Z. Yang, K.A. Beauchemin, and L.M. Rode, Agriculture and Agri-Food Canada, Lethbridge, AB, T1J 4B1 (403-327-4561)

Effects of using alfalfa cubes containing fibrolytic enzymes in dairy cow diets were evaluated in a 4x4 Latin square design using four cannulated lactating cows. Diets consisted of 45% concentrate, 45% alfalfa cubes, and 10% barley silage (DM basis). The four diets were: a) control; b) cubes treated with 1g of enzyme mixture/kg hay (LH); c) cubes treated with 2 g of enzyme mixture/kg hay (HH); and d) both concentrate and cubes treated with 1g of enzyme mixture/kg total DM (HT). The enzyme mixture was obtained from Biovance Technol. Inc. (Omaha, NE). Dry matter intake was not affected by the enzyme additive (control vs enzyme; 20.4 vs 20.7 kg/d). Enzymes did not significantly increase ruminal digestibility of DM (control vs enzyme; 41.3 vs 46.1%), but, total tract DM digestibility was increased (control vs enzyme; 59.8 vs 63.5%; P<0.05). As a result, cows fed diets with added enzymes produced 4% (LH, 24.6 kg/d, P<0.30) or 7% (HH, HT, 25.5 kg/d, P<0.05) more milk than control cows (23.7 kg/d) with no effects on milk composition. Rumen pH was similar for all diets but rumen VFA concentration was numerically higher in cows fed enzyme diets compared to the control diet (130 vs 122 mM). These results indicate that adding a fibrolytic enzyme either to forage or concentrate enhanced feed digestibility and milk production. The magnitude of response to enzyme treatment of feed was affected more by enzyme level than by whether the enzyme was added to forage or concentrate.

#20 FEEDING VALUE FOR LAMBS OF CANOLA MEAL ARISING FROM BIODIESEL PRODUCTION. T.A. McAllister[1], K. Stanford[2], G.L. Wallins[1], M.T.J. Reaney[3], and K.-J. Cheng[1], Agriculture and Agri-Food Canada Research Centres, [1]Lethbridge, AB T1J 4B1 (403- 327-4561) and [3]Saskatoon, SK S7N 2R4 (306-975-7014); and [2]Alberta Agriculture, Food and Rural Development, Agriculture Centre, Lethbridge, AB T1J 4C7 (403-381-5150)

Canola meals prepared from canola screenings (S-CM) and from seed which had heated during storage (H-CM) were compared against commercial canola meal (C-CM) for feeding value. Oil, CP, and ADIN contents in S-CM, H-CM, and C-CM, respectively, were 11.8%, 24.6%, and 1.3%; 22.7%, 31.5%, and 43.6%; and 1.7%, 6.4%, and 2.1%. *In situ*, DM and protein disappearance rates, soluble protein fraction, and effective rumen degradability of protein were lower ($P<0.05$) in H-CM than in S-CM or C-CM, but not ($P>0.05$) if residual oil was ether-extracted. Four isonitrogenous (15% CP) barley-based diets, containing S-CM, H-CM, C-CM, or C-CM supplemented with canola oil (OC-CM), were fed to lambs. In DM, OM and NDF digestibilities, the diets ranked S-CM > H-CM ë OC-CM > C-CM ($P<0.05$). Digestion and retention of N were lower ($P<0.05$) in lambs fed H-CM than in lambs fed other diets. Feed efficiency of lambs fed H-CM was improved ($P<0.05$) as compared to lambs fed S-CM or C-CM. Dressing percentages were higher ($P<0.05$) with H-CM, S-CM, and OC-CM diets than with C-CM. Other than minor changes in fatty acid composition of subcutaneous fat with H-CM and OC-CM, carcass traits were not altered by treatment. Feeding S-CM or H-CM did not adversely affect animal performance or feed utilization.

#21 FEEDING DIFFERENT LEVELS OF PHOSPHORUS AND NITROGEN TO STEERS.

J.P. Fontenot, N.B. Frank, and R.K. Shanklin, Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (540-231-5136)

In order to minimize excretion of nitrogen and phosphorus, major environmental concerns, an experiment was conducted to determine the minimum levels of nitrogen and phosphorus required by growing beef steers fed high-roughage diets. Forty eight individually-fed steer calves (BW 470 kg) were allotted to 6 diets in a 2 x 3 factorial. Two phosphorus levels (0.19 and 0.27%) and 3 crude protein levels (7.4, 8.5 and 9.9%), dry matter basis, were fed during a 112-day trial. Bodyweights and blood samples were taken every 28 d. Daily gains and feed/gain were not consistently affected by dietary phosphorus level. Blood serum phosphorus was higher (P<.05) for the cattle fed the higher P diets after 28 d, and generally, this pattern continued for the duration of the trial. Daily gains increased and feed/gain decreased linearly (P<.01) with dietary crude protein levels. Blood urea values increased linearly (P<.05) with crude protein level in the diet. These values were very low for the cattle fed the low-protein diets throughout the trial, usually below 5 mg/dL. Perhaps the degradable N levels were too low in the low-protein diets for normal rumen fermentation. The dietary phosphorus will need to be lower for the level to affect performance.

#22 THE ROLE OF RUMINAL FERMENTATION IN REGULATION OF VOLUNTARY DRY MATTER INTAKE OF HIGH PRODUCING DAIRY COWS.

M. Oba and M.S. Allen, Dept. of Animal Science, Michigan State University, East Lansing 48824 (517-432-1386)

Effects of NDF digestibility of corn silage and dietary NDF level on intake and feeding behavior were evaluated using 8 multiparous dairy cows in a duplicated 4 x 4 Latin square design. Experimental diets consisted of either brown midrib (bm3) corn silage or its isogenic normal counterpart at 2 levels of dietary NDF (29% and 38%). *In vitro* NDF digestibility (30 h) was higher for bm3 corn silage by 9.4 units. Feeding behavior of animals was monitored continuously for 4 d each period using a computerized data acquisition system. DMI increased for the low NDF and high NDF digestibility treatments. However, an interaction between NDF digestibility and dietary NDF% was observed for meal size in DM (P<0.08) and for interval between meals (P<0.08), implying that different mechanisms regulating DMI dominate depending on fermentation characteristics in the rumen. Meal size (DM) was smaller and the interval between meals tended to be shorter for the low digestible NDF (LFD) treatment than for the high digestible NDF (HFD) treatment when fed in high NDF diets. We speculate that the high NDF diet with LFD did not allow cows to consume adequate energy per meal due to physical fill, resulting in shorter intermeal intervals. However, the opposite effects were observed for the low NDF diets; meal size (DM) was smaller, and the interval between meals tended to be shorter for the HFD treatment compared to the LFD treatment. The higher fermentability of the HFD treatment resulted in lower ruminal pH and it is expected that VFA were more rapidly absorbed, resulting in faster cessation of eating. Increased clearance of VFA from the blood might have allowed shorter intervals between meals. Ruminal fermentation may affect DMI by altering meal patterns of animals.

	Low NDF		High NDF		Statistic		
	HFD	LFD	HFD	LFD	F	FD	FxFD
DMI (kg/d)	23.6	22.8	22.0	20.5	0.002	0.05	NS
Meal bouts/d	11.1	10.6	10.3	11.1	NS	NS	NS
Meal size (kg DM)	2.2	2.3	2.2	2.0	NS	NS	0.08
Interval between meals (min)	90.4	98.2	98.8	87.8	NS	NS	0.08
Rumen pH	5.62	5.78	5.73	5.90	0.06	0.01	NS

HFD: High fiber digestibility; LFD: Low fiber digestibility; F: Effect of dietary NDF level; FD: Effect of fiber digestibility; FxFD = Interaction of dietary NDF level and fiber digestibility

#23 EFFECTS OF FORAGE SOURCE IN HIGH GRAIN DIETS ON TIME SPENT RUMINATING, RUMINAL PH AND FIBER DIGESTION.

[2]M. Krause, [1]K.A. Beauchemin, [1]L.M. Rode, [1]B.I. Farr, and [2]P. Nørgaard, [1]Agriculture and Agri-Food Canada, Research Center, Lethbridge, AB, T1J 4B3 and [2]The Royal Veterinary and Agricultural University, DK-1870, Frederiksberg C, Denmark (tel. 403-317-2235; fax. 403-382-3156)

A study was conducted to investigate the potential benefits of using barley straw rather than barley silage in high grain diets fed to feedlot cattle. Steers were given *ad libitum* access to diets consisting of 95% concentrate (mostly barley) and either 5% barley silage or barley straw on a dry matter basis. Diets were fed once daily. Replacing silage with straw resulted in 1 h/d more rumination time (5.4 vs 4.4 h; P<.05). There was no effect of diet on ruminal pH. Mean pH measured over 24 h was 6.1. Ruminal pH dropped shortly after feeding, reaching an average low of 5.8 about 9 to 16 h after feeding. However, mean diurnal pH profiles for diets did not reflect the fact that the lowest pH (ranging from 4.8 to 6.3) did not occur simultaneously for all animals. Lowest pH was influenced more by animal variation than dietary differences. On average, ruminal pH was <6.2 about 44% of the time, and <5.8 about 19% of the time, suggesting that fiber digestion was likely partially inhibited for a substantial period each day. Replacing silage with straw increased the molar proportion of acetate (P=.007). Acid detergent fiber digestibility was higher for diets containing straw than for diets containing silage (P<.01), indicating an improvement in ruminal function. Higher rumination time observed for cattle fed diets containing straw supports this hypothesis, although a concurrent increase in ruminal pH was not observed. These results indicate that using straw in diets fed to feedlot cattle may be an economically feasible strategy for minimizing digestive disturbances and maximizing diet digestibilty. However, mean ruminal pH was not a reliable indicator of rumination time or fiber digestion. Sub-clinical acidosis in cattle fed once daily is more accurately assessed by measuring the pH decline approximately 12 h after feeding rather than mean pH.

#24 FEEDING BEHAVIOUR OF FEEDLOT CATTLE MONITORED WITH RADIO FREQUENCY TECHNOLOGY.

D. Gibb*[1], R. Wiedmeier[2], and T.A. McAllister[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB T1J 4B1 (403-327-4561) and [2]Utah State University, Logan UT (801-797-2151)

A multiplex radio frequency system (GrowSafe Systems Ltd, Airdrie, AB) was used to monitor individual attendance at the feed bunk of 72 yearling exotic-cross steers (422 kg initial weight) during a step-up transition from backgrounding diet to finishing diet. The system allows simultaneous reading of multiple transponders, via an antenna in the feed bunk which detects passive radio frequency Allflex USA (Dallas, TX) ear tags at 6-s intervals whenever an animal has extended its head into the feed bunk. The time, location and duration of bunk visits were monitored by computer for four pens of 18 steers over a 45 d trial. A new feeding bout was defined as a return to the feed bunk after at least a 2-min absence. A barley grain/barley silage based ration was provided *ad libitum* for the first 35 d of the trial . At 3 to 4 d intervals, barley grain was increased from 50% to 60%, 80%, and 87% of the diet (DM basis). After 8 d on the 87% grain ration, cattle were restricted to 95% of their previous 5 day's intakes. Frequency of feeding bouts and daily time at the bunk decreased linearly ($P<0.001$) as barley grain increased. Restrictive feeding reduced frequency of visits ($P<0.10$) but not duration ($P>0.30$) at the feed bunk. More cattle visited the feed bunk between 0600 to 1200 h and between 1200 to 1800 h than between 1800 to 2400 h and 2400 to 0600 h ($P<0.001$). This system is proving successful at monitoring feeding behaviour of individual cattle in a setting that is typical of a commercial feedlot.

#25 BIOTRANSFORMATION OF ANTI-NUTRITIONAL FACTORS BY RUMEN MICROBIOTA. **A.H. Smith[1,2], A.A. Odenyo[3], P.O. Osuji[3], and R.I. Mackie[1], [1]Dept. of Animal Sciences, University of Illinois, Urbana, IL 61801 (217-333-8809); [2]Agricultural Research Council, Irene, South Africa; [3]International Livestock Research Institute, Addis Ababa, Ethiopia**

Leguminous trees are useful as fodder in arid regions, but their benefits are limited by anti-nutritional factors. Rumen inoculum from animals on legume supplemented diets *(Sesbania sesban, Leucaena pallida, L. diversifolia,* mixed *Acacia* species*)* was compared to an inoculum from sheep fed a hay diet. There was no difference in rate and extent of mimosine, tyramine and ferulic acid transformation. To determine whether organisms able to transform the anti-nutritional factors are ubiquitous in rumen populations, the experiment was repeated in Illinois with steers fed a lucerne diet. Further experiments demonstrated that fungi were not involved in tyramine metabolism. However, if protozoal or bacterial populations were inhibited, tyramine metabolism was reduced. Rumen fluid from the animals was screened for the ability to modify a range of specific anti-nutritional factors as sole carbon and energy source. Growth was observed in media containing the following target compounds: L-mimosine; 2,3-DHP; tyramine; ferulic acid; p-coumaric acid; catechol; vanillic acid; hesperidin, and sarsasaponin. The cultures in which growth was observed were preserved and stored at -86°C. The cultures will be used to obtain isolates of ruminal bacteria which have the ability to modify anti-nutritional factors.

