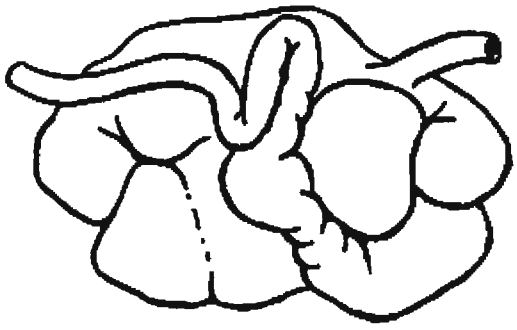
Abstracts

Conference on Rumen Function

Volume 22, 1993



42 Years of Interaction

1951-1993

22nd Biennial Conference on Rumen Function
Chicago, Illinois
November 9-11, 1993

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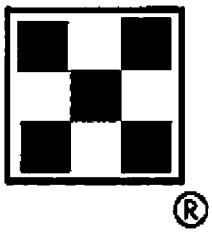

22nd BIENNIAL

CONFERENCE ON RUMEN FUNCTION

1951-1993

Welcome to the 22nd Biennial Conference on Rumen Function. The Rumen Function Conference has been meeting in the Congress Hotel since November 1951. The Conference originally focused on the problem of bloat, and this aspect of rumen function was a central theme until 1961. Since this time, the Conference has broadened its program to other factors which influence rumen fermentation and physiology.

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

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

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

Sincerely,

James B. Russell
Research Microbiologist
USDA

Agenda

November 9, 1993

8:00 - 11:30 pm Mixer in Great Hall.

November 10, 1993

Microbiology-Physiopathology Panel

8:00 Brief introduction by James B. Russell.

8:10 #12 NATURAL VARIABILITY AND DIURNAL FLUCTUATION OF BACTERIOPHAGE POPULATIONS IN THE RUMEN. A V Klieve, R A Swain and J V Nolan. Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, N.S.W. 2351, Australia.

8:30 #7 CLONING AND SEQUENCING OF AN INDIGENOUS PLASMID, PBAW301, FROM *Ruminococcus flavefaciens* R13e2. Tammy May*, Philip E. Vercoe, and Bryan A. White. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801.

8:50 #5 DNA SEQUENCE AND PRELIMINARY TRANSCRIPTIONAL ANALYSIS OF ENDO-BETA-1,4- GLUCANASE GENES FROM *Ruminococcus flavefaciens* FD-1. Philip E. Vercoe*, Jennie L. Finks, Donn H. Spight, and Bryan A. White, Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801.

9:10 #14 DEVELOPMENT OF A DNA PROBE FOR THE DETECTION OF RUMINAL *Streptococcus bovis*. D. A. Odelson[1], L. Nelms[1], S. F. Kotarski[2], and R. B. Hespell[3], [1]Dept. of Biology, Central Michigan University, Mt. Pleasant MI, Center for Microbial Ecology, E. Lansing, MI, [2]The Upjohn Co., Kalamazoo, MI, and [3]USDA-ARS, Peoria, IL.

9:30 #9 DEVELOPMENT OF A RIBOTYPING METHOD FOR THE STUDY OF GASTROINTESTINAL MICROECOLOGY: APPLICATION TO LACTOBACILLI IN THE WEANLING-PIGLET. Denis O. Krause*, Bryan A. White, Robert A. Easter, and Roderick I. Mackie, Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801.

9:50 #8 THE MECHANISM OF ENERGY SPILLING IN *Streptococcus bovis*. G.M. Cook and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853.

10:10 #13 UTILIZATION OF A VARIETY OF XYLANS BY *Butyrivibrio fibrisolvins* STRAIN H17C. R.B. Hespell and M.A. Cotta, USDA/ARS, Natl. Cent. Agric. Utilzn. Res., Peoria, IL (309-681-6270).

10:30 Brief break.

10:50 #6 INFLUENCE OF GROWTH SUBSTRATE ON BIOENERGETIC GROWTH EFFICIENCY OF ANAEROBIC CELLULOLYTIC BACTERIA. H. J. Strobel and K. A. Dawson. Department of Animal Sciences, University of Kentucky, Lexington, KY 40546.

11:10 #1 ANTIBIOSIS BETWEEN RUMEN BACTERIA AND FUNGI Burk A. Dehority and Patricia A. Tirabasso, Department of Animal Science Ohio Agricultural Research and Development Center, The Ohio State University Wooster, OH 44691.

11:30 #16 WHITE ROT FUNGI TO IMPROVE UTILIZATION OF GRASS LIGNOCELLULOSE. D. E. Akin, A. Sethuraman, W. H. Morrison III, S. A. Martin, and K.-E. L. Eriksson, Russell Research Center, ARS-USDA, Athens, GA 30613; Department of Biochemistry and Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602.

11:50 #3 THE ROLE AND REGULATION OF LYTIC ACTIVITY IN *Fibrobacter succinogenes*. J. E. Wells and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853.

12:10 Lunch.

1:30 #11 DUAL POOL LOGISTIC EQUATION FOR ANALYSIS OF IN VITRO FERMENTATION OF MIXED SUBSTRATES BY COMPUTERIZED GAS PRESSURE MEASUREMENTS. P. Schofield and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853.

1:50 #15 COMPARISON OF MICROBIAL FERMENTATION IN DUAL FLOW CONTINUOUS CULTURE AND THE RUMEN OF DAIRY COWS. H. R. Mansfield, M. I. Endres, and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108.

2:10 #4 ESTIMATION OF OPTIMAL RUMEN NH_3 CONCENTRATION USING A DIFFUSION MODEL AND *Coleospora agminosa* gen. nov. sp. nov. M. J. Fron and D. M. Schaefer. Meat and Animal Science Dept., Universib of Wisconsin, Madison, WI 53706.

2:30 #10 RUMINAL METABOLISM OF NITROPROPANOL; MICROBIAL CONTRIBUTION. R. C. Anderson, M. A. Rasmussen, and M. J. Allison. USDA, ARS, National Animal Disease Center, Ames, IA 50010.

2:50 #2 MICROBE ALTERATIONS ASSOCAITED WITH RUMINAL SULFIDE PRODUCTION IN CATTLE FED A HIGH SULFATE DIET. D. H. Gould, B. A. Cummings, D. W. Hamar, Dept. of Pathology, Colorado State University, Fort Collins, CO 80523 and D. R. Caldwell, Dept. of Molecular Biology, University of Wyoming, Laramie, WY 82071.

3:10 Brief break

Nutrition-Agronomy Panel

3:30 #20 VARIATION AMONG INDIVIDUAL SMOOTH BROMEGRASS PLANTS FOR KLASON LIGNIN AND CELL-WALL PHENOLIC ACIDS. H. G. Jung and M. D. Casler, USDA-ARS, US Dairy Forage Research Center Cluster, St. Paul, MN 55108 and Dept. of Agronomy, University of Wisconsin, Madison, WI 53706.

3:50 #17 ALFALFA NITROGEN AND CARBOHYDRATE PARTITIONING AS INFLUENCED BY NITROGEN AS INFLUENCED BY NITROGEN FERTILIZATION. J. H. Cherney, D. J. R. Cherney, and J. L. Ford, III, Departments of Soil, Crop, and Atmospheric Sciences and Animal Science, Cornell University, Ithaca, NY 14853.

4:10 #22 VARIATION IN EXTRACTABLE PECTIC SUBSTANCES OF ALFALFA STEMS WITH MATURITY. M. B. Rymph, P. J. Van Soest, B. A. Lewis, and L. E. Chase, Dept. of Animal Science, and Dept. of Nutritional Sciences, Cornell University, Ithaca, NY 14853.

4:30 #18 FORAGE DRY MATTER AND NITROGEN DISAPPEARANCE AS INFLUENCED BY NITROGEN FERTILIZATION AND COW DIET. D. J. R. Cherney, J. Siciliano-Jones, J. H. Cherney, and J. L. Ford, III, Departments of Animal Science and Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY 14853, and Agway Inc., Tully NY 13159.

4:50 #21 EFFECT OF SHORT TERM FEEDING OF AEROBICALLY DETERIORATED CORN SILAGE ON ANIMAL PERFORMANCE. E. M. Kreck and L. Kung, Jr., Dept. of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717.

5:10 #24 FORMATION OF QUINONE METHIDE INTERMEDIATES DURING RUMINAL FERMENTATION OF CORN STOVER FRACTIONS. V.J.H. Sewalt, W.G. Glasser and J.P. Fontenot, Departments of Animal and Poultry Science, and Forest Products and Wood Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

5:30 #28 THE EFFECT OF ROUTE OF ADMINISTRATION OF ISOLATE 407A (UC-12497) ON FEED INTAKE AND SELECTED RUMINAL VARIABLES OF BEEF STEERS IN AN ACUTE ACIDOSIS INAPPETENCE MODEL. Beth Hibbard[1], Joseph A. Robinson[2], Richard C. Greening[2], Walter J. Smolenski[2], Robert L. Bell[2] and James P. Peters[3], The Upjohn Company, Kalamazoo, MI 49001. (616) 385-6630. [1] Worldwide Animal Health Clinical Research and Product Development, [2] Animal Health Biostatistics and Environmental Research, [3] Currently with A.L. Laboratories, One Executive Dr., Fort Lee, NJ 07024.

5:50 Dinner, at your disgression.

November 10, 1993

8:15 Brief introduction by James B. Russell.

8:30 Invited Speaker. CELLULASES AND HEMICELLULASES: ARCHITECTURE AND APPLICATIONS. G. P. Hazlewood, Institute of Animal Physiology & Genetics Research, Cambridge Research Station, Babraham, Cambridge CB2 4AT, U.K.

9:30 Formal poster presentation in Great Hall.

November 11, 1993

Nutrition-Agronomy Panel Cont'd

8:30 #25 RELATIONSHIP BETWEEN EATING BEHAVIOR AND RUMINAL pH FOR DAIRY COWS CONSUMING LOW OR HIGH FIBER DIETS. R.G. Dado and M.S. Allen, Department of Animal Science, Michigan State University, East Lansing, MI 48824.

8:50 #30 LIPOLYSIS OF TRIGLYCERIDE BY RUMINAL MICROORGANISMS. D. L. Palmquist and Donna J. Kinsey, Dept. of Dairy Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691.

9:10 #19 URINARY EXCRETIONS OF PURINE AND BENZOIC ACID DERIVATIVES AS AN INDICATION OF MICROBIAL PROTEIN SUPPLY AND CELLULOSIC FEED INTAKE IN SHEEP. X.B. Chen, A.T. Mejia, J.H. Pagella. D.J. Kyle and E.R. Orskov. Rowett Research Tnstitute, Bucksburn, Aberdeen AB2 9SB, UK.

9:30 #23 DUODENAL DIGESTA FLOW IN GRAZING CATTLE RECEIVING DIFFERENT LEVELS OF DIPHENYLIODONIUM CHLORIDE. F. Ysunza and W. C. Ellis, Department of Animal Science, Texas A&M University, College Station, TX 77843.

9:50 Brief break.

10:10 #29 RUMEN FERMENTATION AND DEGRADATION OF DRY MATTER AND CRUDE PROTEIN OF A LEGUME *Canavalia ensiformis* AND CELLULOSE. J. Rivera, J.A.Riley and G. Rios, Dept.of Nutrition, FMVZ. UADY. Post Code 4-116, Merida, Yucatan 97100, Mexico.

10:30 #26 COMPARATIVE FIBRE DEGRADATION *IN VITRO* BY BACTERIA AND FUNGI IN COW AND BUFFALO RUMEN, USING STRAW BASED DIETS. D.Mslakar and T.K.Walli, National Dairy Research Institute, Karnal-132001, India.

10:50 #27 FLOW RATE OF DIETARY NAN, MICROBIAL NAN AND ALFA AMINO N AT ABOMASUM IN CROSSBRED CALVES FED CONCENTRATES OF VARYING RDP/UDP RATIOS. O.H.Chaturvedi and T.K.Walli, National Dairy Research Institute, Karnal-132001, India.

11:10 General meeting.

Podium Presentations

Microbiology-Physiopathology Panel

1 ANTIBIOSIS BETWEEN RUMEN BACTERIA AND FUNGI. Burk A. Dehority and Patricia A. Tirabasso Department of Animal Science Ohio Agricultural Research and Development Center, The Ohio State University Wooster, OH 44691 (216) 263-3908.

Bacterial numbers, fungal numbers and cellulose digestion were followed over time in vitro using a purified cellulose medium with and without antibiotics. Tubes were inoculated with a 1:10 dilution of whole rumen contents (WRC). Without antibiotics, fungal numbers decreased to essentially zero within 24 h. In contrast, bacterial numbers increased over 50-fold by 24 h and then decreased gradually up to 72 h. Cellulose digestion was 37, 51, 58 and 70% at 24, 30, 48 and 72 h, respectively. In those fermentations with added antibiotics, bacterial numbers decreased markedly by 24 h and remained very low throughout the 72 h fermentation. Fungal numbers increased slightly after 30 h (3-fold) increasing by 32-fold after 72 h. Cellulose digestion was 1, 3, 17 and 47% after 24, 30, 48 and 72 h, respectively. Similar results were obtained using ground alfalfa as a substrate. In further studies, the in vitro fermentation of purified cellulose was stopped after 18-20 h, and the microbial population killed either by aeration or autoclaving. Antibiotics were added to half the tubes and all tubes were reinoculated with a 1:10 dilution of WRC. After 72 h, considerable cellulose digestion had occurred in the tubes without antibiotics; however, those tubes with added antibiotics digested little if any additional cellulose. The extent of this inhibition was subsequently found to increase in proportion to the length of the initial fermentation period, suggesting the production of a heat stable inhibitory factor(s) by the bacterial fermentation.

2 MICROBE ALTERATIONS ASSOCAITED WITH RUMINAL SULFIDE PRODUCTION IN CATTLE FED A HIGH SULFATE DIET. D. H. Gould, B. A. Cummings, D. W. Hamar, Dept. of Pathology, Colorado State University, Fort Collins, CO 80523 and D. R. Caldwell, Dept. of Molecular Biology, University of Wyoming, Laramie, WY 82071 (303)-491-7455.

A carbohydrate rich, short fiber diet with a high sulfate level was used to study polioencephalomalacia (PEM) in cattle. PEM is a disease affecting ruminants which is characterized by central nervous system impairment, blindness, brain lesions, and death in severely affected animals. This disease may be caused by a number of factors and is commonly associated with thiamine deficiency. The form of experimental PEM induced by the high sulfate diet is associated with high rumen sulfide concentrations in animals that are not thiamine deficient. The types and numbers of rumen bacteria were monitored in pairs of cattle fed diets with and without sulfate before and after the onset of PEM. Sulfate had little effect on the physical parameters of the bacterial populations, but a marked increase in the sulfate-reducing activity of the rumen fluid of cattle exposed to high sulfate levels was observed in vitro. The numbers of apparent sulfate-reducing bacteria enumerated on modified Baar's medium were not increased in cattle fed the high sulfate diet. Bacteria exhibiting sulfate-reducing activity have been isolated from the rumen fluid and factors affecting their activity have been investigated in pure and mixed cultures.

3 THE ROLE AND REGULATION OF LYTIC ACTIVITY IN *Fibrobacter succinogenes*.

J. E. Wells and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508).

Much of the bacterial protein which is produced in the rumen turns over before it passes to the lower gut. This turnover has been attributed to protozoal predation, anaeroplasmic or phage infection, and bacterial starvation. Early work with pure cultures of ruminal bacteria indicated that there was often a decline in optical density during the stationary phase, but the regulation of autolysis was never defined. The cellulolytic bacterium, *Fibrobacter succinogenes*, lyses when exogenous sugar is depleted, and the rate of cell lysis was accelerated by the glycolytic inhibitor, iodoacetate. Iodoacetate had a greater effect on exponentially growing cells than stationary cultures, and continuous culture experiments indicated that the rate of iodoacetate-induced lysis was directly proportional to growth rate. When the cultures were growing rapidly, the iodoacetate-induced rate of lysis was 50% faster than the growth rate. Iodoacetate and the protonophore, TCS, both accelerated lysis, decreased intracellular ATP and dissipated the membrane potential. The ionophore, monensin, also promoted lysis, but only ATP was decreased. Exponentially growing, iodoacetate-treated cultures or stationary cells lysed even faster if the serine proteinases inhibitor, PMSF, was added. Based on these results, *F. succinogenes* appears to overproduce autolysins which are normally degraded by serine proteinases. Proteinase export and autolysin turnover is regulated by an ATP-dependent process that is eliminated by compounds which deplete the ATP pool.

4 ESTIMATION OF OPTIMAL RUMEN NH_3 CONCENTRATION USING A DIFFUSION MODEL AND *Coleospora agminosa* gen. nov. sp. nov.

M. J. Fron and D. M. Schaefer. Meat and Animal Science Dept., Universib of Wisconsin, Madison, WI 53706. (608-263-4317).

A mathematical model was developed to explain the wide range of optimal NH_3 concentrations estimated for the rumen and predict the NH_3 concentration present at the center of a floc of cells based on substrate utilization and diffusion rates. Ammonia was depleted within the floc as the floc thickness increased and initial NH_3 concentration decreased. A unique organism was obtained from anaerobic roll tube enumerations on complex agar and was observed to actively associate with starch granules. A new genus is proposed (*Coleospora agminosa*) which is capable of forming spores, possesses a sheath and displays flocculant growth. Nitrogen utilization rates and exponential growth rates were determined for this organism with starch or glucose substrates over a range of initial NH_3 concentrations (0.1-10 mM) to verify the diffusion model. Linear N utilization rates were highest when 3 mM initial NH_3 was provided and were five fold higher when glucose was the energy substrate rather than starch; however, the rates plateaued at the same NH_3 concentration. Larger flocs and slower growth rates were observed with starch utilization. Similar "optimal" NH_3 concentrations were estimated with starch and glucose energy substrates which may be the result of diffusional resistance to NH_3 transport within the floc.

5 DNA SEQUENCE AND PRELIMINARY TRANSCRIPTIONAL ANALYSIS OF ENDO-BETA-1,4- GLUCANASE GENES FROM *Ruminococcus flavefaciens* FD-1.

Philip E. Vercoe*, Jennie L. Finks, Donn H. Spight, and Bryan A. White, Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-2091).

Three genes encoding endo-beta-1,4-glucanases from *Ruminococcus flavefaciens* FD-1 have been subjected to DNA sequence analysis. Two of these recombinant clones, pBAW101 and pBAW201 (in the phagmid vector pBluescript SK-), contain the endoglucanase genes celB (gene product hydrolyses SigmaCell, acid swollen cellulose, carboxymethylcellulose[CMC] and xylan) and celC (gene product hydrolyses acid swollen cellulose, CMC and xylan) respectively . In the case of pBAW101, initial subcloning indicated that endoglucanase activity expressed was present in a 4.6 kb ClaI fragment (pBAW102). A further subclone, pBAW104, was obtained by digesting pBAW102 with EcoRI, and deleting a 2.4 kb fragment. Activity was still present in pBAW104, indicating that the celB gene was contained within a 2.2 kb insert. The third endoglucanase gene, celD (gene product hydrolyses acid swollen cellulose, CMC and xylan), was cloned into lambdaDash, and EcoRI restriction endonuclease sites were used to subclone 6.7 kb (pBAW007) and 2.3 kb (pBS2.3) fragments directly into pBluescript SK-. The gene encoding the endo-beta-1,4-glucananase in pBS2.3 was designated celD. In vitro transcription/translation studies suggested that celD encodes a 36,000 dalton polypeptide. DNA sequence analysis was performed using synthetic oligonucleotide primers in combination with Sanger's dideoxy chain termination method and the polymerase chain reaction. Open reading frames's encoding CelB, CelC, and CelD were identified from the three DNA sequences. The protein sequences were aligned with other published sequences of cellulase and xylanase genes, to determine the cellulase family to which each belonged. Preliminary transcriptional analyses were performed on native mRNAs to map transcription intitiation sites, and identify possible promoter reglons.