#26 EFFECT OF STEROIDAL SAPONIN FROM *YUCCA SCHIDIGERA* EXTRACT ON THE GROWTH OF RUMINAL BACTERIA. **Y. Wang[1], T.A. McAllister[1], L.J. Yanke[1], P.R. Cheeke[2], and K.-J. Cheng[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB T1J 4B1 (403-327-4561) and [2]Desert King International, Chula Vista CA 91911 (541-737-1917)**

Four ruminal bacterial species, *Prevotella ruminicola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium*, and *Streptococcus bovis*, were exposed to low levels of steroidal saponin (SSAP) from *Yucca schidigera* extract by culturing for five 48 h transfers in ruminal fluid medium containing 0 or 25 μg/ml of SSAP (measured as smilagenin equivalents). Each strain, non-exposed and pre-exposed, was then used to inoculate medium containing 0 or 250 μg smilagenin equivalents/mL, and 24 h growth curves were determined (A_{600}). The effects of SSAP on growth varied among the species tested. Including SSAP in the medium reduced growth of *S. bovis* ($P<0.01$ at 2, 3, 4, 5, 6, and 8 h), *P. ruminicola* ($P<0.05$ at 4, 5, 6, 8, 10, and 24 h), and *R. amylophilus* ($P<0.05$ at 14 and 24 h), but growth of *S. ruminantium* was enhanced ($P<0.05$ at 10, 14, and 24 h). For all 4 species, however, growth curves were similar ($P>0.05$) between pre-exposed and non-exposed cultures. Concentrations of total SSAP (measured as total smilagenin) and soluble SSAP (measured as soluble smilagenin) did not change over time ($P>0.05$) in cultures of any of the 4 species. Prior exposure to low levels of SSAP did not induce adaptation to SSAP in any of the bacteria studied. None were able to degrade steroidal sapogenin from YE, or to deglycosylate steroidal saponin. The effect of SSAP on bacterial growth was species-specific.

#27 THE ROLE OF MICROORGANISMS IN LOCOWEED TOXICITY.

T. May, C.M. Liddell, J.L. Riggs, T. Cook, K. Brown, and E.S. Woodruff, Depts. of Animal and Range Sciences and Entomology, Plant Pathology, and Weed Science, New Mexico State University, Box 30003, Dept. 3-I, Las Cruces, NM 88003 (505-646-2016)

Ingestion of locoweed *(Astragalus* spp. and *Oxytropis* spp.) leads to a syndrome in ruminants known as locoism characterized by vacuolization in soft tissues including the brain. Swainsonine, an indolizidine alkaloid, is the primary toxicant responsible for this condition. This compound is an alpha-mannosidase inhibitor that results in the failure of cellular glycoprotein synthesis. Perhaps a reason managerial, preventative or treatment measures to combat locoism have not been successful is because the plants are not actually producing the swainsonine themselves. A fungus, *Rhizoctonia leguminicola,* is known to produce swainsonine. Research in our laboratories has shown that there is a fungal endophyte in seeds of both *Astragalus* and *Oxytropis* spp. The endophyte appears to be a species of *Alternaria*, a common toxin-producing genus of hyphomycetes. The endophyte is found in the endosperm and embryo of the seed and cannot be removed from the seed by heat or chlorox treatments without killing the seed itself. We have hypothesized that this fungal endophyte of *Astragalus* and *Oxytropis* may be the source of the swainsonine in these plants and may account for the variable occurrence of locoism. Preliminary work has demonstrated putative swainsonine production by this endophyte. Attempts to correlate presence of the endophyte with actual toxin levels in the plant are underway.

#28 THE POTENTIAL FOR SWAINSONINE BIOTRANSFORMATION OR UTILIZATION BY RUMINAL MICROORGANISMS.

M. Morgan and T. May, Dept. of Animal and Range Sciences, New Mexico State University, Box 30003, Dept. 3-I, Las Cruces, NM 88003 (505-646-2016)

Swainsonine is an indolizidine alkaloid found in plant species from the genera *Astragalus* and *Oxytropis*, locoweeds. This compound is a structural analog to mannose. Swainsonine inhibits cellular alpha-mannosidases. Animals that graze locoweed species suffer from neurological effects (locoism), emaciation, and reproductive problems including birth defects and spontaneous abortions. This study investigates the possibility of biodegradation or biotransformation of swainsonine by ruminal bacteria. At least 9 distinct species of mannose-utilizers were isolated from freshly collected bovine ruminal fluid. Growth curves were performed on media containing 4 mg/mL vs 200 µg/mL mannose. All isolates, except #4, grew well on media containing 4 mg/mL mannose reaching optical densities (OD_{550}) of 1.2 to 1.4. Isolate #4 only reached a maximum OD_{550} of 0.7. Rates of growth were calculated and 2 isolates with the highest rates (#6 and #11) were chosen for experiments using swainsonine. This media contained either 0.1 mg/mL swainsonine or 2.4 mg/mL mannose + 0.1 mg/mL swainsonine. Isolates #6 and #11 did display growth on swainsonine reaching OD_{550} of 0.25 and 0.3, respectively. Further experimentation will include measurement of swainsonine loss from cultures and elucidation of the mechanism of swainsonine utilization.

#29 TANNIN-TOLERANT BACTERIA FROM THE GASTROINTESTINAL TRACTS OF BIRDS, RUMINANTS, AND PRIMATES.

T.K. Woolston, A.N. Pell, and K. De Leeuw, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876)

When eaten at high levels, tannins have adverse effects on domestic animals but some wild herbivores can eat high levels of tannins without apparent ill effects. Our goal was to determine whether the gastrointestinal tracts of some herbivorous birds, primates, and ruminants (elk, red-bellied lemur, gorilla, and hoatzin) had bacteria able to tolerate tannins. Ruminal fluid was obtained from anesthetized Rocky Mountain elk by stomach tube. Fresh feces were collected from Ugandan gorillas and Malagasy lemurs. Crop contents of Venezuelan hoatzins were obtained from chicks with a pipette or from sacrificed adults. We enriched for tannin-tolerant bacteria by inoculating ruminal fluid or fecal or crop samples into medium with 1 g/L of crude tannic acid (TA) or quebracho (Q). The ability to grow on various carbohydrate sources was assessed using ruminal fluid medium with 1 g/L carbohydrate. Most isolates grew on galactose, sucrose, glucose, and fructose. The lemur isolates did not grow on cellobiose. The ability to grow on mannitol was variable. Only one isolate from elk was able to grow on alpha-cellulose and none could grow on filter paper. Morphologically the isolates differed significantly in size, shape, and Gram stain. Tannin tolerance was assessed using Q and TA as models for condensed and hydrolyzable tannins. Some isolates could tolerate up to 15 g/L TA. Growth on tannins from Desmodium ovalifolium was also investigated.

#30 THE EFFECTS OF INOCULATION WITH TANNIN-TOLERANT BACTERIA ON FIBER AND NITROGEN DIGESTIBILITY OF LAMBS FED HIGH TANNIN DIETS.

D.O. Molina, A.N. Pell, and D.E. Hogue, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876)

The goal of this study was to evaluate the effects of dosing unadapted lambs with tannin-tolerant bacteria when a high-tannin diet was fed. During the initial phase, a diet containing 40% peanut skins was fed to 2 groups of Suffolk x Finn-Dorset ram lambs that were about 3 months old and weighed 24.2 ± 1.4 kg. All lambs received 150 ml of a culture (A_{600} of 1.0) of a Gram positive rod (a close relative of *Eubacterium cellulosolvens*) that was able to tolerate 0.5 g/L of purified tannins from Desmodium ovalifolium. The control group (7 lambs) was inoculated with autoclaved bacteria and the treatment group (6 lambs) received actively growing bacteria. Lambs received the bacterial treatments daily for 3 weeks. Dry matter and CP intake, and digestibility of DM, CP, and NDF were similar between treatments. Crude protein retention and CP retention/CP intake were higher for animals inoculated with the live bacteria ($P<0.08$). Carry-over effects due to bacterial inoculation were investigated by feeding the animals the same high-tannin diet as during the initial phase but the lambs received no supplemental bacteria. No significant ($P>0.10$) differences in DM and CP intake were found between groups. During the last phase of the experiment, the animals were fed a low-tannin diet and received no bacteria. Dry matter and CP intake and the feed:gain ratio tended to be higher for the control group.

#31 TOTAL RNA EXTRACTION FROM RUMEN BACTERIA EXPOSED TO TANNINS.

P.B. Arcuri, A.N. Pell, and M.L. Thonney, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876)

Tropical legumes have potential as forages but many contain tannins that bind to microbes and inhibit microbial growth. In order to detect fluctuations in rumen microbial populations due to tannins using 16S rRNA probes, quantitative recovery of intact rRNA is required. Tannins interfere with extraction because they bind to nucleic acids via hydrogen bonds. The goal of this project was to develop a method to extract rRNA in the presence of tannins. Cultures of *Ruminococcus albus* 8 and *Fibrobacter succinogenes* S85 were grown in 30 ml of semi-defined medium. Both bacteria were exposed to either 8 g/L or 0 g/L of tannic acid. *R. albus* was also exposed to 1 g/L purified tannin from Desmodium ovalifolium. The cultures were rinsed twice with Tris buffer containing either 6% PVP or 8% PEG to remove tannins prior to cell lysis. Cells were lysed in a bead beater for 3 min in the presence of guanidinium thiocyanate. Total RNA was extracted with the RNAqueous kit from Ambion. Densitometry of the 16S rRNA bands was used to compare the actual yield of RNA from 1 microgram of total RNA determined by spectrophotometry. The bands from *R. albus* washed with PEG or PVP were 73% and 32% as dense, respectively, as those not exposed to Desmodium tannin. When *R. albus* cells exposed to Desmodium tannin were not washed with PEG or PVP, RNA did not migrate from the wells. The recoveries of RNA from *R. albus* and *F. succinogenes* were similar in the presence of tannic acid although differences remained between buffers.

#32 PCR DETECTION OF PYRIDINEDIOL-DEGRADING RUMINAL BACTERIUM, *SYNERGISTES JONESII*, IN THE RUMEN FLUID OF CATTLE.

J. Yang[1], N. Du[2], J.R. Carpenter[2], and D. Borthakur[3], Depts. of Biochemistry[1], Animal Sciences[2] and Plant Molecular Physiology[3], University of Hawaii at Manoa, Honolulu, HI 96822 (808-956-6600)

Synergistes jonesii, a gram-negative rumen bacterium, can catabolize toxic pyridinediols which are produced in the rumen from mimosine present in leucaena fodder and thus protect the ruminants from the harmful effects of this toxin. A PCR based method was developed to detect the presence of this pyridinediol-degrading bacterium in the rumen fluid of cattle. A unique 1.0-kb DNA sequence was identified by sequencing several fragments from a genomic library of *S. jonesii* strain 78-1 and several pairs of primers were designed and used for PCR amplification of specific fragments. This method can detect the presence of 10 to 100 bacteria in a sample. The method was tried on rumen fluid samples with an added known amount of the bacterium. With a simple pretreatment of the mixed sample, the PCR method reached a sensitivity about 100 fold lower than with pure-culture samples. Similar results were obtained using primers designed from the non-conserved regions of the 16S rRNA of *S. jonesii* strain 78-1. To further increase the sensitivity and specificity of the detection, experiments using nested primers are under way.

#33 SMALL SUBUNIT RIBOSOMAL RNA SEQUENCE ANALYSIS OF ANAEROBIC RUMINAL FUNGI ISOLATED IN NEW ZEALAND.

L.A. Thill[1], S.A. Kocherginskaya[1], G.E. Naylor[2], K.N. Joblin[2], and B.A. White[1], [1]Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801 (217-333-2091), and [2]AgResearch, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand

The 18S ribosomal DNA (rDNA) gene sequences from 4 anaerobic ruminal fungi isolated in New Zealand have been determined. One *Neocallimastix* isolate (ROLD) was obtained from the rumen of a sheep and another (12D) from faeces from a feral cow. A *Caecomyces* isolate (B7) and a *Piromyces* isolate (B19) were isolated from faeces of a domesticated cow. Bulk DNA from these anaerobic ruminal fungi was used as a template for polymerase chain reaction (PCR) amplification of the 18S rDNA gene. PCR products were cloned into appropriate PCR cloning vectors and subjected to DNA sequence analysis by automated sequencing at the University of Illinois Biotechnology Center. Both strands were sequenced using sequencing primers based on conserved regions of small subunit ribosomal RNAs. These data were compared with 18S rDNA/rRNA sequences for *Saccharomyces cervisiae* and other anaerobic ruminal fungi found in sequence databases. Phylogenetic trees were inferred using distance data for 1425 umabiguously aligned sequence positions as well as 1567 umabiguously aligned sequence positions, if *Sphaeromonas communis* was not considered. Sequence similarities were very high within the anaerobic ruminal fungi. The phylogenetic trees were generated using Clustal W and were bootstrapped 1,000 times. Regardless of the amount of data used the phylogenetic trees were nearly identical. These data suggest that the anaerobic ruminal fungi are monophyletic.

#34 CHARACTERIZATION OF PIROMYCES SP. (B3) ISOLATED FROM RUMEN OF A REVERINE BUFFALO.

A.K. Samanta, T.K. Walli, V.K. Batish, S. Grover, and A.K. Montany, National Dairy Research Institute, Karnal - 132001, India (0184-255478)

Anaerobic fungi were isolated from rumen of a Murrah buffalo. Out of 10 isolates studied, B3, belonging to *Piromyces* sp. possessed the highest endoglucanase activity. However, the xylanase activity was much higher than the endoglucanase activity. The isolate was monocentric in nature with round and large sporangia (size 1.5 x 1.5 µm) It possessed extensively branched rhizomycelia, with fine branching towards the end. Zoospores also were round in shape (size 0.01 x 0.02 µm) and were monoflagellated (length 0.84 µm). Growth of culture on cellobiose soft agar (0.3%) produced profuse gas and the highest quantity of acetate (14.25 µmol./ml) compared to other isolates. All the isolates failed to produce propionate or butyrate. The culture supernatent of the isolate B3 possessed several hydrolytic enzyme activities viz. endoglucanase (17.5925 mIU/ml), avicelase (90.629 mIU/ml), cellobiase (19.3737 mIU/ml), and xylanase (237.613 mIU/ml). Sonification of the cells failed to release any hydrolytic enzyme, suggesting an absence of extracellular enzymes in the isolate. The pH optima was 6.5 for endoglucanase, 6.0-6.5 for cellobiase, and 6.5 for xylanase. The endoglucanase activity was highest on the 4th d of incubation. The isolate had the moderate level of lysine (7.17) and cystine plus methionine (4.46) as g/100 g total amino acids.

#35 USE OF THE AN-IDENT SYSTEM IN IDENTIFICATION OF RUMINAL BACTERIA.

L.J. Yanke and K.-J. Cheng, Agriculture and Agri-food Canada Research Centre, P. O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1 (403-327-4561); e-mail: yanke@em.agr.ca

Conventional biochemical testing of anaerobes together with gas-liquid chromatography (GLC), is still considered the most accurate, reliable, and widely used approach to identification of ruminal anaerobes. However, detailed phenotypic characterization of bacterial strains can be labour intensive, time-consuming, and with rumen bacteria, requires an experienced worker. Use of the AN-Ident identification system has demonstrated its ability to discriminate between the major genera of rumen bacteria, with a minimum of technical expertise necessary. The system is based on the detection of constitutive enzymes, is growth independent, and requires only a 4 h aerobic incubation. A preliminary similarity identification matrice has been assembled using known bacterial strains from a variety of ruminal sources. The source of the isolates was not seen to have any influence on the results obtained with the AN-Ident system. Use of this matrice with unidentified strains has been found to give rapid identifications with good reproducibility of results and a minimum of supplementary testing required. Over 85% of the isolates could be identified, with only supplementary gram staining required. A further 10% could be identified with GLC analysis of the end-products of glucose fermentation.

#36 QUALITATIVE AND QUANTITATIVE STUDY OF THE EQUINE CAECAL CELLULOLYTIC BACTERIAL POPULATION.

V. Julliand[1], A. de Vaux[1], L. Millet[2] and G. Fonty[2], [1]Laboratoire associé de Recherches Zootechniques INRA-ENESAD, BP 1607, 21036 Dijon Cédex, [2]Laboratoire de Microbiologie, INRA, CR de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France (330473624245)

Although the microbial degradation and fermentation of plant cell wall polyssacharides is a major function of the horse hindgut, the composition and structure of the fibrolytic microflora is not very well known. The aim of the present work was to quantify the caecal cellulolytic microflora of 3 ponies and 3 donkeys fed a hay based diet and to determine its composition. The cellulolytic bacterial population was quantified using a selective culture medium (Most Probable Number) and specific oligonucleotide probes. The total bacterial population was quantified using a non-selective medium (Roll tube method) and an universal probe. The numbers of cellulolytic bacteria were similar in donkeys and ponies (c.a. 10^7 bact. ml^{-1} caecal contents) and represented less than 4 p 100 of the total anaerobes. Quantification with probes showed the predominance of *Ruminococcus flavefaciens* (1 p 100 of total bacterial 16S rRNA). *R. albus* and *Fibrobacter succinogenes* were also detected but in lower proportions. The cultivated cellulolytic population was characterized by *R. flavefaciens* ressembling strains (chain of cocci). However major differences appeared between equine and ruminal type strains of *R. flavefaciens* (007 and FD1) and *R. albus* (7) in their ability to use sugars and polysaccharides and in their profile of end-products of cellulose and cellobiose fermentation.

#37 CULTIVATION OF CELLULOLYTIC COCCI FROM BUFFALO RUMEN.

S. Iqbal, R. Singh, and S.P.S. Sodhi, Dept. of Veterinary Physiology, Punjab Agricultural University, Ludhiana, India 141004 (0091-161-401960, ext. 228)

Six rumen fistulated male buffaloes were used as experimental animals. M-8 medium was adopted for the cultivation of rumen bacteria using the roll tube Hungate anerobic technique. Individual colonies of the rumen bacteria were isolated and transfered into the broth medium for further investigation. The rumen isolates were studied for their morphological characteristics, fermentation reactions, and volatile fatty acids (VFA) production. VFA were determined with the help of the gas liquid chromatography. Morphological characteristics of some of the cultures revealed that they were gram negative in reaction, cocci in shape, occuring singly or in short chains, capsulated, nonmotile, and nonsporulated. These cultures fermented glucose, fructose, sucrose, cellobiose, and cellulose with the production of formic and acetic acids. The isolated did not ferment galactose, xylose, rhamnose, trehalose, raffinose, starch, glycrol, mannitol, and salicin. Hence they were identified as the different strains of *Ruminoccus albus*. However, the morphological features of some of the remaining isolates showed that they were gram positive, cocci in shape, occuring in chains, capsulated, nonmotile, and nonsporulated. These isolates grew on sugars like glucose, arabinose, cellobiose and cellulose producing formic, and acetic acids. These isolates did not grow on substrates viz. galactose, xylose, rhamnose, mannose, trehalose, raffinose, starch glycerol, mannitol, and salicin. Thus they were identified as the different strains of *Ruminococcus flavefaciens*.

#38 ECOLOGICAL ROLE OF A SUCCINATE-DEGRADING BACTERIUM FROM THE RUMEN.

T.L. Weddington, D.M. Hopkins, H.J. Strobel, and K.A. Dawson, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606-257-7552)

Studies were conducted to isolate, characterize and evaluate the ecological role of a succinate-degrading bacterium from the rumen of a heifer fed a hay -based diet. A gram-negative, rod-shaped anaerobic bacterium (TL7) that stoichiometrically produced one mole of propionate per mole of succinate degraded was isolated from an enrichment culture containing succinate. Succinate was the only compound found to support the growth of this organism. Yeast extract was required for growth in a semi-defined medium and volatile fatty acids enhanced growth but were not required for growth. The ability of TL7 to release radiolabeled carbon dioxide from end labeled succinic acid was compared with that of *Selenomonas ruminantium* strains HD4 and H18. The affinity (Km) of TL7 and the *S. ruminantium* strains for succinate were similar (4.76, 4.16 and 1.04 mM, respectively). However, the theoretical maximum decarboxylation rate was greater for TL7 than either *Selenomonas* strain (354 vs. 2.77 and 18.1 moles/mg protein/h, respectively). Degradation of filter paper cellulose by *Fibrobacter succinogenes* strain S85 was enhanced when strain TL7 was included in the culture. Propionate was the major end product in cultures containing both TL7 and S85 while succinate and acetate were the predominant end products in cultures containing only S85. The relative high specific activities of the decarboxylase enzyme systems in TL7 suggest that similar succinate-decarboxylating organisms may make a major contribution to succinate metabolism in the rumen.

#39 EFFECT OF RUMEN CELLULOLYTIC BACTERIAL CONCENTRATIONS ON *IN SITU* DIGESTION OF FORAGE CELLULOSE.

B. A. Dehority and P. A. Tirabasso, Dept. of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691 (330- 263-3909)

By feeding a diet containing 57% purified cellulose to sheep in a switchback design trial, it was possible to increase the concentrations of rumen cellulolytic bacteria as compared to feeding an alfalfa-corn diet containing 17% cellulose. No differences were found for 24h in situ cellulose digestion from alfalfa between the two diets. However, there was an overall mean difference in pH between the diets (P<.01). Measurements were taken at 0, 3, 6, 9 and 24 h and pH for the high cellulose diet was significantly lower at 0, 9 and 24 h. Thus, the lack of an increase in cellulose digestion with an increase in the concentration of cellulolytic bacteria was confounded with pH. Adding 3% sodium bicarbonate to the high cellulose diet and feeding twice a day resulted in pH values which were not different from the low cellulose diet , i.e., high = pH 5.95 and low = pH 5.90. When the high cellulose diet was fed, the concentration of total rumen bacteria increased (P<.06); concentration of cellulolytic bacteria increased (P<.03) and % of cellulolytics increased (P<.04). In situ digestion of alfalfa cellulose (30h) was not affected by diet (P>.6). These data suggest that increasing the concentration of cellulolytic bacteria almost 10-fold, from $8x10^8$/ml to $74x10^8$/ml, has little if any effect on the *in vivo* rate of intact forage cellulose digestion. Possibly all digestion sites are saturated at a concentration of $8x10^8$/ml or rate of activity for the cellulase enzymes may be the limiting factor.

#40 A COMPARATIVE STUDY OF THE *FIBROBACTER* POPULATION IN THE RUMEN OF DIFFERENT CATTLE BREEDS FED A LOW-QUALITY ROUGHAGE.

B.G. Greyling, A.H. Smith, and E.H. Osler, Agricultural Research Council, ANPI, P/Bag X2, Irene, 1675, South Africa

A physiological study of Nguni (*Bos taurus africanus*), Simmentaler (*B. taurus*) and Brahman (*B. indicus*) cattle fed a low-quality roughage was complemented by a study on the *Fibrobacter* population of the rumen. Quantification of rumen populations with 16S rRNA targeted oligonucleotides (S-D-Bact-0338-a-A-18; S-G-Fibr-0225-a-A-21) using DIG (Digoxigenin) labelled probes and 4-nitro blue tetrazolium chloride (NBT) as the detector substrate was evaluated. Hybridization signals obtained from the DIG-labeled probes could, like radioactive signals, be quantified since a linear relationship (R-squared=0.99) was demonstrated between hybridization signals and amount of rRNA blotted on the membrane. The method was found to be reproducible with a coeffecient of 14.2% when tested on 9 identical rumen samples. The residual means square of the replicate samples were not significantly different (P=0.97). The average rRNA index of *Fibrobacter* in whole rumen contents of the Nguni and Simmentaler cattle was 2.2% and 2.3%, respectively, which was lower than that of the Brahman cattle, which was 3.3% (P=0.69). In accordance with the previously published data on *Fibrobacter*, considerable variation with regard to the rRNA indexes existed between animals within the same group and between days.

#41 EFFECT OF DIET ON RUMEN MICROFLORA AND MICROFAUNA IN BUFFALO CALVES.