6 INFLUENCE OF GROWTH SUBSTRATE ON BIOENERGETIC GROWTH EFFICIENCY OF ANAEROBIC CELLULOLYTIC BACTERIA.

H. J. Strobel and K. A. Dawson. Department of Animal Sciences, University of Kentucky, Lexington, KY 40546 (606-267-7554).

Anaerobic bacteria often preferentially use a limited number of substrates when more than one carbon source is present in the environment. However, it is not always apparent why certain substrates are preferred over others. Recent experiments suggest that preferences may sometimes be associated with changes in maintenance energy expenditures. Cellobiose was the preferred substrate in cultures of Ruminococcus albus or Clostridium thermocellum containing the disaccharide plus glucose. Cell yields in continuous culture were at least 40% greater when cellobiose replaced glucose as the energy source. The increased cell yields on cellobiose could be partially explained by a greater conservation of metabolic energy via cellobiose phosphorylase activity. However, maintenance coefficients of glucose-grown cultures were four to five fold higher than in cellobiose-limited cultures. In both cellulolytic organisms, glucose had a detrimental effect on the efficiency of growth at low dilution rates. These studies suggest that oligosaccharides resulting from cellulose degradation are the preferred substrate for cellulolytic organisms and that oligomers support more efficient bacterial growth.

7 CLONING AND SEQUENCING OF AN INDIGENOUS PLASMID, PBAW301, FROM *Ruminococcus flavefaciens* R13e2.

Tammy May*, Philip E. Vercoe, and Bryan A. White. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801(217-333-2091).

An indigenous plasmid is a logical tool for developing a gene transfer system in *Ruminococcus*. Past attempts at electroporation of *Ruminococcus* using various broad host range plasmids with antibiotic resistance markers have been mostly unsuccessful. A small, cryptic plasmid, pBAW301, was isolated from *R. flavefaciens* R13e2. Despite the low-copy number of pBAW301, a restriction map was generated which allowed cloning into *Escherichia coli*. This plasmid was linearized with *Mbo*I and cloned into the *Bam*HI site of the *Lactobacillus-E. coli* shuttle vector, pBS19 (Em^r, Cm^r). Using $CaCl_2$ transformation, this construct was introduced into *E. coli* DH5-alpha yielding the clone, pBAW302. DNA sequence analysis was then undertaken using synthetic oligonucleotide primers, the Sanger-dideoxy sequencing method, and the polymerase chain reaction. The insert, pBAW301, was determined to be 1,776 bp and the sequence verified the originally observed restriction sites: *Acc*I, 1 site; *Mbo*I, 1 site; and *Taq*I, 2 sites. Furthermore, several unique restriction sites have been detected. These sites should be helpful in construction of a *Ruminococcus* shuttle vector from pBAW301. Several open reading frames have been identified and computer sequence analysis has been used to identify potentially important coding regions of the plasmid. All electroporation attempts to date to introduce both pBS19 or the clone, pBAW302, into *R. albus* have been unsuccessful.

8 THE MECHANISM OF ENERGY SPILLING IN *Streptococcus bovis*.

G.M. Cook and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508)

Non-growing (chloramphenicol-treated or nitrogen-limited) *S. bovis* cells fermented glucose at a rapid rate, and the rate of glucose consumption was 10 fold greater than the maintenance rate of growing cells. Because this non-growth energy dissipation was eliminated by N,N-dicyclohexylcarboiimide (DCCD), an inhibitor of the F_1F_0 ATPase and increased by 3,3',4',5-tetrachlorosalicylanilide (TCS), a protonophore, it appeared that a futile cycle of protons through the cell membrane was spilling energy. When the energy-spilling cells were treated with iodoacetate, the rate of energy spilling decreased, but there was little decline in membrane potential (Dp). Based on this non-Ohmic relationship, it did not appear that the rate of proton flux was being regulated directly by Dp. When the rate of proton flux (amperage) was estimated from the Dp (voltage) and rate of heat production (wattage) using the electrical formula amperage = wattage/voltage, the H^+/ATP stoichiometry of the F_1F_0 ATPase was approximately 2 at all rates of energy spilling. Based on Ohm's law (resistance = voltage/amperage), the resistance of the cell membrane to proton conductance increased 15 fold as the rate of energy spilling was decreased by iodoacetate. The rate of energy spilling was highly correlated with the intracellular ATP content of the cells, but further work is needed to identify the relationship between energy spilling and intracellular adenylate pools.

9 DEVELOPMENT OF A RIBOTYPING METHOD FOR THE STUDY OF GASTROINTESTINAL MICROECOLOGY: APPLICATION TO LACTOBACILLI IN THE WEANLING-PIGLET.

Denis O. Krause*, Bryan A. White, Robert A. Easter, and Roderick I. Mackie, Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-244-2526).

Traditionally ecological studies of the gastrointestinal tract have been conducted by culturing specific bacterial groups on selective or semi-selective media and speciating bacterial isolates using biochemical and physiological techniques. These methods are extremely labor intensive and lack resolution. More recently ribosomal RNA (rRNA) from various bacteria have been sequenced to determine phylogenetic relationships between bacteria. Probes for the study of the microbial ecology of the gastrointestinal tract can be designed from these rRNA sequences but are very often species or sub- species specific and do not adequately cover the diversity of the specific group of organisms of interest. Restriction fragment length polymorphisms (RFLP's) can be used as a method of studying the ecology of bacteria in the gastrointestinal tract. All prokaryotes have 5S, 16S and 23S rDNA sequences located on the chromosome. Regions of these genes are highly conserved between and among species. By probing chromosomal DNA with highly conserved ribosomal sequences, RFLP's can be obtained that can be subjected to multivariate analysis procedures and estimates of phylogeny can be made. Lactobacilli are a taxonomically heterogenous group of organisms which have traditionally been classified using biochemical and physiological characteristics. However, the systematics of *Lactobacillus* species are poorly understood at the molecular level. RFLP's have been used to study the ecology of lactobacilli isolated from the gastrointestinal tract of the weanling-piglet. Methodology describing this ribotyping technique, and resulting RFLP patterns obtained will be presented. Diversity indices calculated from RFLP analysis of gastrointestinal lactobacilli can be compared and correlated with diversity indices calculated from classical speciation techniques.

10 RUMINAL METABOLISM OF NITROPROPANOL; MICROBIAL CONTRIBUTION.

R. C. Anderson, M. A. Rasmussen, and M. J. Allison. USDA, ARS, National Animal Disease Center, Ames, IA 50010 (515-239-8200).

Ruminal microbes metabolize nitropropanol (NPOH), the toxic constituent of various milkvetchs; however, this metabolism is variable and often insufficient to prevent livestock poisonings. Our goal is to learn more about the microbial detoxification of NPOH. Ruminal microbes reduce NPOH to aminopropanol and addition of ferrous and sulfide ions increases the rate of this reaction. Cell-free extracts prepared from mixed populations of ruminal microbes utilize low potential electron donors such as methyl viologen to mediate the reduction of NPOH. Involvement of specific nitro-reductases has not yet been determined; however, successive transfers of a mixed population in batch cultures consisting of a rumen fluid medium containing milkvetch hay resulted in increased rates (>10 fold) of NPOH metabolism. With alfalfa hay, the addition of NPOH was needed for the adaptation to occur and to maintain the increased activity in successive transfers. This adaptation suggests a selection for competent NPOH metabolizing microbes.

#11 DUAL POOL LOGISTIC EQUATION FOR ANALYSIS OF IN VITRO FERMENTATION OF MIXED SUBSTRATES BY COMPUTERIZED GAS PRESSURE MEASUREMENTS. P. Schofield and A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876).

Cellulose digestion *in vitro* was measured by following total gas production with computer-interfaced pressure sensors. Mixtures of bacterial and processed cellulose (Cellulon and alpha cellulose) give complex gas production curves with abrupt slope changes. These curves cannot be modelled satisfactorily with single or dual pool equations of the first order exponential structure commonly used to analyze fiber digestion. A dual pool logistic equation, with a single lag term, is able to model these complex curves. This equation is derived on the assumption that digestion rates are governed both by fiber levels and by microbial mass. The equation also fits single substrate data extremely well and may provide a means to analyze multiple kinetic pools in natural forages.

#12 NATURAL VARIABILITY AND DIURNAL FLUCTUATION OF BACTERIOPHAGE POPULATIONS IN THE RUMEN. A.V. Klieve, R.A. Swain and J.V. Nolan. Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, N.S.W. 2351, Australia (067) 73 2942.

The effects of phages on ruminal bacterial populations and consequent effects on nutrition and animal production are poorly understood. By examining phage DNA in rumen fluid it is possible to estimate ruminal bacteriophage numbers by using pulsed field gel electrophoresis and laser densitometry. With this method phage numbers were estimated to be between 3×10^9 and 1.6×10^{10} particles ml^{-1} of rumen fluid. The phage population of the rumen appears to have two major components, a broad region comprising DNA from many phages (presumptively including temperate phages), and discrete bands of DNA from one or a few phage genotypes (presumptively representing lytic phage blooms). A study to determine the extent of natural fluctuations in ruminal phage populations was undertaken. Phage DNA banding patterns and total phage DNA ml^{-1} of rumen fluid were found to be highly variable between; (a) species of ruminants, (b) animals at pasture and in pens, (c) animals penned together and on the same diet, and (d) the same animal sampled at the same time of day on consecutive days. When a single animal, fed once daily an oaten chaff-alfalfa (70:30) diet, was sampled at two-hourly intervals the phage population changed only slowly, with no apparent change between some two-hourly intervals. A marked diurnal variation in the phage population (up to ten-fold increase) in total phage DNA ml^{-1} of RF occurred 6-8 hours after feeding in these animals. This surge in phage activity is currently under examination.

In conclusion, the phage population of the rumen is highly dynamic. The numbers and fluctuations in the phage population suggest that phages may play an important role in turnover of microbial cells and supply of protein to the animal.