S. Singh and R. Singh, Dept. of Veterinary Physiology, College of Veterinary Science, Punjab Agricultural University, Ludhiana (Punjab) 141004 India

Four healthy rumen fistulated male buffalo calves varying in weight from 100-125 kg and in age of 14-16 months were used as an experimental animals. All animals were subjected to 4 different kinds of diet. Diet A was conventional ration consisting of wheat straw, green fodder, concentrate, vitablend, and mineral mixture. Diet B was subabul diet containing subabul concentrate, wheat straw, and green fodder. Diet C was wheat straw treated with urea, molasses, and salt in ratio of 2:6:1. Diet D was exclusively wheat straw. All animals were kept for 21 d on each diet for adaptation of rumen microbial population. First rumen liquor sample was taken before feeding (zero hour) and subsequent samples were obtained at 0.5, 1, 2, 4, and 6 h interval of post-feeding for enumeration of total and viable counts of rumen bacteria and rumen protozoa. Results revealed that total and viable bacterial counts showed an initial post-prandial decline followed by rising trend and attained peak levels at 4 h post-feeding under all 4 diets while total rumen protozoal count showed a rising trend and attained peak count at 4 h post-feeding under all 4 dietary regimes. Diet A sustained maximum concentration of total rumen bacteria. Diet B exhibited highest population of proteolytic, amylolytic, lipolytic, viable bacteria, and total rumen protozoal count. Diet C showed highest count of cellulolytic bacteria while Diet D indicated lowest population of rumen microbes. Thus, dietary differences and post-prandial intervals had significant effects on rumen microbial population.

#42 VARIATION OF SULFATE REDUCING BACTERIA POPULATIONS IN BOVINE RUMEN.

M.G.Delgado[1] and R.L. Sager[2], [1]Private practitioner, [2]Instituto Nacional de Tecnologia Agropecuaria, EEA San Luis. CC 17. 5730. Villa Mercedes, San Luis, Argentina (54-657-22616)

To evaluate changes in the Sulfate Reducing Bacteria (SRB) population that can be relevant to the pathogenesis of Polioencephalomalacia (PEM) a group of 10 Angus steers received different dietary levels of total sulfur at different times. During the first month of the trial the steers received 0.33 % S/DM from alfalfa hay and well water containing 0.750 g SO_4/l and in the second month 1.16 % S/DM mainly from well water containing 3.75 g SO_4/l. At the end of each feeding period, individual rumen fluid samples were taken by rumen puncture to determine most probable number of SRB (MPN) using 2 enrichment liquid media; BRS-10 and MB. These cultures were inoculate into RGCA and MB media to evaluate sulfide production, absence of growth in TSA, and thioglycolate broth and presence of desulfoviridin pigment characteristic of *Desulfovibrio* strains. ATCC *Desulfovibrio* and *Desulfotomaculum* strain were also analyzed. The MPN of SRB during the first feeding period were 950 bact./ml for BRS-10 medium y 1095 bact./ml for MB medium, respectively. Several SRB were isolated from these media but none of them were *Desulfovibrio* or *Desulfotomaculum*. The MPN of SRB for the second feeding were 2620 bact./ml and 20,897x103 bact./ml for BRS-10 and MB media, respectively. *Desulfovibrio* sp. with high sulfide production capacity were isolated at this time period. Results indicate that there are several SRB in the bovine rumen, but with high sulfur levels their numbers are increased and *Desulfovibrio* predominates. Although these steers did not developed nervous disease, the increased SRB numbers and capacity to produce sulfide indicate that *Desulfovibrio* may play an important role in the pathogenesis of PEM.

#43 EFFECTS OF A MICROBIAL ADDITIVE, LEVUCELL® SC, ON GROWTH AND METABOLISM OF A RUMINAL ACETOGENIC BACTERIAL STRAIN *IN VITRO*.

F. Durand-Chaucheyras[1,2], G. Fonty[1], and G. Bertin[2], [1]Laboratoire de Microbiologie, INRA, CR Clermont-Ferrand Theix, 63122 Saint-Genès Champanelle , [2]SANTEL-groupe Agritek, 85 rue Anatole France, 92300 Levallois-Perret, France (330473624241)

The effect of a microbial additive, Levucell® SC (Saccharomyces cerevisiae, CNCM I-1077, Institut Pasteur, France) was investigated on the acetogenic strain Ser 5 isolated from the rumen of a lamb. Strain Ser 5 was cultured in a minimal medium containing (G+) or not (G-) 3 g/l of glucose, in presence (YE+) or in absence (YE-) of 3 g/l of yeast extract. Alive or heat-killed SC cells were added at different concentrations (log10(N)=7 or 8 cells/ml), to bacterial cultures. The medium was incubated H_2-CO_2 (80:20, 202 kPa) . Hydrogen utilization and acetate production were measured in 7 d bacterial cultures. In medium G-YE-, hydrogen utilization by Ser 5 increased after the addition of log10(N)=7 cells/ml of live SC (respectively, 2.88 and 61.88 mM of hydrogen consumed); the effect was greater with log10(N)=8 cells /ml (147.89 mM). Autoclaved yeasts were as efficient as live yeasts. Acetate production by Ser 5 was consequently stimulated (0.2 mM in control, 15.5 mM with log10(N)=7 cells/ml, 42.4 mM with log10(N)=8 cells /ml). In medium G-YE+, H_2 utilization by the bacteria was much higher (123.35 mM) than in medium G-YE-. The addition of live or autoclaved SC increased, but in a lesser extent, H_2 utilization (respectively, 140.37, 207.96, 149.56, and 211.78 mM with log10(N)=7 live ,log10(N)=8 live, log10(N)=7 dead, and log10(N)=8 dead cells/ml) and acetate production by Ser 5. In medium G+YE+ incubated with H_2-CO_2, live yeast cells were able to outcompete the acetogenic strain for glucose utilization and then diverted it to hydrogen consumption. Acetate production from O-methylated compounds such as ferulic, syringic and vanillic acids, was stimulated in presence of log10(N)=8 cells/ml of Levucell® SC in medium YE+ incubated under 100% CO2.

#44 EFFECT OF NONIONIC SURFACTANT ON FERMENTATION CHARACTERISTICS AND ENZYME ACTIVITY DURING *IN VITRO* INCUBATION OF BARLEY GRAIN.

D.R. ZoBell[1]*, T.A. McAllister[2], J. A. Shelford[3], M. Goto[4], R. Wiedmeier[5] and K.-J. Cheng[2]. [1]Alberta Agriculture, Food and Rural Development, Lethbridge, AB (403-381-5351); [2]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB (403-317-2240); [3]Dept. of Animal Science, University of British Columbia, Vancouver, BC (604-822-6578); [4]Faculty of Bioresources, Mie University, Tsu, Japan (0592-31-1211), Utah State University, Logan UT (801-797-2151)

A derivative of polyoxyethylenesorbitan was included at varying concentrations (0, 0.01, 0.20, or 1.0%, w/v) in a 48 h *in vitro* incubation of ground barley grain in diluted ruminal fluid. Data collected on fermentation characteristics were analyzed by nonlinear regression and orthogonal polynomial contrasts (SAS Institute, Inc., 1990) to determine any linear, quadratic, or cubic effects of the nonionic surfactant (NIS) on ruminal fermentation. Quintuplicate measurements of each variable were made after 8, 24, and 48 h of incubation at 39°C. Total gas production and starch degradation were unaltered (P<0.05) by NIS, but NIS linearly decreased rate of gas production and lag time (P = 0.019 and P = 0.0007, respectively). At 24 h, propionate and total volatile fatty acid concentrations increased quadratically (P<0.05), the acetate:propionate ratio decreased quadratically (P<0.05), and starch degradation decreased linearly (P<0.05) with increasing NIS. Endoglucanase activity and bacterial protein concentrations were linearly increased (P<0.05) by NIS at all time points, as was alpha-amylase activity at 8 and 48 h. Nonionic surfactants may favourably alter the products of fermentation and increase the production of carbohydrases by ruminal bacteria.

#45 BACTERIA FROM CATTLE FED CONCENTRATES BECOME RESISTANT TO MONENSIN BUT NOT NISIN. T.R. Callaway, R.P. Lana, and J.B. Russell, Section of Microbiology, Cornell University, and Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Monensin is an ionophore that inhibits gram-positive bacteria, and the shift from gram-positive to gram-negative species in the rumen decreases fermentation losses. Bacteriocins like nisin have a similar mode of action. They inhibit gram-positive bacteria and promote the flux of ions across the cell membrane. The effect of ionophores on rumen microbial ecology has been confounded by the fact that most ruminal bacteria are gram-variable. However, ruminal population shifts can be monitored via potassium depletion. When ruminal bacteria from cattle fed timothy hay were treated with monensin, the concentration needed to cause half maximal potassium depletion was 177 nM. Nisin was slightly less effective, and the half maximal potassium depletion constant (Kd) was 393 nM. When cattle were fed 45% corn meal and 55% timothy hay, the Kd values for monensin and nisin were 400 and 410 nM, respectively. Cattle fed 90% corn meal had a very high Kd for monensin (1148 nM), but the Kd for nisin was still less than 350 nM. Based on these results: 1) monensin sensitivity decreases as dietary concentrate increases, 2) bacteria from cattle fed concentrates remain sensitive to nisin, and 3) ruminal bacteria can have different resistance mechanisms for monensin and nisin.

#46 THE ADVERSE EFFECT OF NITROGEN LIMITATION AND EXCESS CELLOBIOSE ON *FIBROBACTER SUCCINOGENES* S85. G. Maglione and J.B. Russell, Section of Microbiology and Cornell University and Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Fibrobacter succinogenes S85 cultures that were cellobiose-limited, converted cellobiose to succinate and acetate, produced little glucose or cellotriose, maintained an intracellular ATP of 4.1 mM and a membrane potential of 140 mV for 24 h, did not lyse at a rapid rate once they had reached stationary phase, and had a most probable number (MPN) of viable cells that was greater than 10^8 per ml. When the cellobiose concentration was increased 6-fold (5 to 30 mM), ammonia was exhausted, and the cultures left 10 mM cellobiose. The cellobiose-excess cultures produced succinate and acetate while they were growing, but there was little increase in fermentation acids after the ammonia was depleted and growth ceased. The stationary phase, cellobiose-excess cultures had a lysis rate that was 7-fold faster than the cellobiose-limited cultures, and the MPN was only 3.3×10^3 cell per ml. The stationary phase, cellobiose-excess cultures had 2.5 times as much cellular polysaccharide as the cellobiose-limited cultures, but the intracellular ATP and membrane potential were very low (0.1 mM and 40 mV, respectively). Methylglyoxal, a potentially toxic end-product of carbohydrate fermentation, could not be detected, and fresh inocula grew rapidly in spent medium that was supplemented with additional ammonia. Stationary phase, cellobiose-excess cultures converted cellobiose to glucose and cellotriose, but the apparent Km of cellotriose formation was 15-fold lower than the Km of glucose production (0.7 versus 10 mM).

#47 COMPLEMENTATION OF AN *ESCHERICHIA COLI* ADENYLATE CYCLASE MUTANT WITH A GENE FROM *PREVOTELLA RUMINICOLA* D31D.

T.R. Whitehead[1], M.A. Cotta[1], and M.B. Wheeler[2], [1]USDA/ARS, National Center Agricultural Utilization Research, Peoria, IL 61604 (309-681-6272), and [2]Dept. of Animal Sciences, University of Illinois, Urbana, IL 61801

Regulation of gene expression by anaerobic and other bacteria involves a variety of mechanisms, including the process of catabolite repression. Synthesis of cyclic AMP (cAMP) by the enzyme adenylate cyclase is involved in catabolite repression in aerobic and facultative anaerobic bacteria. However, the presence of cAMP in anaerobic bacteria has been questioned. Previous work in our laboratories has demonstrated that cAMP is virtually undetectable in a variety of ruminal and other anaerobic bacteria, even under growth conditions where catabolite repression-like regulatory phenomena were demonstrated. The only exception observed was with the ruminal bacterium *Prevotella ruminicola* D31d, which produced increased levels of cAMP during early growth and early stationary phases. In order to further study the presence of cAMP in this strain, attempts were made to clone the gene for adenylate cyclase. The *E. coli* strain SP850, which is deficient in adenylate cyclase, cannot ferment lactose and produces colorless colonies on MacConkey agar, was used as the host for cloning the P. ruminicola D31d gene. A genomic bank from D31d was prepared with pUC18 and introduced into SP850 by electroporation. One clone was isolated that produced dark red colonies when plated onto MacConkey agar, indicative of lactose fermentation. The clone contained a plasmid with a 2.3-kb insert. The clone is currently being analyzed for cAMP production and the DNA insert is being sequenced. Presence of cAMP in the *E. coli* clone and DNA sequence analyses of the insert should provide evidence for the isolation of an adenylate cyclase gene from *P. ruminicola* D31d, which would be the first such gene cloned from an anaerobic bacterium.

#48 ACID TOLERANCE OF *ESCHERICHIA COLI* O157:H7 UNDER AEROBIC CONDITION.

E.S. Woodruff and T. May, Dept. of Animal and Range Sciences, New Mexico State University, Box 30003, Dept. 3-I, Las Cruces, NM 88003 (505-646-2016)

Escherichia coli O157:H7 has emerged as a food-borne pathogen of significant public health concern. Outbreaks involving acidic foods have drawn attention to acid tolerance properties of E. coli O157:H7. The mechanisms for acid tolerance are not well understood; the rpoS gene may be involved. Proteins regulated by rpoS have been associated with stationary-phase acid tolerance in *E. coli* K-12. We are interested in looking at the acid tolerance properties in *E. coli* O157:H7. The bacterial strains used were: *E. coli* K-12 MC4100 (ATCC35695), *E. coli* K-12 TM9700 (rpoS-), *E. coli* O157:H7 (ATCC43895), and *E. coli* O157:H7 FRIK816-3 (rpoS-). These organisms were grown in Luria-Bertani (LB) broth at pH 5, 6, 7, and 8 to log and stationary phase. The organisms were transferred into LB broth (pH 2.5), incubated (2 h), and plated onto LB agar to determine the percentage of survival. Organisms grown to stationary phase and those grown in a slightly acidic environment prior to treatment at pH 2.5 had the highest survivability. Wild-type *E. coli* O157:H7 had the highest levels of acid tolerance when grown to stationary phase prior to the acid treatment. Wild-type *E. coli* K-12 MC4100 and *E. coli* O157:H7 had the highest levels of acid tolerance when grown in an acidic environment (pH 5) prior to the acid treatment. Acid tolerance was dependent on the growth phase and the pH of the medium.

#49 COMPETITIVE COLONIZATION OF SHEEP BY *E. COLI* O157:H7. N.A. Cornick, T.A. Casey and H.W. Moon, Veterinary Medical Research Institute, Iowa State University and National Animal Disease Center, Ames, IA 50010 (515-294-6236)

Ruminants are considered a reservoir of *E. coli* O157:H7 but little is known about how animals become colonized. We developed a competitive colonization model in sheep to determine whether *E. coli* O157:H7 colonize ruminants more effectively than other pathotypes of *E. coli.* Six sheep were simultaneously inoculated with 10^{10} or 10^{7} CFU of 5 different strains of *E.coli*: 2 strains of O157:H7, 2 enterotoxigenic (ETEC) strains, and 1 enteropathogenic strain. At 2 d post inoculation (pi) all strains were detected in the feces of all animals. At 2 weeks pi 11/12 and 12/12 animals were still shedding the 2 O157:H7 strains but less than half of the animals were shedding the other 3 strains. At 2 months (m) the 2 strains of *E. coli* O157:H7 given at 10^{10} CFU were detected in 2/6 and 4/6 animals at $\leq$50 CFU/g. When these O157:H7 strains were given at 10^{7} CFU 2/6 animals were still shedding 2 m pi ($\leq$100 CFU/g). This is in contrast to 1 ETEC strain which was shed ($\leq$50 CFU/g) at 2 m pi by 3/6 animals given the 10^{10} dose but 0/6 animals given the 10^{7} dose. The other 2 strains were not detected at 2 m pi. These results suggest that *E. coli* O157:H7 colonize the alimentary tract of ruminants more effectively than other pathotypes of *E. coli.*

#50 REGULATION OF GLYCOGEN FORMATION IN CONTINUOUS CULTURE BY THE RUMINAL BACTERIUM *PREVOTELLA BRYANTII* $B_1$4. H.J. Strobel and C.R. Jones, Dept. of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606-323-4762)

Prevotella species are some of the most common bacteria in the rumen. Earlier studies have shown that *Prevotella bryantii* accumulates large amounts of glycogen during growth on maltose via a UDP-glucose-dependent pathway that had not been previously described in eubacteria. In addition, significantly more polysaccharide was formed at low vs high growth rates. Therefore, it was of interest to determine whether the synthesis of glycogen-forming enzymes, namely glycogen synthase and UDP-glucose pyrophosphorylase, was related to this growth rate-dependent glycogen accumulation. When *P. bryantii* $B_1$4 was grown on maltose in continuous culture at dilution rates ranging from 0.05 to 0.60 per h, the lowest specific enzyme activities were observed at the highest dilution rate. However, there was not a straight-forward relationship between growth rate and enzyme activities at lower dilution rates. These results suggest that the regulation of glycogen formation by *P. bryantii* is complex and involves other factors beside the control of enzyme synthesis. Preliminary results suggest that intracellular levels of metabolites may also contribute to the regulation of glycogen synthesis. Since ruminal bacteria presumably experience alternate periods of rapid and slow growth, growth rate-dependent regulation of glycogen synthesis could have important implications in the ruminal environment.

#51 INITIAL CHARACTERIZATIONS OF PROTEOLYTIC BACTERIA TOLERANT TO CONDENSED TANNINS.

C.S. McSweeney, B. Palmer, and D. O. Krause, CSIRO Tropical Agriculture, Indooroopilly, QLD 4068, Australia (61-07 3124-2723)

Ruminant production in the arid tropics is almost always limited by adequate protein supply to the rumen. Supplementary protein can be made available via shrub-legumes high in protein (eg. *Calliandra calothyrsus*), but these plants often contain condensed tannins which complex with protein thus making it unavailable to ruminal microorganisms. Tannin-phenolics are often inhibitory to ruminal bacteria but tolerant bacteria able to ferment amino acids in the presence of tannins may be of benefit to the animal. We isolated a range of bacteria from sheep or goats fed *Calliandra*, some of which were able to form clearing zones on proteinaceous agar medium overlayed with *Calliandra* tannin. Nineteen bacteria were assessed and could be divided into 3 groups: 1) proteolytic bacteria (azocasein hydrolysis assay) able to ferment carbohydrates but make little ammonia, 2) proteolytic bacteria unable to ferment carbohydrates and fermented amino-acids to ammonia at high rates, and 3) non-proteolytic bacteria able to ferment carbohydrate and fermented amino-acids to ammonia at high rates. These bacteria represented several morphotypes and could be differentiated with genetic fingerprints.

#52 ISOLATION AND CHARACTERISATION OF MUTANTS OF *PREVOTELLA ALBENSIS* (FORMERLY *PREVOTELLA RUMINICOLA*) M384 WITH DECREASED PEPTIDASE ACTIVITY.

N.D. Walker and R.J. Wallace, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK, AB21 9SB. (+44-1224-712751)

Washed cells of *P. albensis* M384 were treated with ethyl methanosulfonate and plated on minimal medium to which was added sterile filter paper discs each impregnated with different 4-methoxynaphthylamide dipeptidyl peptidase (DPP) substrates, which are toxic when hydrolysed. Zones of clearing were observed with wild-type cells. With mutagenised cells, several resistant colonies grew in the clear zone. These were isolated and their peptidase activities measured. All DPP activities were decreased. In some instances di- and tripeptidase activities were also affected. No significant difference in activity was observed between whole and sonicated cells, indicating that none were permease mutants. All grew well in minimal medium containing methionine and ammonium sulfate as N sources, and had a decreased rate of ammonia production when cultured alone and with other ammonia-producing bacteria in the presence of Trypticase. A penicillin enrichment strategy for the isolation of mutants unable to use methionine-containing peptides yielded an isolate which again appeared to have generally decreased peptidase activities rather than a single peptidase deletion.

#53 THE GINGIPAIN-LIKE (PrtA) ACTIVITY OF *PREVOTELLA BRYANTII* STRAIN $B_1$4.

H.M.F. Madeira and M. Morrison, Dept. of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583-0908 (402-472-6405)

Biochemical and mutational analysis of *Prevotella bryantii* strain $B_1$4 has shown that the enzyme responsible for much of the Gly-Arg-MNAse (PrtA) activity in cell extracts is similar to gingipain, a novel family of extracellular, calcium-dependent, cysteine proteases isolated so far only from strains of *Porphyromonas gingivalis*. The current work is aimed at the purification of the gingipain-like activity(ies) from *P. bryantii*. Cells from a 60-liter culture were anaerobically harvested and disrupted by sonication. The cytoplasmic and periplasmic proteins were concentrated by ammonium-sulfate precipitation and following dialysis, were loaded onto a Q-Sepharose column. Proteins were eluted with a linear gradient of NaCl (0-750 mM) and total PrtA activity was resolved into 4 major peaks ($PrtA_1$ to $PrtA_4$). The 2 peaks with the greatest specific activities ($PrtA_1$ and $PrtA_3$) were each subjected to Arg-Sepharose affinity chromatography, which has proven to be an effective purification step for the *P. gingivalis* enzymes. However, both $PrtA_1$ and $PrtA_4$ appear to possess only weak affinity for the resin; although there was a 1.5-fold increase in the specific activity of $PrtA_4$ following Arg-Sepharose chromatography, the yield was low (<0.01% initial activity). Activities of $PrtA_2$, $PrtA_3$, and $PrtA_4$ were significantly reduced when assays were done in the presence of oxygen, while $PrtA_1$ activity was not affected. The multiple peaks of PrtA and the differences in oxygen sensitivity suggest that multiple gingipain-like enzymes are produced by strain $B_1$4, which may be released from membrane fragments by sonication. However, some degree of enzyme fragmentation by proteolysis can not be ruled out.

#54 SEQUENCING AND CLONING OF A FAMILY I GLUTAMATE DEHYDROGENASE FROM *RUMINOCOCCUS FLAVEFACIENS* FD-1.

D.A. Antonopoulos, R. Aminov, B.A. White, and R.I. Mackie, Dept. of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801 (217-333-8809)

In prior work, activities of three major enzymes involved in ammonia assimilation (glutamine synthetase [GS], glutamate synthase [GOGAT], and glutamate dehydrogenase [GDH]) were demonstrated in crude extracts from the ruminal microorganism *Ruminococcus flavefaciens* FD-1. An oligonucleotide primer based on the N-terminal sequence of a purified NADP-dependent glutamate dehydrogenase (GDH) was designed by maximizing its overall length tempered by increasing degeneracy of the sequence. An optimal sequence was deduced and this primer was then used in conjunction with the Universal Genome Walking Kit (Clontech) to amplify a 460 bp fragment by the polymerase chain reaction (PCR). This fragment was subsequently cloned into competent *Eschericia coli* cells and sequenced. Comparison of the resultant nucleotide sequence with the National Center for Biotechnology Information (NCBI) database yielded high scoring homologies with other GDH sequences from a variety of organisms. Primers were designed using GeneWorks 2.5.1 computer application in conjunction with the Universal Genome Walking Kit for further upstream and downstream sequencing. The subsequent downstream fragment was cloned and sequenced. Sequence overlap with the prior cloned fragment yielded a consensus 932 bp fragment which exhibited high scoring homology with Family I GDH sequences. Translation of the nucleotide sequence yielded three characteristic motifs found in Family I GDH amino acid sequences. Concurrent work is being conducted on the complete sequencing and cloning of the GDH gene as well as investigations of potential regulatory mechanisms.

#55 THE GLUTAMINE SYNTHETASE (GlnN) OF *PREVOTELLA BRYANTII* B_14. Z. Wen and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln, NE 68583-0908 (402-472-6405)

Production of the toxic metabolite methylglyoxal during glucose metabolism by *Prevotella* spp. is associated with growth limiting concentrations of ammonia rather than phosphate limitation. Better understanding of nitrogen regulation in *Prevotella* spp. should provide further insight into factors affecting cell viability and yield. Glutamine synthetase (GlnN) is often the primary enzyme of ammonia assimilation when nitrogen is limiting, and is detectable in *Prevotella bryantii* B_14, a methylglyoxal-producing strain. The highest specific activity was observed when cells were grown in growth-limiting concentrations of ammonia (1 mM). However, GlnN specific activity was considerably lower than typically observed with enteric bacteria, and did not increase dramatically in response to nitrogen limitation. Western immunoblot analysis with anti-GlnN antibodies also indicated that GlnN protein abundance does not change greatly in response to nitrogen limitation. The structural gene *(glnN)* was isolated from a plasmid library of *P. bryantii* strain B_14 genomic DNA by *E. coli* mutant complementation. The GlnN protein belongs to enzyme Family III, and Northern blot analysis showed that the *glnN* transcript was greatest in nitrogen-limited cells. Methionine sulfoxamine (MSX), a potent GS inhibitor, resulted in the termination of growth within one doubling time of its addition, irrespective of the ammonia concentration in the growth medium. However, MSX did not greatly affect growth rate or yield when trypticase was provided as the nitrogen source. Although the results demonstrate that GlnN activity in *P. bryantii* is required for glutamine biosynthesis and its expression is nitrogen-regulated, the requirement for GlnN in relation to ammonia assimilation remains equivocal. One way to address this issue is to construct *gdh* mutants of *P. bryantii,* and assess how growth is affected at different concentrations of ammonia.