#13 UTILIZATION OF A VARIETY OF XYLANS BY *Butyrivibrio fibrisolvins* STRAIN H17C. R.B. Hespell and M.A. Cotta, USDA/ARS, Natl. Cent. Agric. Utilzn. Res., Peoria, IL (309-681-6270).

The ability of *B. fibrisolvens* H17c to utilize xylan derived from oat spelts, larchwood, birchwood, a corn cell culture broth (corn xylan), 4-0-methyl glucuronoxylan (4MG), and two xylan fractions prepared from corn cobs (soluble and insoluble fractions after neutralization of base extracted xylan) was determined. H17c cultures were grown anaerobically in a complex medium with 0.3% (w/v) xylan as the growth substrate. Logarithmic rates of disappearance during the first 12 h of growth ranged from -0.176 h^{-1} for the soluble corn cob xylan to -0.068 h^{-1} for the insoluble fraction of corn cob xylan. No clear relationship could be established between solubility or degree of substitution on the rate of utilization for all xylans. For the wood xylans (larchwood, birchwood, and 4MG) there was increase in the rate of disappearance with increasing uronic acid content. Solubility appeared to be an important determinant of degradation rate for the oat and corn derived xylans. The degree of substitution may also play an important role since treatment of corn xylan with oxalic acid to remove arabinose side groups markedly enhanced the rate of disappearance of this substrate. Disappearance of uronic acid and neutral sugar components are also discussed.

#14 DEVELOPMENT OF A DNA PROBE FOR THE DETECTION OF RUMINAL *Streptococcus bovis*. D. A. Odelson[1], L. Nelms[1], S. F. Kotarski[2], and R. B. Hespell[3], [1]Dept. of Biology, Central Michigan University, Mt. Pleasant MI, Center for Microbial Ecology, E. Lansing, MI (517-774-7585), [2]The Upjohn Co., Kalamazoo, MI, and [3]USDA-ARS, Peoria, IL.

The ruminal starch-degrading bacterium *Streptococcus bovis* was used for the production of a species-specific nucleic acid probe. To do this, DNA isolated from strains JB1, K27FF4, and 21096C was used for separate polymerase chain reaction (PCR) amplification of several variable regions of the 16S ribosomal RNA. PCR products of the V1, V3 and V6 variable regions were approximately 90, 300 and 100 base pairs, respectively. Southern hybridization experiments utilizing these PCR products as probes showed different hybridization banding patterns. This pattern was also different for each *S. bovis* strain. Both of these variations were independent of the strain produced PCR product that was utilized as a probe. In slot blot hybridization experiments only the V_1 variable region PCR product showed specificity for *S. bovis*, including fresh ruminal isolates. This probe did not hybridize with DNA from *Bacteroides succinogenes, Butyrivibrio fibrisolvens, Eubacterium ruminantium, Lactobacillus vitulinus, Megasphaera elsdenii, Prevotella ruminicola, Ruminococcus bromii, Selenomonas ruminantium, Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, or Staphylococcus aureus.* Additionally, this probe did not hybridize with human clinical isolates of *S. bovis*. Optimal hybridization conditions are being established for use in enumerating *S. bovis* in mixed samples. Future investigations are directed towards monitoring the number of *S. bovis* in the rumen and fermentation bioreactors .

#15 COMPARISON OF MICROBIAL FERMENTATION IN DUAL FLOW CONTINUOUS CULTURE AND THE RUMEN OF DAIRY COWS. H. R. Mansfield, M. I. Endres, and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108 (612-624-6216).

Four fermenters and four cannulated lactating dairy cows were used in a replicated Latin square to compare fermentation and microbial ecology of the in vitro and in vivo systems. Diets contained 32% corn silage, 19.8% alfalfa-grass hay, and 48.2% concentrate, and diets were arranged in a 2 x 2 factorial with two levels of nonfibrous carbohydrate (NFC:40%, 25%) and two levels of degradable intake protein (DIP:70%, 50%). Concentrations (cells/ml) of viable bacteria were greater (P = .012) in vitro compared with in vivo averaging 5.04 x 10^9 and 2.75 x 10^9, respectively. Cellulolytic bacterial concentrations were lower (P = .038) in vitro (4.09 x 107) compared with in vivo (6.15 x 10^7), but concentrations of proteolytic and amylolytic bacteria were similar (P > .05) averaging 5.05 x 10^7 and 1.79 x 10^8, respectively. Protozoal concentrations were greater (P = .0001) in vivo (3.72 x 105) compared with in vitro (.0028 x103). Total VFA concentration exhibited a square x NFC interaction (P < .05) indicating that cows fed 40% NFC had lower total VFA (91.7 mM) than fermenters supplied with 25% NFC (100.2 mM) or 40% NFC diets (99.6 mM). However, concentration of total VFA in the rumen of cows fed 25% NFC (98.2 mM) was similar (P > .05) to concentration in vitro. These preliminary data indicate that differences exist between fermentations in continuous culture and in vivo and that these effects may be diet dependent.

#16 WHITE ROT FUNGI TO IMPROVE UTILIZATION OF GRASS LIGNOCELLULOSE. D. E. Akin, A. Sethuraman, W. H. Morrison III, S. A. Martin, and K.-E. L. Eriksson, Russell Research Center, ARS-USDA, Athens, GA 30613 (706-546-3482); Department of Biochemistry (706-542-4453) and Department of Animal and Dairy Science (706-542-1065), University of Georgia, Athens, GA 30602.

Intact 10-mm sections of bermudagrass internodes were incubated with *Phanerochaete chrysosporium* and it cellulase-less mutants 3113 and 85118, *Phellinus pini*, and *Ceriporiopsis subvermispora*. Untreated and fungal-treated stem residues were then analyzed by a series of methods, *C. subvermispora* improved the quality of biomass the most consistently and substantially, giving increase in in vitro digestion and VFA production of 80% over untreated stems. *C. subvermispora* removed ester-linked p-coumaric and ferulic acids, and middle lamella of parenchyma cells was particularly affected. Lignin from secondary and middle lamella layers of sclerenchyma walls appeared to altered with improved digestion of these cells.

Nutrition-Agronomy Panel

#17 ALFALFA NITROGEN AND CARBOHYDRATE PARTITIONING AS INFLUENCED BY NITROGEN AS INFLUENCED BY NITROGEN FERTILIZATION. J. H. Cherney, D. J. R. Cherney, and J. L. Ford, III, Departments of Soil, Crop, and Atmospheric Sciences and Animal Science, Cornell University, Ithaca, NY 14853 (607-255-0945).

Our objective was to determine the influence of nitrogen fertilization (0, 112, 224, 336 kg N/ha supplied as ammonium nitrate or potassium nitrate) on carbohydrate and nitrogen partitioning in alfalfa (*Medicaqo sativa* L.). Three field replicates of each fertilizer rate were used for each fertilizer type. Two first-growth cuttings (bud stage; 1 wk apart) were harvested beginning on May 27. Nitrogen solubility was not affected by fertilizer type. Alfalfa fertilized with 336 kg N/ha had slightly lower NDF than alfalfa fertilized with O kg N/ha N (33% vs. 34% NDF). Nitrate, CP, soluble CP (DM basis), and CP solubility increased with increased N fertilization. Plant maturity stage was not affected by fertilizer level. As expected, the later cutting of alfalfa was higher in NDF (37% vs. 31%) and lignin (5 . 4% vs. 4 . 4%), and lower in nitrate (. 16% vs. . 20%), and CP (24% vs. 25%). Low fiber and high CP can cause nutritional problems for ruminants. Nitrogen in dairy manure applied to alfalfa may adversely affect alfalfa quality by delaying maturity and increasing N uptake. .

#18 FORAGE DRY MATTER AND NITROGEN DISAPPEARANCE AS INFLUENCED BY NITROGEN FERTILIZATION AND COW DIET. D. J. R. Cherney, J. Siciliano-Jones, J. H. Cherney, and J. L. Ford, III, Departments of Animal Science and Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY 14853, and Agway Inc., Tully NY 13159 (607- 255-0604).

Alfalfa (*Medicaqo sativa* L.) and reed canarygrass (*Phalaris arundinacea* L.) were ruminally incubated for 6 and 24 h in nylon bags to determine the influence of N fertilization, harvest date, and level of fat in cow diet on dry matter disappearance (DMD), N disappearance (NDIS), and N concentration of the residue (NRES). High fat (8% vs. 5%) resulted in lower DMD at 24 h for both alfalfa (79.5% vs. 84.1%) and grass (63.9% vs. 70.3%). Alfalfa incubated with high fat had higher NRES (1.40% vs. 1.03%) and lower NDIS (92.0% vs. 96.0%) than alfalfa incubated with medium fat. Fat in the diet did not affect grass NDIS. Harvest date did not influence measured parameters for alfalfa (l wk apart). The later harvest date for grass, 6 wk later than the first harvest date, resulted in lower DMD, higher NRES, and lower NDIS at 6 and 24 h. High levels of N fertilization on alfalfa (336 kg/ha) resulted in lower DMD than unfertilized alfalfa at 6 and 24 h (61.6% vs. 70.3% at 6 h and 79.5% vs. 84.0% at 24 h), but NDIS was not affected. Grass N fertilization (112 kg/ha) did not affect DMD or NDIS. Decreased DMD and high NDIS of N fertilized alfalfa may result in high ruminal N to carbohydrate ratios, increasing loss of N as ammonia. This problem may be increased by high fat in the diet.

#19 URINARY EXCRETIONS OF PURINE AND BENZOIC ACID DERIVATIVES AS AN INDICATION OF MICROBIAL PROTEIN SUPPLY AND CELLULOSIC FEED INTAKE IN SHEEP. X.B. Chen, A.T. Mejia, J.H. Pagella. D.J. Kyle and E.R. Ørskov. Rowett Research Tnstitute, Bucksburn, Aberdeen AB2 9SB, UK. (Tel. +44 224 712751, FAX +44 224 716687).