#56 PARTIAL PURIFICATION OF ENDOGLUCANASE OF *PIROMYCES* SP. (B3) ISOLATED FROM RUMEN OF A REVERINE BUFFALO. T.K. Walli, A.K. Samanta, Y.S. Rajput, V.K. Batish, S. Grover, and A.K. Mohanty, National Dairy Research Institute, Karnal-132001, India, (0184-255478)

Based on the highest endoglucanase activity, isolate B3, belonging to *Piromyces* sp. was selected out of the 10 fungal isolates obtained from the rumen of a Murrah buffalo for partial purification of the enzyme. At the end of 4 d of incubation on cellobiose soft agar (0.3%), the crude enzyme from the extracellular culture supernatent was fractioned with ammonium sulfate (60% saturation). The precipitated enzyme caused binding with DEAE sepharose and the gel filtration with suprose-12 column also failed to give enough enzyme recovery. On hydroxylapatite column, the enzyme gave 2.97 fold purification with specific activity of 0.3532 IU/mg (12.12% recovery). However, purification on mono Q column (FPLC) led to the separation of 4 isoenzymes of endoglucanase with 50% recovery of the enzyme and 1.9 to 4.87 fold purification. The relative abundance of these isoenzymes were 18.2, 17.1, 21.8, and 43.3 for 4 respective peaks and on SDS-PAGE, endoglucanase gave 4 activity bands, having MW of 100 kDa, 66 kDa, 39.8 kDa, and 33 kDa, respectively.

#57 A CELLULOSE-BINDING PROTEIN FROM THE GRAM-POSITIVE BACTERIUM *RUMINOCOCCUS ALBUS* BELONGS TO THE PIL-FAMILY OF PROTEINS AND IS INVOLVED WITH SURFACE ASSOCIATED PROTEIN COMPLEXES.

M.A. Larson, R.S. Pegden, R.J. Grant, and M. Morrison, Dept. of Animal Science, University of Nebraska, Lincoln, NE 68583-0908 (402-472-6405)

The *cbpC* gene, which encodes a low molecular mass cellulose-binding protein in *Ruminococcus albus* strain 8, has been cloned and sequenced by a combination of PCR and genomic walking procedures. The deduced *cbpC* sequence encodes a protein of 169 amino acids with a calculated molecular weight of 17,655 Da, and can be divided into 2 major domains. The amino-terminal third of *cbpC* is typical of the Pil-family surface associated proteins, which are most often involved with the formation of type 4 fimbriae in gram-negative, pathogenic bacteria. The remainder of the *cbpC* sequence shares homology with a highly conserved, tandemly-repeated motif present in a surface antigen protein of *Rickettsia* spp., which is thought to be critical in cell adhesion and invasion. Kyte-Doolittle hydropathy plots of *cbpC* and other type 4 fimbrial proteins are very similar, which further supports the similarity among these proteins in terms of structure and function. Northern blots of RNA isolated from cellobiose-grown cells showed that the *cbpC* transcript is approximately 640 nucleotides in length, and its abundance appears to be increased by the inclusion of either ruminal fluid, phenylpropionic acid or phenylacetic acid to the growth medium. Genomic walking procedures identified a second open reading frame beginning 177 bp downstream of the *cbpC* coding sequence, which also bears a high degree of sequence identity to type 4 fimbrial proteins. Both Southern blot and Western immunoblot analyses revealed that *R. albus* strains SY3, 7, and B199 all possess *cbpC* homologs. The molecular biology underpinning synthesis and assembly of surface-associated protein complexes by *R. albus* appears to be distinct from the cellulosome paradigm, and more consistent with a general secretory pathway most commonly found in gram-negative bacteria.

#58 ADHERENCE TO CELLULOSE BY *RUMINOCOCCUS ALBUS* STRAIN 8.

S.K. Reddy and M. Morrison, Dept. of Animal Sciences, University of Nebraska, Lincoln, NE 68583 (402-472-9382)

Cellulose degradation by many strains of *Ruminococcus albus* is either dependent upon or stimulated by the provision of both phenylacetic acid (PAA) and phenylpropionic acid (PPA) in growth media. However, the mechanistic details underpinning these requirements remain uncharacterized. *Ruminococcus albus* strain 8 was cultured in EM-cellobiose medium supplemented with either 5% (vol/vol) ruminal fluid, PAA and PPA (25 μM final concentration), or no additions. After six transfers through the respective media, cells were grown to mid-log phase (OD_{600} 0.3-0.7), harvested by centrifugation, washed, and resuspended in media to provide an initial OD_{600} of 2.0. A suspension of crystalline cellulose (20%wt/vol) was then added to the cells and after one hour, the OD_{600} of the supernatant fraction was measured. After appropriate corrections, the percent adhesion was determined by the difference between initial and final OD_{600} values. The relative adhesion values (%) were 65.6 ± 2.54, 73.0 ± 3.37, and 79.5 ± 3.74 for cells cultured in the presence of no supplements, PAA and PPA, and ruminal fluid, respectively. Therefore factors in ruminal fluid, including PAA and PPA, appear to positively affect adherence. We have begun to isolate spontaneously arising, adherence defective mutants of *R. albus* 8. Mutant enrichment was achieved by growing cells on EM-cellobiose medium containing ruminal fluid, adding cellulose to the washed cell suspension, then retrieving those cells still present in the fluid fraction. After six cycles of selection (stage 1), the adhesion value for this mutant population was reduced 60% relative to the wild type parental cells (to 32%). Three more cycles of enrichment (stage 2 mutant population) did not further reduce the adherence value. Three successive transfers of the stage 1 mutant population in EM-cellobiose medium increased the adhesion value only marginally (from 32 to 40%).

#59 CELLULOSE-BINDING PROTEINS (GLYCOPROTEINS) INVOLVED IN ADHERENCE OF *FIBROBACTER SUCCINOGENES* S85 TO CELLULOSE. E.E. Egbosimba*, J. Miron, and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1 (519-824-4120)

Bacterial surface proteins that mediate adherence of *Fibrobacter succinogenes* S85 to cellulose were identified. Antiserum raised against whole cells of the wild type bacterium, adsorbed with cells of an adhesion deficient mutant, Ad4, was used to probe SDS-PAGE separated cellulose-binding proteins (CBPs) present in outer membrane and periplasm by Western blotting. Three glycoproteins with molecular masses of 240, 220, and 180 kDa and a non-glycosylated 120 kDa outer membrane CBP, identified as endoglucanase 2 (EG2), reacted strongly with the antiserum. The 120 kDa CBP was also present in the periplasm along with 50, 45, and 40 kDa CBPs that failed to react with the antiserum. When antiserum pre-adsorbed with Ad4 and made monovalent by digestion with papain, was used to treat whole cells of S85, their capacity to bind to cellulose was blocked by 95%, while antiserum against the 180 and 120 kDa CBPs treated in the same fashion blocked binding by 70% and 35%, respectively. Preimmune serum only caused a 5% inhibition. Zymogram analysis of the CBPs, showed that 180 kDa CBP is a xylanase. No known enzymatic activity has so far been ascribed to the 240 and 220 kDa CBPs. These results show that the 240, 220, and 180 kDa outer membrane CBPs of *Fibrobacter succinogenes* may have a major role in adhesion of cells to cellulose. The 120 kDa protein, probably has a minor role.

#60 CELLULAR LOCALIZATION OF NUCLEASE ACTIVITY IN *FIBROBACTER SUCCINOGENES* S85. S.R. MacLellan and C.W. Forsberg, Dept. of Microbiology, University of Guelph, Guelph, Ontario, N1G 2W1, (519-824-4120)

Previous attempts to transform *Fibrobacter succinogenes* S85 have proven unsuccessful. Expression of a non-specific, highly active nuclease, DNase A, may be a significant barrier to the uptake of DNA. In this study, localization experiments were conducted to determine the cellular location of nuclease activity. Glutamate dehydrogenase, cellodextrinase, and endoglucanase 2 were used as marker proteins and nuclease activity was monitored by a microtitre plate-based assay and by fluorometric methods. Approximately 74% of the nuclease activity was present in the outer membrane and periplasmic fractions while the remainder was associated with the protoplasmic (cytoplasm and cytoplasmic membrane) fraction. Activity within the extra-protoplasmic fractions appeared to be mainly soluble. In contrast, a significant portion of the activity in the protoplasm remained in association with the cytoplasmic membrane. Nuclease activity remained stable in subcellular fractions that were incubated for 8 days at 37°C, 4°C and –20°C. On the basis of these results, it seems unlikely that the physical techniques used to remove or reduce nuclease activity successfully in other bacterial strains prior to transformation will prove applicable to *F. succinogenes* S85.

#61 TEMPERATURE AND PH PROFILES OF RUMINAL ENZYME FRACTIONS FROM ANIMALS FED INCREASING PROPORTIONS OF CORN IN A CORN/CORN SILAGE DIET.

V.L. Nsereko, L.M. Rode, K.A. Beauchemin, A.F. Furtado, Z. Mir, and P.S. Mir, Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, T1J 4B1 (403-327-4561)

Three Jersey steers, fitted with rumen cannulae were fed a diet of corn silage and steam rolled corn at 379 (A), 620 (B), or 790 (C) g steam rolled corn/kg^{-1} dry matter. Digesta, collected from each animal 2 h after feeding was fractionated into a solid associated phase (SAP) and an extracellular liquid phase (ELP). pH and temperature profiles were determined over pH 3.0 to 7.0, and 39 to 70°C, using synthetic substrates dyed with remazolbrilliant Blue R. Optimum conditions for SAP xylanase and amylase activities were pH 5 to 6, 45°C and pH 5 to 5.5, 50°C, respectively, and amylase activities of SAP and ELP fractions increased with each increment in corn. The SAP xylanase activities reduced with each increase in corn. In contrast the ELP xylanase activity was 3.8 and 3.6 fold higher for diet C than for diets A and B, respectively. pH optima were similar for SAP CMCase, xylanase, and amylase; however, temperature optima for CMCase were 60, 50, and 39°C for animals fed diets A, B, and C, respectively. Optimal SAP CMCase activity was highest on diet A, and lowest on diet C. In contrast, ELP CMCase activity was highest on diet C, representing 2.7 and 4.5 fold that of animals fed diets B and A, respectively. The results suggested that characteristics of SAP and ELP fibrolytic enzymes, particularly CMCase can differ, and these characteristics may vary with diet.

#62 RUMINAL POLYSACCHARIDASE ACTIVITIES IN ANIMALS FED INCREASING PROPORTIONS OF CORN IN A CORN/CORN SILAGE DIET

V.L. Nsereko, L.M. Rode, K.A. Beauchemin, A.F. Furtado, Z. Mir, and P.S. Mir., Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada, T1J 4B1 (403-327-4561)

Three Jersey steers, fitted with permanent rumen cannulae were fed a diet of corn silage and steam rolled corn at 379 (A), 620 (B), or 790 (C) g steam rolled corn/kg^{-1} dry matter. Digesta, collected from each animal, 2 h after feeding was fractionated into a solid associated phase (SAP), an extracellular liquid phase (ELP) and a free cell phase (FCP). Activities (total) of the fractions were assayed using synthetic substrates by measuring reducing sugars (RS) released at 39°C, pH 6.5. The SAP accounted for most of the avicelase (0.92 – 0.95) and amylase (0.70– 0.78) activities in all animals, and the ELP contained most of the residual amylase activity ($P<0.01$). Xylanase activity was predominantly in the SAP, representing 0.94 and 0.97 of total activity in animals fed diets A and B, respectively, but only 0.72 of that in the animal fed diet C, with the ELP accounting for most of the residual activity ($P<0.01$). CMCase and amylase activities in the SAP increased with each increment in corn ($P<0.01$). The SAP CMCase activity was but positively correlated to the SAP amylase activity ($r^2 = 0.998$; $P<0.05$) and negatively correlated to the SAP xylanase activity ($r^2 = 0.999$; $P<0.01$). The results suggest that the SAP contained most of the ruminal fibrolytic and amylolytic activity, and that ruminal amylase and CMCase activities were positively correlated.

#63 DIGESTION KINETICS OF CORN GRAIN. Y.-K. Chen, P. Schofield, A.N. Pell, and L.E. Chase, Dept. of Animal Science, Cornell University, Ithaca, NY14853 (607-255-2876)

The goal of this study was to partition corn grain into A, B1, and B2 fractions and to measure the digestion rate of each fraction. Seventeen corn samples with varying levels of NDF were extracted with 80% ethanol to obtain the ethanol insoluble residue (EIR). Aliquots of the supernatant were assayed for sugar content with the anthrone method. To obtain fiber residues for fermentation (B2), samples were dialyzed in 8M urea and Termamyl overnight, and then digested in ND solution and Termamyl. Whole grain, EIR, and B2 residues of all samples were fermented *in vitro* and gas production was measured. Gas production of the ND soluble fraction (A+B1) was calculated as the difference in gas production between the whole grain and its NDF. Gas data were fitted to the single-pool logistic equation. Ethanol extracted 7.1±1.2% of DM from corn grain but the average sugar content was only 2.6±0.3% because the EIR contained substances other than sugar. The B2 fraction was 10.6±0.7% of DM and highly digestible (94.6±1.4%). The B2 contributed 9.5% of the total gas production from corn. The lag time, digestion rate, and gas production for B2 were 3.6±0.9 h, 0.063±0.006 h^{-1}, and 3.3±0.2 ml/100mg DM. The lag time, digestion rate, and gas production for A+B1 fraction were 2.4±0.7 h, 0.093±0.019 h^{-1}, and 31.2±1.6 ml/100mg DM, respectively. Because the A fraction is small and there is little variation in the B2 fraction, emphasis should be on the digestion kinetics of the B1 fraction of corn grain samples.

#64 *IN VITRO* DIGESTION KINETICS OF TROPICAL FORAGES. F.I. Juarez, P. Schofield, and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14850 (607-255-2876)

In order to use the Cornell Net Carbohydrate and Protein System or level 2 of the N.R.C. Nutrient Requirements of Beef Cattle (1996) in the tropics, the digestion rates of the A, B1, and B2 fractions of tropical forages must be known. Our goal was to use gas curve subtraction to determine the rates for the A, B1, and B2 carbohydrate fractions. Samples of whole forage (WF), residue insoluble in 90% ethanol (EIR), and isolated NDF (ND) were fermented *in vitro* and gas production was measured. The gas volume from the following fractions, A=WF-EIR; B1=EIR-ND; and B2=ND, was determined. Four tropical grasses (Andropogon, Brachiaria, Cynodon, and Panicum) fertilized with either 0 or 100 kg N/ha/year were studied. Forages were cut at 35 or 42 d of regrowth. The data were fit with the single-pool exponential model with lag. The volume (ml gas/100 mg DM), rate (%/h) and lag (h) were: WF (22.8; 5.3; 2.1); A (3.2; 13.3; .5); B1 (1.5; 24.5; .2); and B2 (18.3; 6.6; 5.2). Fertilized forages produced less gas than the unfertilized ones. Volumes and rates varied by species. Andropogon and Panicum produced the most gas for WF and A. The gas volumes from B1 and B2 did were not differ among species. Rates for WF and B2 were lower for Cynodon than for the other species. For A and B1, Andropogon and Brachiaria had the highest rates. Overall, Andropogon produced the greatest gas volume and had the fastest rate while Cynodon produced the least gas at the slowest rate. Panicum and Brachiaria were intermediate.

#65 SOYHULL DIET SUPPRESSION OF *IN SITU* DIGESTION KINETICS.

R. Uctuk, D.E. Johnson, and G. Bernal, Dept. of Animal Science, Colorado State University, Fort Collins, CO 80521 (970-491-7833 or 491-3080) email: ridvan@lamar.colostate.edu

Two ruminally-cannulated steers (average weight 796 kg) were used to determine *in situ* digestion rate of DM, NDF, and ADF for alfalfa and soyhull in a crossover design. Dacron bags containing 7 g of alfalfa or soyhull were incubated ruminally for 0,3,6,9,12,18,24,30,36,48,72, and 96 h. Ruminal pH was measured for alfalfa vs soyhull diet. Effects of feeding 100% alfalfa diet vs 100% soyhull diet to the steers on the DM, NDF, and ADF digestion rate of alfalfa and soyhull in the dacron bags were measured. Measured *in situ* disappearance rate of DM, NDF, and ADF from soyhull or alfalfa was lowest when the host animal was fed soyhull. Rates of alfalfa DM, NDF and ADF digestion were depressed 77, 73, 78%, respectively, when the animals were fed soyhull ($P<.05$). Conversely feeding alfalfa as compared to soyhull to the host animal resulted in 300% or greater increases in the *in situ* rate of digestion of soyhull DM, NDF, and ADF. This shows that an alfalfa diet has a positive effect on the digestion rates of soyhull; and conversely, a soyhull diet has a negative effect on the rate of digestion of alfalfa DM, NDF, and ADF. Ruminal pH was 0.82 higher for alfalfa diet than soyhull diet. The higher pH due to the alfalfa diet may be the reason for the positive effects of alfalfa on the digestion rate of soyhull DM, NDF, and ADF in the dacron bags.

#66 EFFECT OF BM3 MUTATION ON RATE AND EXTENT OF NEUTRAL DETERGENT FIBER DIGESTION OF CORN SILAGE GROWN IN MULTIPLE ENVIRONMENTS.

Y. Ying and M.S. Allen, Dept. of Animal Science, Michigan State University, East Lansing, MI 48824 (517-432-1386)

Effect of the brown midrib (bm3) mutation on *in vitro* rate and extent of NDF digestion of corn silage was evaluated. Twelve forage samples of Cargill hybrid 6727 BMR corn and its near isogenic normal corn were collected from 3 locations per year in 1994 and 1995. The locations were Aurora, IL, Covington, OH, and Leola, PA in 1994 and Aurora, IL, Carrolton, MO, and Mt. Vernon, IN in 1995. Corn forages were chopped on the same day and ensiled in 9.7 x 29 cm PVC mini silos. After 30 days of ensiling, samples were dried at 55° C and ground with a Wiley mill (1 mm) for analysis. Samples were analyzed sequentially for NDF, ADF, and sulfuric acid lignin. Samples were then incubated in rumen fluid and NDF residues were determined immediately following incubation for 0, 1.5, 3, 6, 9, 12, 18, 24, 36, 48, 72, 96, and 120 h. Data were analyzed with the non-linear model procedure of JMP using the following model: NDF disappearance = $100-(I+(100-I)*e^{-k(time-lag)})$. Dry matter content of bm3 corn silage was 12% lower than normal when chopped on the same date. No difference was observed for NDF content between the treatments but lignin content was reduced 36% for the bm3 treatment. Indigestible residue decreased 40% and rate of digestion of potentially digestible NDF increased 32% for the bm3 treatment. No effect of treatment was observed for lag time. Digestibility of NDF at 30 h was 46% greater for the bm3 treatment when predicted by the model. Enhanced NDF digestion of bm3 can be attributed to both increased rate of digestion and decreased indigestible NDF fraction.

	BMR	Normal	Significance (P =)
DM (%) 55° C	28.9	32.7	< .01
NDF (% of DM)	43.3	42.5	NS
Lignin (% of DM)	1.74	2.73	< .01
Indigestible NDF (% of NDF)	20.7	34.4	< .01
Rate of digestion (%/h)	3.84	2.89	< .01
Lag (h)	2.08	2.51	NS
NDF digestibility (30 h, %)	52.1	35.6	< .01

#67 EFFECTS OF CORN GRAIN PARTICLE SIZE AND MATURITY AT HARVEST ON FEEDING BEHAVIOR AND RUMINAL PH OF HOLSTEIN HEIFERS BEFORE AND AFTER CALVING.

Y. Ying, M.S. Allen and M.J. VandeHaar, Michigan State University, East Lansing, MI (517-347-0450)

The effects of corn grain particle size and maturity on feeding behavior of Holstein heifers before and after calving were examined. Eight ruminally and duodenally cannulated pregnant Holstein heifers were utilized in a replicated 4 x 4 Latin square design balanced for carryover effects with 21-d periods. Corn grain was harvested from one field of approximately 3 ha which was divided into 12 subplots of 18 rows. Alternate subplots were harvested as high moisture corn at 67% DM and ensiled in a bag silo. The remaining subplots were harvested at 83% DM and artificially dried to 86% DM. Dry corn was mixed, divided in half and ground finely (810 μm) or cracked (4442 μm). High moisture corn was ground finely (1996 μm) or coarsely (5632 μm) prior to feeding. Two experiments were conducted using the same animals before calving (beginning approximately 5 months prior to calving) and after calving (beginning approximately 1 month after calving). The experiment was conducted both before calving (mean DMI = 1.61% of BW) and after calving (mean DMI = 3.33% of BW) because DMI might change the relative passage rates and therefore the digestibility of the treatments. Diets contained 62% forage and 36% corn for the experiment before calving and 49% forage and 38% corn grain for the experiment after calving. The forage was the same alfalfa silage for both experiments. Feeding behavior and ruminal pH were monitored continuously by computerized data acquisition system for 4 d each period. Increased maturity resulted in an additional meal per day both before and after calving. The animals consumed approximately 11.2 and 10.2 meals per day for dry and high moisture corn, respectively for both experiments. High moisture corn tended to increase meal size after calving only ($P<.08$) and had no effect on DMI either before or after calving. High moisture corn resulted in lower mean ruminal pH and increased variance in ruminal pH for both experiments. Particle size had no effect ($P>.10$) on meal patterns, daily DMI, or ruminal pH in these experiments. Maturity at harvest might have a greater effect on feeding behavior and ruminal pH than particle size for corn grain.

#68 EFFECT OF FATTY ACIDS AND THEIR Ca-SALTS ON RUMINAL FERMENTATION, DIGESTION, AND MICROBIAL GROWTH DURING INCUBATION OF GRASS HAY *IN VITRO*.

U.M. Yang and H. Fujita, Dept. of Animal Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro-shi 080, JAPAN (1-330-263-3795)

This study was designed to clarify conditions relating to the inhibitory effects of grass lipids on ruminal fermentation at the initial stage of incubation. Lipid-free grass hay was the control substrate. Substrates were incubated for 24 h with or without addition of linolenic and stearic acids and their Ca-salts. Ruminal fermentation, digestion, and microbial growth were retarded ($P<0.05$) by 6% linolenic acid, but not by stearic acid or the Ca-salts of linolenate or stearate. Ruminal nitrogen degradation was inhibited by linolenic acid but not by stearic acid or the Ca-salt of linolenic acid. These results suggested that inhibitory effects of linolenic acid in grass lipids on ruminal fermentation, digestion, and microbial growth were significant. Polyunsaturated fatty acids produced during hay making, and lipolysis at the initial period of ruminal incubation have remarkable effects on the ruminal fermentation.

#69 PREDICTION OF VFA PROPORTIONS FROM NUTRIENT INTAKE AND AVAILABILITY **[1]K. A. Beauchemin, [2]M.R. Weisbjerg, [2]T. Hvelplund, [2]B.M. Bibby, and [3]P. Nørgaard, [1]Agriculture and Agri-Food Canada, Research Center, Lethbridge, AB, TIJ 4B3; [2]Danish Institute of Animal Science, DK-8830, Tjele, Denmark; and [3]The Royal Veterinary and Agricultural University, DK-1870, Frederiksberg C, Denmark (tel. 403-317-2335; fax. 403-382-3156)**

A study was conducted to determine the accuracy of published techniques for predicting molar proportions of volatile fatty acids (VFA) in dairy cows, and to develop more accurate predictions by accounting for ruminal availability of dietary nutrients. Data on VFA proportions for dairy cows were compiled from 33 publications (147 diets, 958 cows). Intake of DM, OM, CP, starch, sugar, water soluble carbohydrates (WSC), NDF, ADF, cellulose, and fat were recorded. Ruminal degradation characteristics of CP, starch, and NDF were calculated from degradation characteristics of individual ingredients. Ruminal digestion of DM, OM, CP, starch, sugar, WSC, NDF, ADF, cellulose, and fat was estimated from intake and ruminal digestion coefficients. A stoichiometric approach to estimating VFA proportions was evaluated (Murphy, 1984). Correlation coefficients (r) for observed and predicted acetate, propionate, and buyrate proportions were very low; .03, .20, and -.23, respectively. Estimates of molar proportions of individual VFA from percentage concentrate in the diet (Lescoat and Sauvant, 1994) also showed large discrepancies between observed and predicted values. Multiple regression equations were developed to predict individual VFA proportions from nutrient intake and availability. When only nutrient intake variables were used, predictions of acetate, propionate, and butyrate proportions were poor (r^2 = .18, .28, .35, respectively). Incorporating estimates of ruminal availability of nutrients vastly improved predictions of ruminal VFA proportions (r^2 = .61, .65, .73, respectively).