Aromatic metabolites in urine of ruminants are primarily in the form of hippuric acid, the glycine conjugate of benzoic acid (BA). BA is the excretory metabolite of 3-phenylpropionic acid and, to a lesser extent, of cyclohexanecarboxylic acid absorbed from the rumen. These two compounds are microbial fermentation products of a range of hydroxycinnamic acids and alicyclic acids in feeds. Purine derivatives (PD) excreted in urine, allantoin, uric acid, xanthine and hypoxanthine, originate mainly from the metabolism of absorbed microbial purines. This experiment examined the excretion of purine and benzoic acid derivatives in sheep receiving different feeds and its relationship with feed intake. Sixteen sheep were offered *ad libitum* one of four diets (l) straw, (2) barley, (3) lucerne nuts and (4) a mixed ration containing hay and concentrate. After a 2-week adaptation period, urine was collected for 4 days. Feed DM intake varied from ().35-1.72 kg/d between diets and between animals. PD excretion (sum of all four compounds) ranged from 7.1 - 22.6 mmol/d, and was correlated with DM intake across all diets and all animals (r^2=0.73, n=16). Aromatic metabolites in urine, determined using reversed-phase HPLC, were predominantly hippuric acid. Small quantities of BA, but not its glucuronic acid conjugate, was present. For the barley diet, total BA (sum of hippuric and BA) excretion was low (3.3±1.0 mmol/d) and not related to feed intake. For the other 3 diets, total BA excretion ranged from 12.1 - 27.4 mmol/d and was highly correlated with the DM intake (r^2=0.88, n=12). Interestingly, the response of BA excretion to DM intake was similar in the 3 diets. The results show that measurements of urinary PD and BA can be used in combination to provide an indication of the supply of microbial protein (a function of total fermentable energy intake) and the type of feeds consumed (cellulosic vs. concentrate). The excretion of BA, when linked with information of the content of its precursors in feeds, may provide a simple measure of the intake of cellulosic feedstuffs.

#20 VARIATION AMONG INDIVIDUAL SMOOTH BROMEGRASS PLANTS FOR KLASON LIGNIN AND CELL-WALL PHENOLIC ACIDS. H. G. Jung and M. D. Casler, USDA-ARS, US Dairy Forage Research Center Cluster, St. Paul, MN 55108 and Dept. of Agronomy, University of Wisconsin, Madison, WI 53706 (612/625-8291).

Smooth bromegrass plants from three breeding populations and a wild-type collection were analyzed for components of lignification that are thought to be related to ruminal degradability of forage cell walls. Eighty individual plants were harvested at the vegetative stage. Samples were analyzed for Klason lignin concentration by a two-stage sulfuric acid hydrolysis. Ester- and ether-linkedp-coumaric (PCA) and ferulic (FA) acids were determined by alkaline hydrolysis. Among these 80 individual plants, Klason lignin concentration ranged from 48 to 82 g/kg. Esterified PCA and FA ranged from 0.19 to 1.06 and 1.10 to 2.22 g/kg, respectively. The ether-linked phenolic acid concentrations were similar (0.40-0.81 and 1.19-2.20 g/kg for PCA and FA respectively). Content of ester- and ether-linked PCA, and etherified FA was weakly, but significantly, correlated with Klason lignin concentration (r=.35, .26 and .28, P<0.05, respectively). Based on this large degree of variation we have begun a breeding program to improve forage quality of smooth bromegrass through selection for components of lignification.

#21 EFFECT OF SHORT TERM FEEDING OF AEROBICALLY DETERIORATED CORN SILAGE ON ANIMAL PERFORMANCE. E. M. Kreck and L. Kung, Jr., Dept. of Animal Science and Agricultural Biochemistry, University of Delaware, Newark, DE 19717 (302-831-2524).

The metabolism of lactate and water soluble carbohydrates by aerobic organisms such as yeasts and molds are thought to be the cause of increased pH, DM loss, and poor quality feed once silage is exposed to air. However, there is limited documentation that aerobically spoiled silage decreases feed intake or animal performance. Short term feeding studies using mid-lactation cows and growing lambs were conducted to assess negative affects of feeding spoiled silage.

Twelve cows were fed a balanced TMR which included spoiled corn silage (Aerobic) or fresh corn silage (Control). The DMI of the Aerobic group dropped 35% when fed silage that had spoiled for 4 d (Control 27.4, Aerobic 16.9 kg/d, DMB). These cows seemed to adapt to spoiled silage as DMI increased when fed 7-d spoiled silage (Control 24.6, Aerobic 25.8 kg/d, DMB): pH = 3.69 Control, 5.96 Aerobic; yeast counts (cfu/g silage) = 10^3 Control, 10^7 Aerobic; total lactate (%, DMB) = 4.66 Control, 0.21 Aerobic; Acetate (%, DMB) = 1.12 Control, 0.30 Aerobic. There were no changes in milk production, composition, or body weights. There was no difference in DMI or body weights in two similar sheep trials even after feeding silage that had spoiled for 6 d: pH = 3.81 Control, 7.15 Aerobic; yeast counts (cfu/g silage) = 10^5 Control, 10^8 Aerobic. Control and Aerobic groups that were offered fresh or 7-d spoiled silage preferred the later.

#22 VARIATION IN EXTRACTABLE PECTIC SUBSTANCES OF ALFALFA STEMS WITH MATURITY. M. B. Rymph, P. J. Van Soest, B. A. Lewis, and L. E. Chase, Dept. of Animal Science, and Dept. of Nutritional Sciences, Cornell University, Ithaca, NY 14853 (607-255-4478).

Pectic substances were extracted from alfalfa (*Medicago sativa* L.) stems of known maturities by sequential extraction with 85% acetone:water, 0.05 M potassium EDTA (K) and then 0.05 M Na_2CO_3 (N). Extraction filtrates were directly assayed for galacturonic acid (GalA) or were precipitated with ethanol and the precipitate assayed for neutral sugars (S). Extractable GalA content of immature (I) vs. mature (M) alfalfa stems was significantly higher (6.22% vs. 3 .99%; $p< 0.01$). Proportions of S varied by extraction and in some cases by maturity.

		--% of Sample DM--			S as % of Total S within K or N*			
Sample	Age	GalA	NDF	saLig	Ara-K	Gal-K	Ara-N	Gal-N
5/08	I	6.09	22.22	2.74	45.5	42.5	62.8	30.9
6/12	M	3.70	57.71	9.58	39.5	47.2	56.2	32.3
6/30	I	6.21	34.18	4.33	48.8	38.9	66.9	25.1
7/24	M	3.92	64.11	11.39	38.0	46.3	62.5	26.4
8/12	I	6.36	35.49	4.77	43.9	44.2	65.2	26.3
9/02	M	4.36	54.83	8.98	40.8	45.7	64.2	26.3

* S analysis of extraction run 1 ethanol precipitates

#23 DUODENAL DIGESTA FLOW IN GRAZING CATTLE RECEIVING DIFFERENT LEVELS OF DIPHENYLIODONIUM CHLORIDE. F. Ysunza and W. C. Ellis, Department of Animal Science, Texas A&M University, College Station, TX 77843 ([409] 845 5214).

Diaryliodonium compounds have shown effectiveness in suppressing amino acid fermentation and increasing escape of dietary amino acids from ruminal degradation. Previous results suggested that feeding low levels of diphenyliodonium chloride (DIC) may alter ruminal fermentation, increasing digesta flow to the small intestine in grazing cattle. Six cannulated heifers, 287 kg average body weight (BW), grazing ryegrass pasture were assigned to receive 0, 10, 20, 40, 80 or 160 mg/d of DIC in a corn based supplement (500 g/d). Duodenal digesta was collected at different times during 4 d, after 10 d of adaptation during three periods. Duodenal flow of organic matter (OMF), true protein (TPF) and free peptides and amino acids (FPAF) were estimated by reference to La, daily dosed as nitrate in the supplement. Heifers receiving 80 mg/d of DIC had above 50% higher OMF, TPF and FPAF than the rest of the animals ($P<.02$). Regression models performed against level of DIC showed cubic responses of OMF, TPF and FPAF ($P<.02$). Flow tended to decrease slightly with increasing levels of DIC up to 40 mg/d, before increasing notably with 80 mg/d. Results suggest that DIC affects flow of protein and amino acids to the small intestine in grazing cattle. Additional research is being conducted on intake and digestibility responses.

#24 FORMATION OF QUINONE METHIDE INTERMEDIATES DURING RUMINAL FERMENTATION OF CORN STOVER FRACTIONS. V.J.H. Sewalt, W.G. Glasser and J.P. Fontenot, Departments of Animal and Poultry Science, and Forest Products and Wood Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (703)231-5136.

Experiments were conducted to determine whether formation of quinone methide intermediates from lignin occurs during ruminal fermentation of corn stover, as indicated by nucleophilic addition reaction with S-containing reducing agents. Corn stover leaf and stem fractions harvested at full maturity were incubated in buffered ruminal fluid without reducing agents, or with cysteine- HCl, Na_2S, cysteine-HCl plus Na_2S, or $(NH_4)_2SO_4$ (S-control); and in only buffer with or without cysteine-HCl plus Na_2S. Sulphur content was determined in the residual fiber. In vitro NDF degradation after 48 h was not affected by adding reducing agents, but solubilization of NDF ($P=.070$) and DM ($P=.055$) in buffer alone tended to be enhanced, and S content of residual NDF was elevated ($P<.001$). In a subsequent experiment corn stover fractions of varying lignin compositions harvested at two maturities (early dent and full maturity) in two subsequent years, were incubated in buffered ruminal fluid under addition of mixed reducing agents (cysteine-HCl and Na_2S), and incorporation of S into the undegraded fiber was determined. Degradation of NDF was correlated with extent of S-incorporation ($r=-.54$), and was highly correlated with lignin methoxyl content of NDF ($r=-.84$). Apparently, quinone methides are formed at a higher rate during fermentation of fiber containing more lignin methoxyl groups, which needs to be taken into account in addition to cell wall composition when comparing relative ruminal degradability of forages.