#70 TRACE ELEMENTS STATUS IN THE DIFFERENT FRACTIONS OF RUMEN LIQUOR UNDER DIFFERENT DIETARY REGIMS. **S. Singh and R. Singh, Dept. of Veterinary Physiology, College of Veterinary Science, Punjab Agricultural University, Ludhiana (Punjab) 141004, India**

Four healthy rumen fistulated male buffalo calves varying in weight from 100-125 kg and in age of 14-16 months were used as an experimental animals. All animals were subjected to 4 different kinds of diet. Diet A was coventional ration consisting of wheat straw, green fodder, concentrate, vitablend, and mineral mixture. Diet B was subabul diet containing subabul concentrate, wheat straw and green fodder. Diet C was wheat straw treated with urea, molasses, and salt in ratio of 2:6:1. Diet D was exclusively wheat straw. All animals were kept for 21 d on each diet for adaptation of rumen microbial population. Rumen liquor samples were collected at weekly interval for analysis of trace elements. Copper, zinc, iron, cobalt, and manganese were determined in whole rumen liquor, strained rumen liquor, and centrifuged rumen liquor. The concentration of trace elements were estimated by using Atomic Absorption Spectrophotometer (Model AA6, Varian Techtron, M/s Varian Techtron Pvt. ltd., Melbourne, Australia). Results revealed that concentration of all trace elements were maximum in whole rumen liquor followed by strained rumen liquor and lowest in centrifuged rumen liquor. Copper and cobalt status was maximum on diet A followed by diets B, C, and D. However, zinc, iron, and manganese levels were highest on diet B followed by A, C, and D.

#71 ALTERED DIETARY ENERGY UTILIZATION BY LACTATING DAIRY COWS FED HIGH LEVELS OF WHOLE COTTONSEED. **J.R. Carpenter, H.A. Rachuonyo, and R.Y. Niino-DuPonte, Dept. of Animal Sciences, CTAHR, University of Hawaii at Manoa, Honolulu, HI 96822 (808-956-8393)**

Whole cottonseed (WCS), is a unique feed for lactating dairy cows where heat stress is high and DM/fiber intake is limited. Twenty-four Holstein cows averaging 575 kg BW, 50 DIM, 35 kg/d/ milk, and average body condition score of 2.6 (on 5 point system) were blocked by these variables then randomly assigned to either: Trt 1 (control), diet with no cottonseed products; Trt 2, diet with 15% WCS (DM basis); and Trt 3, diet with 30% WCS (DM basis). Diets were balanced to provide 19% CP, 1.75 Mcal/kg NEl, 75% TDN, similar Ca to P ratios and a minimum of 19% ADF. Twice a day, alfalfa hay was fed *ad libitum* and .91 kg of alfalfa cubes were fed to each cow with her concentrate mix (.4 kg/kg milk). There were no differences (P>.05) in DM, OM, and CP intake, and daily milk, protein, and solids-not-fat production among treatments. Cows fed Trt 3 lost more (P<.05) weight (.-03, .06, .-26 kg/d) and body condition (+.16, +.06, and -.06), and both WCS fed groups produced more (P<.01) fat (.9, 1.3, 1.5 kg/d), 3.5% FCM (29.3, 36.8, 38.1 kg/d), energy corrected milk (29.6, 35.6, 36.5 kg/d), and total solids (P<.05) (3.8, 4.3, 4.3 kg/d) than cows fed the control diet. NEl consumption averaged 39.64 Mcal/d (25.8% of this for maintenance). Percent of NEl used for lactation was significantly higher for the 2 WCS diets (64.8 and 66.2%) than the control (50.3%) diet . Energy loss was 2.55 times higher for animals not fed WCS. These data suggest that WCS greatly improves energy balance, can be effectively used at levels higher than normally recommended (4 to 5 kg/d), and should be considered a key ingredient in alfalfa hay based diets for early lactating dairy cows in hot climates.

#72 EFFECT OF *YUCCA SCHIDIGERA* EXTRACT ON RUMINAL FERMENTATION *IN VITRO*. **Y. Wang[1], T.A. McAllister[1], L.J. Yanke[1], P.R. Cheeke[2], and K.-J. Cheng[1], [1]Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB T1J 4B1 (403-327-4561) and [2]Desert King International, Chula Vista CA 91911 (541-737-1917)**

Ruminal fluid (RF) from dry dairy cows fed either 100% alfalfa hay or 70% barley grain/30% barley silage was diluted 1:1 with buffer containing 0 (control) or 20 mg/mL of YE to prepare inoculum for an *in vitro* incubation. Substrate for alfalfa RF was ground alfalfa hay; for barley RF, rolled barley grain was used. Incubations in 35-ml serum vials at 39°C were terminated after 0, 1, 2, 4, 6, 8, 12, and 24 h. Gas production and total VFA were increased by YE with barley ($P<0.05$ from 0 to 10 h), but were reduced ($P<0.01$) by YE with alfalfa. However, the ratio of acetic to propionic acids was greatly reduced by YE ($P<0.01$) with both diets. Ammonia concentrations were substantially reduced by YE ($P<0.01$), irrespective of diet. With either diet, total steroidal saponins (expressed as smilagenin equivalents) did not change ($P>0.05$) over 24 h. In contrast, soluble steroidal saponins concentrations declined with time ($P<0.01$), more rapidly with alfalfa than with barley ($P<0.05$). Yucca extract stimulated ruminal digestion of barley, repressed that of alfalfa, and decreased protein degradation with both feeds. Ruminal microbes did not degrade steroidal sapogenins, but did deglycosylate steroidal saponins. Supplementary YE could be beneficial for ruminants fed high barley diets.

#73 EFFECT OF SESBANIA SPECIES ON RUMINAL PROTOZOA IN ETHIOPIAN AND SCOTTISH SHEEP.

B. Teferedegne[1,2], P.O. Osuji[2], A. Odenyo[2], R.J. Wallace[1], and C.J. Newbold[1], [1]Rowett Research Institute, Aberdeen AB21 9SB, UK (+44-1224- 712751) and [2]International Livestock Research Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia

Sesbania sesban, a multipurpose leguminous tree from sub-saharan Africa, was found previously to transiently depress the number of protozoa in the rumen of sheep in the UK and to inhibit protozoal activity *in vitro*. In the present study 9 ILRI accessions of *S. sesban* and one accession of *S. goetzei* were ranked according to their ability to inhibit protozoal bacteriolytic activity, measured by the degradation of 14C-labelled *Selenomonas ruminantium in vitro*. The 4 accessions identified as the most inhibitory (15036, 15022, 15019, and 1198, all *S. sesban*) were fed to sheep in Debre Zeit (DZ), Ethiopia. Twenty sheep received 500 g of sululta hay plus 200 g of wheat bran per day. Groups of 4 sheep were supplemented with 200 g/d of each *S. sesban*; 4 control sheep received no supplement. No effects on protozoal numbers were observed. The effect of the plants on protozoal activity *in vitro* was re-examined with rumen fluid collected in DZ and Aberdeen(Ab), UK. The antiprotozoal effect of *S. sesban* (Ab vs DZ, percent inhibition in the breakdown, compared to the control no addition, of S. ruminantium when added at 10 g/l was, 86 vs 49; 100 vs 47; 100 vs 62, and 84 vs 62 for accessions 15036, 15022, 15019, and 1198, respectively) was less evident in rumen fluid collected in DZ than Ab. Washed protozoa prepared from sheep in DZ and Ab remained sensitive to *S. sesban*. Apparently the difference in the effectiveness of S. sesban in suppressing protozoal activity in whole rumen fluid, both *in vivo* and *in vitro*, in DZ and Ab, was not due to any decreased susceptibility of the protozoa per se. Bacterial metabolism of the antiprotozoal component of *S. sesban* is a likely explanantion.

#74 EFFECT OF 9,10-ANTHRAQUINONE ON MICROBIAL METHANE PRODUCTION, RUMINAL FERMENTATION, AND NUTRIENT DIGESTION IN SHEEP.

K.M. Koenig[1], L.M. Rode[1], L. Kung, Jr.[2], and L. Neill[1], [1]Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1 (403-327-4561) and [2]Dept. of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717-1303 (302-831-2522)

Four ruminally cannulated sheep were used in an experiment designed as a 4 x 4 Latin Square with a 2 x 2 Factorial arrangement of treatments to determine the effect of feeding 9,10-anthraquinone (AQ), a potential methane inhibitor, on ruminal methane, sulphide and volatile fatty acid (VFA) production in sheep fed diets with and without sulphate supplementation. In addition, the influence of AQ on microbial populations and nutrient digestion was measured. The basal diet consisted (dry matter basis) of 71% alfalfa hay pellets and 29% barley grain-based concentrate. The 4 dietary treatments were control, 33 ppm AQ, 0.4% sulphate, and 0.4% sulphate plus 33 ppm AQ. The diets were fed at a level of 80% of *ad libitum* intake and were allocated at 2 h intervals. Feed intake, dry matter digestibility, and rumen pH were not affected by the supplementation of AQ or sulphate ($P>0.05$). Anthraquinone had no affect on methane production in rumen contents incubated for 4 h, nor did it affect the reduction of sulphate to sulfide in the rumen ($P>0.05$). Total VFA and molar concentrations of VFA were not affected by AQ or sulphate ($P>0.05$). Total rumen bacterial numbers were reduced numerically to less than half of the control values when sheep were supplemented with AQ or sulphate. Anthraquinone caused a reduction in the number of celluloytic bacteria ($P<0.05$) but this effect was negated when AQ was supplemented with sulphate. There was no affect of the treatments on rumen protozoal numbers. The data indicated that AQ supplemented at 33 ppm had no effect on methane production and rumen fermentation although it appeared to influence bacterial populations within the rumen.

#75 EFFECT OF FREEZING AND EXTRACTION METHOD ON POLYSACCHARIDE-DEGRADING ACTIVITIES OF RUMEN CONTENT.

A.N. Hristov, T.A. McAllister, and K.-J. Cheng, Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB T1J 4B1 (403-327-4561)

Three preparations of whole ruminal contents from eight cattle were further processed by five methods prior to determination of carboxymethylcellulase (CMCase), xylanase, and amylase activities. Cattle were fed either a 90%forage/10% concentrate or a 70% concentrate/30% forage diet. Ruminal content was studied whole (WRC), or as rumen fluid enriched (ERF) with microorganisms loosely attached to fed particles or as a mixture of fluid and solid fractions (1:1, ml:g) blended together for 30-s (WRCB). Selected enzyme activities were measured in fresh samples as well as samples that had been frozen for 48 h. Samples were processed in three manners: 1) sonication three times for 30-s per time, 2) treatment with lysozyme, and 3) treatment with lysozyme + EDTA. Amylase activity was higher ($P<0.05$) and CMCase and xylanase activities were lower ($P<0.05$) in samples from the those fed 90% concentrate as compared to 70% concentrate. Xylanase and CMCase activities in frozen samples were 83% and 90% ($P<0.05$), respectively, of those measured in non-frozen samples but freezing increased amylase activity by 25% ($P>0.05$). Across processing methods, enzyme activities were highest ($P<0.05$) in WRCB, followed by ERF and then by WRC ($P<0.05$). Xylanase and CMCase activities following sonication were 4% higher and 7% lower, respectively, than those measured after lysozyme treatment ($P<0.05$). It was concluded that freezing and sonicating are suitable methods for processing ruminal content samples for determination of polysaccharide-degrading enzymes.

#76 TANNIN-PROTEIN INTERACTIONS IN SOLUTION STUDIED BY FLUORESCENCE QUENCHING.

P. Schofield and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-0604)

Tannins are polyphenolic secondary compounds found in many tropical legumes. They inhibit microbial growth, probably because of their strong protein binding capacity, and may have both negative and positive nutritional effects depending on tannin type and dosage. We have studied the interaction of both hydrolysable (tannic acid) and condensed tannins (myrtle, quebracho) with lysozyme by labeling the protein N-terminus with fluorescein isothiocyanate to produce a fluorescent enzyme (LF) which retains full activity. The fluorescence of LF is strongly quenched when tannins bind to the protein. The concentrations of tannic acid, myrtle, and quebracho tannins needed to produce 50% enzyme inhibition of LF (1.0, 2.5, 5.0 μg/mL respectively) were similar to those required for 50% fluorescence quenching (2.5, 2.5, 3.5 μg/mL). Tannic acid quenching of LF was reduced about 5 fold by addition of 0.2 M NaCl and was eliminated by addition of 20% DMSO. This same solvent also blocked tannin-induced enzyme inhibition. For tannic acid, quenching was reduced as the temperature was raised from 4 to 40° C. Quenching curves (Stern-Volmer plots) as a function of tannin concentration curved upwards sharply. One possible interpretation may be a strongly cooperative binding process. These results suggest that fluorescence quenching experiments may provide useful data on tannin-protein interactions in solution.

Author index by abstract number

The Organizing Committee

Michael S. Allen, Chairman
Jeffrey L. Firkins, Nutrition and Agronomy
Michael A. Cotta, Microbiology and Physiopathology
Mark A. Rasmussen, Treasurer
Jane Leedle, Secretary

would like to thank

Major Donors

USDA, Agricultural Research Service
Purina Mills, Inc.

and Other Donors

Chr. Hansen BioSystems
Diamond V Mills
Fort Dodge Animal Health
Hoffman LaRoche

for providing

the proceedings, mixer, refreshments, poster boards and postage.