#25 RELATIONSHIP BETWEEN EATING BEHAVIOR AND RUMINAL pH FOR DAIRY COWS CONSUMING LOW OR HIGH FIBER DIETS. R.G. Dado and M.S. Allen, Department of Animal Science, Michigan State University, East Lansing, MI 48824 (517-336-1386).

Feeding behavior and ventral rumen pH of 12 lactating Holsteins were measured continuously with a data collection system. Cows received a 25% (LF) or 35% (HF) NDF diet in each of two, 21 d periods. Objectives were to evaluate rumen pH changes in relation to eating activity. Five variables were calculated for each of 550 meals: pH at the start of the meal (STpH), minimum pH following the meal but prior to the next meal (MINpH), decline in pH after eating (STpH - MINpH; dpH), time between end of each meal and MINpH (dT), and rate of pH decline (dpH/dT). Backward elimination multiple linear regression was used to determine which feeding parameters significantly influenced pH ($P < 0.01$). STpH differed between treatments (LF = 6.35; HF = 6.64) and was related positively to the length of the intermeal interval (IMI) prior to the current meal (PreIMI) (model $R^2 = 0.74$). Following meals, pH declined for over 97% of all meals (dpH = 0.27 $\pm$ 0.20; mean $\pm$ SD); was influenced by treatment (LF = 0.32; HF = 0.24); was related positively to STpH, meal size, and the IMI following the current meal (PostIMI); and negatively to the length of time between the previous rumination period and the current meal (model R2 = 0.37). dpH/dT (0.030 $\pm$ 0.059 units/min) was related positively to STpH and meal size, and negatively to PostIMI (model $R^2 = 0.27$). Within-day changes in rumen pH are related to eating activity.

#26 COMPARATIVE FIBRE DEGRADATION *IN VITRO* BY BACTERIA AND FUNGI IN COW AND BUFFALO RUMEN, USING STRAW BASED DIETS. D.Mslakar and T.K.Walli, National Dairy Research Institute, Karnal-132001, India (0184-21832).

In a 2x3x4 factorial design experiment, the 2 sources of rumen inoculumn were, a crossbred cow and Murrah buffalo, fed a ration of straw ant concentrate in equal proportions. The 3 dietary combinations as the substrates for the 48h *in vitro* stuties were formed by mixing ground wheat straw and concentrate in the ratio of 60:40, 50:50 and 40:60. The 4 antibiotic treatments consisted of Tl:whole rumen fluide (WRF) without any antibiotic, T2: WRP + Penicillin G + Streptomycin (ment to inhibit bacterial growth), T3: WRF + Cycloheximide (to inhibit fungal growth), T4: WRF + Penicillin G + Streptomycin + Cycloheximide (to inhibit both bacterial as well as fungal growth). The bacterial counts were significantly higher (P/0.05) for buffaloes (217.23x10 /ml) than for cow (191.46x10 /ml), and the fungal counts were significantly higher (P/O.OS) for cow (35.00x10 /ml) than for buffaloes (22.4x10 /ml), however, the fibre degradation was similar in both the species. When the antibiotic against bacteria was used (T2), the bacterial and fungal counts were 34.42x10 /ml and 82.00x10 /ml respectively, however, when the antibiotic against fungi was used (T3), the corresponding bacterial and fungal counts were 306.16x10 /ml and 5.25x10 /ml. The variation among the 4 antibiotic treatments was significant (P/0.01) with respect to bacterial and fungal population and fibre degradation. The variation in fibre degradation was, however, non-significant between T2 and T3, the values being 17.09 and 18.95 per cent respectively.

#27 FLOW RATE OF DIETARY NAN, MICROBIAL NAN AND ALFA AMINO N AT ABOMASUM IN CROSSBRED CALVES FED CONCENTRATES OF VARYING RDP/UDP RATIOS. O.H.Chaturvedi and T.K.Walli, National Dairy Research Institute, Karnal-132001, India (0184-21832).

A 4x4 latin sq design experiment was conducted on 4 crossbred calves, each fitted with a rumen and an abomasal cannuls. The animals were fed green maize, and wheat straw as roughages and the 4 types of concentrate mixtures, having decreasing CP content viz. 23.37, 20.96, 18.37 and 15.92 per cent and the variable RDP/UDP ratio, viz., 80:20, 70:30, 60:40 and 50:50 (using variable proportion of groundnut cake, cottonseed cake and maize gluten) for the treatments T1, T2, T3 and T4 respectively, and receiving 120, 100, 90 and 80 per cent CP of the NRC (1988) recommendations. Inspite of the decreasing order in N intakes from T1 to T4, the flow rate of NAN and alfa amino N at abomasum (as g/d) did not show any variation among the treatments. However, when expressed as per cent of N intake these N fractions showed a significant variation ($P<0.01$) among the treatments, the values for NAN flow rate being 79.39 ± 0.10, 91.04 ± 0.07, 102.93 ± 0.16 and 112.87 ± 0.96 per cent and the values for alfa amino N being 64.42 ± 0.11, 74.59 ± 0.11, 84.45 ± 0.34 and 92.17 ± 0.87 per cent, for the treatments T1, T2, T3 and T4 respectively. While the dietary plus endogenous NAN flow rate (g/d) showed an increasing ($P<0.01$) trent, the values being 15.05 ± 0.41, 23.13 ± 0.55, 33.46 ± 1.12 and 40.69 ± 1.07g from T1 to T4, respectively; the corresponding values for microbial NAN showed a decreasing ($P<0.01$) trend, being 51.85 ± 2.30, 43.29 ± 1.00, 32.75 ± 1.07 and 25.63 ± 0.77g.

#28 THE EFFECT OF ROUTE OF ADMINISTRATION OF ISOLATE 407A (UC-12497) ON FEED INTAKE AND SELECTED RUMINAL VARIABLES OF BEEF STEERS IN AN ACUTE ACIDOSIS INAPPETENCE MODEL. Beth Hibbard[1], Joseph A. Robinson[2], Richard C. Greening[2], Walter J. Smolenski[2], Robert L. Bell[2] and James P. Peters[3], The Upjohn Company, Kalamazoo, MI 49001. (616) 385-6630. [1] Worldwide Animal Health Clinical Research and Product Development, [2] Animal Health Biostatistics and Environmental Research, [3] Currently with A.L. Laboratories, One Executive Dr., Fort Lee, NJ 07024.

An acute acidosis inappetence model was used to evaluate various methods of administering *Megasphaera elsdenii* isolate 407A to beef cattle. This study used 72 Angus crossbred steers (279 ± 2.3 (SE) kg body weight) in a randomized complete block design with repeated measures across a 21-day observation period. There were six treatment groups in 12 blocks. Following the switch from a 50% to a 90% concentrate diet (day 1), animals that received 407A intraruminally or orally had higher dry matter intake ($P<.10$) and ruminal pH ($P<.10$), and lower ruminal lactate concentration ($P<.10$) than non-treated controls or steers administered heat-inactivated 407A. Ruminally cannulated steers had lower dry matter intake than non-cannulated steers receiving the same treatment. There were no differences (alpha=.10) in ruminal acetate concentrations. The 407A dosed animals had higher ($P<.10$) ruminal butyrate concentrations than non-treated and heat-inactivated 407A groups at several timepoints. Heat-inactivated 407A steers had higher ruminal butyrate concentrations ($P<.10$) than controls on day 2. In summary, treatment with 407A, administered either intraruminally or orally, was generally effective at preventing acute acidosis in this model.

#29 RUMEN FERMENTATION AND DEGRADATION OF DRY MATTER AND CRUDE PROTEIN OF A LEGUME *Canavalia ensiformis* AND CELLULOSE. J. Rivera, J.A.Riley and G. Rios, Dept.of Nutrition, FMVZ. UADY. Post Code 4-116, Merida, Yucatan 97100, Mexico. (99) 47-15-43.

The rumen fermentation and rate of disappearance of DM and CP of *Canavalia ensiformis* and rate of disappearance of cellulose (filter paper) was investigated using the dacron bag technique in a 3x3 latin square design. Three castrated criollo male goats mean body mass 20 (+ 2 kg SE) were used. Goats were fed long elephant grass at the rate of 46 g DM/kg M.d and one of the following supplements offered at the rate of 32 g DM/kg M075.d. The supplement treatments were: Tl.raw canavalia; T2. A mixture (50%-50%) of raw canavalia and sorghum-soya bean meal (SBM); T3. A mixture of sorghum-SBM. Molasses (15%) was added to all supplements. 4 g supplement DM/dacron bag were used. The soluble fraction of DM of canavalia was high ($P<0.05$) in Tl (45.4%) than in T2 (34.4%) and T3 (29.8%). Compering Tl and T3 in terms of effective degradation of the DM, Tl (70%) was significantly ($P<0.05$) high than T3 (60%). The soluble fraction of CP of Tl (68.5%) was significantly high ($P<0.05$) than in T3 (34%). The effective degradation of CP in Tl was higher (84%) than in T3 (64%). the effective disappearance of DM and CP was due to a higher solubility of the DM and CP of canavalia rather than the fractional rate of disappearance.The rate of disappearance of the cellulose between treatments was not different. This suggest that canavalia has not toxic effects on the microbios of the rumen. The results suggest that canavalia could be considered as a protein supplement to ruminants.

#30 LIPOLYSIS OF TRIGLYCERIDE BY RUMINAL MICROORGANISMS. D. L. Palmquist and Donna J. Kinsey, Dept. of Dairy Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691(216-263-3795).

Only unesterified, soluble long-chain fatty acids are toxic to ruminal microorganisms. It has long been assumed that the rate of lipolysis of glycerides in the rumen is rapid and not rate-limiting for subsequent metabolism of long-chain fatty acids. There have been few studies to quantify rates of lipolysis, or to compare lipolytic rates of glycerides from different feed ingredients. Ruminal bacteria grown under typical conditions contain no triglyceride. We have applied this principle to assess the disappearance of triglycerides from in vitro incubations of ruminal content. Ruminant content from a steer fed alfalfa hay was filtered through two layers of cheesecloth; 20 ml was diluted with 30 ml buffer and incubated with 0.5 g alfalfa meal and 0.05 g of test fat adsorbed on 1.0 g of Solka floc. Incubations were in triplicate at 39°C in a rotary shaking incubator for 0-24 hr. Samples were centrifuged, and the supernatant was discarded. Cholesterol (50 mg) was added as an internal standard, the pellet was extracted (hexane:isopropanol, 3:2), washed with water to remove isopropanol, and the hexane fraction was dried under nitrogen. The cholesterol and triglycerides were dissolved in 10 ml methylene chloride and separated and quantified by HPLC. Lipolytic rate was strongly influenced by melting point of the fat source. Animal-vegetable blend (iodine value 92) lipolysis was a rapid first-order process (0.33 hr-l), whereas lipolysis of tallow and hydrogenated tallow (IV = 45 and 30, respectively) was much slower and apparently zero order (about 20% disappearance in 12 hr for tallow and less than 10% for hydrogenated tallow). This approach is simple and straightforward for estimating potential rates of lipolysis by ruminal content.

Poster Session

Microbiology-Physiopathology Panel

#31 EFFECT OF TIME OF FEEDING ON THE INCIDENCE OF ALFALFA BLOAT IN CATTLE. W. Majak and J.W. Hall, Agriculture Canada, 3015 Ord Rd. Kamloops, BC V2B 8A9 (604-554-5205).

It is often claimed that the risk of bloat may be reduced by waiting until the dew is off before allowing cattle to graze alfalfa. Two feedlot and two grazing trials were carried out to compare the effects of early (0700 h or 0800 h) and late (1130 h or 1200 h) feedings on the occurrence of bloat. The trials had two period cross-over designs with 3 to 5 cattle per group. In the feedlot trials, animals were fed 50 kg per head per day of alfalfa herbage cut within a half hour of feeding. In the grazing trials, animals were allowed to graze a paddock of alfalfa for 4 h daily and were held in pens at other times. The alfalfa was in the vegetative to mid-bud stages of growth. Observations of bloat were made 0.75 to 2.0 h after feeding started. A further feedlot trial was carried out to measure rumen chlorophyll prior to feeding. Bloat was observed from 2 to 17 times more often when cattle were fed early than when they were fed late. No consistent differences were found between the two feeding times in feed dry matter, ADF, NDF, chlorophyll, total N or soluble N. Rumen chlorophyll was higher before the early than before the late feeding. This suggests that feeding in the late morning reduces the predisposition of cattle to bloat by increasing the 24 hour clearance from the rumen.

#32 FACTORS AFFECTING LACTATE UTILIZATION BY THE RUMINAL BACTERIUM *Megasphaera elsdenii*. H. M. Waldrip and S. A. Martin, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706-542-1065).

The objective of this study was to determine the effects of an *Aspergillus oryzae* fermentation extract (Amaferm) as well as other factors on lactate utilization by the ruminal bacterium *Megasphaera elsdenii* B159. Addition of Amaferm or a filter-sterilized Amaferm filtrate stimulated L-lactate uptake by both *M. elsdenii* and the ruminal selenomonad strain H18. Growth of *M. elsdenii* in medium that contained DL-lactate (2 g/L), Trypticase, and yeast extract was only slightly stimulated by the addition of 5% (vol/vol) Amaferm filtrate after 24 h. However, growth of *M. elsdenii* in a similar medium lacking Trypticase and yeast extract was increased over twofold by the addition of either 2 or 5% (vol/vol) Amaferm filtrate. These results suggest that Amaferm provides growth factors (i.e., amino acids, B vitamins) to support growth of *M. elsdenii* on lactate. There was no inhibition of L-lactate uptake when lactate-grown cells of *M elsdenii* were incubated with excess (10 mM) glucose, sucrose, or maltose. In addition, when cells were grown on glucose, sucrose, or maltose rather than lactate there was little difference in L-lactate uptake, suggesting that L-lactate transport in *M. elsdenii* is not subject to catabolite repression by these soluble sugars. Both K+ and Na+ had little effect on L-lactate uptake. Uptake was unaffected at extracellular pH values between 6.0 and 8.0, whereas pH values of 5.0 and 4.0 increased uptake. In addition, L-lactate uptake was inhibited between 34 and 61% by protonophores. These results suggest that protons may be involved in driving L-lactate uptake by *M elsdenii* B159.

#33 SURVIVAL OF A GENETICALLY MODIFIED BACTEROIDES THETAIOTAOMICRON (BTX) IN VITRO WITH RUMEN CONTENTS. T.R. Whitehead and M.A. Cotta, USDA/ARS, Natl. Cent. Agric. Utilzn. Res., Peoria, IL (309-681-6273).

A xylanase gene originally cloned from the ruminant anaerobic bacterium *Prevotella ruminicola* was inserted into the chromosome of the human colonic bacterium *Bacteroides thetaiotaomicron* 5482. The resultant strain, termed BTX, overproduced xylanase activity by 500-fold as compared to the original *P. ruminicola* strain. This organism could potentially serve as a model for introduction of a genetically modified organism into the rumen. The BTX strain was tested for the ability to survive in rumen contents obtained from a fistulated steer. A selective plating technique using a defined medium and chondroitin sulfate as the carbon source was used to follow the BTX strain following addition to the rumen contents, while total bacterial counts were determined with a complex medium containing rumen fluid. In the absence of added feed material, BTX numbers declined in a parallel manner as compared to total ruminal bacterial numbers. Addition of alfalfa cell walls allowed total cell numbers to increase, while BTX concentration decreased over time. Addition of chondroitin sulfate, a component of intestinal membranes, allowed BTX numbers to increase over time as compared to total bacterial numbers. These results suggest that the BTX strain can survive under rumen-like conditions if an appropriate carbon source is available.

#34 THE ROLE OF THE RUMINAL ANAEROBIC FUNGUS *Neocallismastix patriciarium* IN LIGNIN DEGRADATION. C.S. McSweeney, A. DuLieu, Y. Katayama* and J.B. Lowry, CSIRO Division of Tropical Animal Production, Private Bag No. 3 PO, Indooroopilly, Qld, 4068, Australia (+61-7-377-0820), *Cooperative Research Centre, Tokyo Noko University, Koganei, Japan.

Studies with plant fragments and radiolabeled lignocellulose indicated that rumen fungi are able to solubilise or degrade lignin but the mechanism of biodegradation has not been determined. This current study was undertaken to provide evidence that the anaerobic rumen fungus *Neocallimastix patriciarium* could cleave lignin bonds, and linkages between lignin and polysaccharide. The fungus did not degrade or transform model lignin compounds including alpha and beta aryl ether linked dimers, pinoresinol and biphenyls when fermented with carbohydrates or as the main carbon source for growth. Klason lignin, purified from spear grass (*Heteropogon contortus*) was colonised by the fungus but not degraded. Ester- and ether-linked phenolics in Mitchell grass (*Astrebla sp.*), spear grass and sorghum stem were readily released by the fungus as free acids and soluble ether-linked phenolics respectively. It is concluded that *Neocallimastix patriciarium* produces aryl esterases that solubilises lignin fragments but there is no evidence of etherase or lignolytic activity that depolymerises lignin.

#35 THE EFFECT OF PROTAMINE UPON GROWTH OF *Prevotella ruminicola*. H. M. F. Madeira and M. Morrison, Dept. of Animal Sciences, University of Nebraska, Lincoln, NE 68583. (402-472-9382).

Mechanistic and molecular details of peptide utilization by *Prevotella ruminicola* are lacking. Interesting features include i) size dependence of peptides for use as a nitrogen source and; ii) toxicity associated with the peptide salmine. Such features suggest that the peptide transport system(s) of *P. ruminicola* differ from those of the enteric bacteria; and that at least some peptides might enter the cell in an unhydrolyzed, and toxic, state. We have begun to examine the effect(s) of the protamine salmine chloride upon growth of *P. ruminicola*. When grown with 5 millimolar NH4Cl, the minimal inhibitory concentrations of salmine were 2 to 5 micromolar for strains $B_1$4 and D31d. Both strains $B_1$4 and D31d were then grown with 5 millimolar NH4Cl plus varying amounts of trypticase peptone, with or without 5 micromolar salmine. The results indicate that in the presence of peptides, strain $B_1$4 is more sensitive to salmine than strain D31d. However, increasing peptide concentrations minimized (strain $B_1$4) or overcame (strain D31d) the inhibition from salmine. This is interpreted as presumptive evidence that salmine uptake and toxicity is mediated by the peptide transport system(s) of *P. ruminicola*. Therefore, one class of salmine resistant mutants should be defective in peptide utilization, providing a selectable phenotype. Other peptides are also being examined as possible selective agents for the isolation of mutants.

#36 DEGRADATION AND UTILIZATION OF ISOLATED SOYBEAN PROTEIN AND CASEIN AS NITROGEN SOURCES FOR GROWTH OF *Prevotella ruminicola* $B_1$4. Kenneth E. Griswold and Roderick I. Mackie. Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801 (217-333-8809).

Manipulation of protein degradation and utilization within the rumen is an area of great interest to all ruminant nutritionists. The majority of degradation studies with rumen bacteria have examined casein as a nitrogen source. Casein is not a common feedstuff of ruminants, so soybean protein was chosen to compare degradation and utilization of two different nitrogen sources by a prominent proteolytic rumen bacteria, *Prevotella ruminicola* $B_1$4. *P.ruminicola* was grown in batch culture at 39°C using a defined medium with either isolated soybean protein or casein as the sole source of nitrogen. Glucose(5 mg/ml) was included as an energy source. Cultures were sampled hourly for twelve hours. Optical density was measured at 600 nm to determine growth rate, and maximum optical density. Samples were analyzed for ammonia, amino acids, soluble and insoluble protein, and RNA. Inclusion of soybean protein resulted in faster growth rate(0.11 vs. 0.075 h^{-1}) and maximum optical density(1.36 vs. 0.85) than casein. Ammonia levels were extremely low for both nitrogen sources over time, decreasing from 0.9 to 0.4 mM for soybean protein and from 1.3 to 0.5 mM for casein. Free amino acid levels were similar for both protein sources, ranging from 0.9 to 1.2 mM over the 12 h sampling period. Soluble protein decreased over time for both protein sources, decreasing from 0.47 to 0.30 mg/ml for soybean protein and from 1.29 to 0.93 mg/ml for casein. Insoluble protein was greater for soybean protein(50 - 77 mg) than for casein (3 -10 mg) over the 12h period. Bacterial dry weight was greater for soybean protein(6 - 20 mg) than for casein(2 - 9 mg) over time. Soybean protein was utilized more efficiently by *P.ruminicola* $B_1$4 than casein.

#37 BINDING OF IONOPHORES TO RUMINAL MICROORGANISMS. J. M. Chow* and J. B. Russell, Dept. of Animal Science and Section of Microbiology, Cornell University, and USDA/ARS, Ithaca, NY 14853 (607-255-4508).

Gram-negative, ionophore-resistant ruminal bacteria and Gram-positive, ionophore-sensitive species which were incubated with 5 uM ^{14}C-monensin or ^{14}C-lasalocid bound similar amounts of lasalocid, but neither group took up large amounts of monensin. The binding was first order at low cell concentrations, but binding was saturable at high cell densities. However, mixed bacteria did not show a preference for lasalocid and took up nearly equal amounts of monensin and lasalocid. Monensin binding was greatly reduced when mixed ruminal bacteria were pre- treated with Tris+EDTA, but Tris+EDTA did not affect the binding of lasalocid. Mixed ruminal protozoa always took up more lasalocid than monensin, but feed particles bound equal amounts of lasalocid and monensin. Because the half saturation constants (mass needed to bind half of the ionophore) of mixed ruminal bacteria, mixed ruminal protozoa, and feed were approximately 1.5, 1.0, and 7.4 g/l, respectively, it appeared that much of the ionophore in vivo is bound by cells and feed, and little ionophore would be left free in ruminal fluid. Since in vivo cell concentrations are often much greater than those found in vitro, these results suggest that in vitro experiments have probably used pharmacological rather than physiological doses of ionophores.

#38 PHOSPHOENOLPYRUVATE CARBOXYKINASE FROM *Rurninococcus flavefaciens* FD-l. L. Schocke[1] and P.J. Weimer,[1,2] Department of Bacteriology,[1] University of Wisconsin-Madison, and U.S. Dairy Forage Research Center,[2] U.S. Dept. of Agriculture, Agricultural Research Service, Madison,WI 53706 (608-264-5320)

Phosphoenolpyruvate (PEP) carboxykinase is the major carboxylation enzyme in Ruminococcus flavefaciens FD- 1 and is thus important in succinate production. Kinetic studies with crude extracts showed dependence of PEP carboxykinase on divalent cations. As Mn^{2+} was effective in 100-fold lower concentration than was Mg^{2+}, it is considered that Mg^{2+} interacts with the substrate GTP and that Mn^{2+} interacts with the enzyme. The enzyme was purified by a scheme that included ammonium sulfate precipitation, desalting on BioGel P6DG, preparative isoelectric focusing and chromatographic buffer exchange. The enzyme has an isoelectric point of approximately 4.1 and a molecular weight of about 68 kD (SDS-PAGE). Lyophilization of the desalted enzyme did not show any effect on its activity.

#39 EFFECTS OF LIVE YEAST CELLS ON ZOOSPORE GERMINATION, GROWTH AND CELLULOLYTIC ACTIVITY OF *NEOCALLIMASTIX FRONTALIS* MCH3. F. Chaucheyras [(1)], G. Fonty [(1)], G. Bertin [(2)] and P. Gouet[(1)]. (1) Laboratoire de Microbiologie, INRA, 63122 Saint-Genes-Champanelle (33.73624000);(2) OHF-Santel, 92300 Levallois-Perret (33.147393222), France.

Several studies have shown that live cultures of yeast used as probiotics can seimulate microbial activities in the rumen. The aim of this work was to investigate the effects of a live yeast strain (*Saccharomyces cerevisiae*), which had been previously shown to increase cellulose degradation in vivo and in Rusitec, on the growth and activity of the rumen fungus *Neocallimastix frontalis* (strain MCH3). In vitro, addition of yeast cells (10^6 to 10^7/ml) to fungal zoospores in a vitamin-deficient culture medium stimulated their germination (from 2-fold to 27-fold) and increased:

l) the fungal biomass estimated with specific oligonucleotidic probes;
2) cellulose degradation (8 to 40-fold with 10 7 yeast cells per ml);
3) the concentration of cellulose fermentation end-products (Hydrogen, Formate, Total Volatile Fatty Acids). In presence of yeast cells, fungal growth and activities were comparable to those measured in control cultures when the fungus was cultivated in the medium containing several B-vitamins. Therefore, these results can provide an explanation of the effect of yeasts observed in vivo, with certain diets, on the microbial population, especially cellulolytic microorganisms, and on plant cell-wall breakdown.

#40 COMPETITION BETWEEN *Ruminococcus flavefaciens* FD-1 and *Fibrobacter succinogenes* S85 FOR UTILIZATION OF CRYSTALLINE CELLULOSE. Y. Shi[1] and P. J. Weimer,[2] [3] Departments of Dairy Sciencel and Bacteriology,[2] University of Wisconsin-Madison; and U.S. Dairy Forage Research Center,[3] U.S. Dept. of Agliculture, Agncultural Research Service, Madison, WI 53706 (608-264-5320)

R. flavefaciens FD-l and *F. succinogenes* S85 are two of the most important cellulolyiic ruminal bactena, but little is known about how they interact in the rumen. Co-inoculation of FD-l and S85 into a cellulose-limited chemostat resulted in a complete takeover of the culture by FD-1 (as determined by a signature membrane associated fatty acids assay method). Inoculation of FD-l (1% v/v) into an established chemostat of S85 (D=0.03 h-l) resulted in an FD-l monoculture after 2 days. Neither species could establish itself when inoculated into an established batch culture of the other species that had fully colonized a limiting amount of cellulose. By contrast, in cellulose-nonlimited batch cultures the adherence and growth of both species were observed if FD- 1 and S85 were inoculated simultaneously or within 12 minutes of one another. Preliminary data suggest that the competitive success of FD-1 under cellulose limitation may be due to its more rapid adherence to cellulose and/or its lower affinity for certain products of cellulose hydrolysis (e.g., cellobiose).

#41 INITIAL ISOLATION AND CHARACTERIZATION OF RUMEN BACTERIA CAPABLE OF GROWING ON SUBSTRATES CONTAINING HIGH LEVELS OF TANNINS. K. Nelson, A.N. Pell, P. Schofield, and B. Giner-Chavez. Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2884).

Rumen fluid was obtained from goats fed on high tannin diets of Desmodium (Desmodium ovalifolium) in Colombia, South America. Rumen microbes were maintained on rumen fluid medium in which unpurified Quebracho (*Loxopterygium lorentzil*) tannin was the added carbon source. Repeated transfer has given two bacteria, both gram negative, and capable of tolerating or growing on high levels of tannins. The first is a diplococcal facultative anaerobe, whose main energy substrates are glucose and cellobiose, and which can tolerate very high levels of both condensed and hydrolyzable tannins. This organism gives white, round glossy colonies on agar plates. The second bacterium is a crescent shaped obligate anaerobe whose growth over 60 hours in rumen fluid and pure culture medium was associated with approximately 30% disappearance of purified condensed tannins (as measured by the butanol-HCI assay). Zones of clearing on agar plates layered with both purified and unpurified condensed tannins have also been obtained. Initial two dimensional thin layer chromatography studies show changes in the band patterns of unpurified tannins after this organism has been cultured in media containing this substrate. Presently HPLC is being used to further investigate the carbon metabolism of this bacterium.

#42 THE EVALUATION OF DIFFERENT LABELLING METHODS OF AN OLIGONUCLEOTIDE PROBE FOR THE DETECTION OF ANAEROBIC FUNGI IN THE RUMEN. L. Millet, G. Fonty an Ph. Gouet. Laboratoire de Microbiologie, INRA de Clermont-Fd-Theix, 63122 Saint-Genes- Champanelle (33-73624000), France.

A nucleic probe enabling the identification, by molecular hybridization, of a genomic fragment specfic to anaerobic fungi of the rumen, was labelled with either ^{32}P (radioactive) or with DIG or ECL systems (non-radioactive probes). The DIG (BOEHRINGER MANNHEIM) allows a 3' end labelling using a dideoxynucleotide coupled with digoxigenin. After membrane hybridization (slot blot), immunodetection of the probe is carried out using alkaline phosphatase conjugated with the anti-digoxigenin antibody and visualized, by autoradiography, with a chemiluminescent substrate (AMPPD). The ECL (AMERSHAM) system is based on the association of fluorescein with a deoxynucleotide. Six to eigth units are attached to the probe. Detection, following membrane hybridization, is achieved by the addition of an anti-fluorescein antibody combined with peroxidase wich act upon a chemiluminescent substrate (luminol). The sensitivity and specificity of this probe was assessed, for all three types of labelling, on DNA samples taken from 1) fungi (*Piromyces communis*) in a pure culture, 2) the rumen content of gnotobiotic lambs devoid of fungi and protozoa or harbouring a known and controlled fungal flora, 3) the rumen content of an adult stag. The detection sensitivity was marginally better with the ^{32}P (0 5 ng vs 1 ng) labelling. On the other hand, the specificity of the probe was the same irrespective of the method of labelling used and of the origin of the DNA samples. The use of non-radioactive oligonucleotide probes can therefore be envisaged for the detection of anaerobic fungi in the rumen.

#43 IN-VITRO INTERACTIONS BETWEEN RUMEN H_2-PRODUCING MICROORGANISMS AND A H_2-UTILIZING ACETOGENIC BACTERIUM. B. Morvan, G. Fonty and Ph. Gouet. Laboratoire de Microbiologie, INRA de Clermont- Ferrand-Theix, 63122 Saint-Genes-Champanelle (33-73624000), France.

The aim of this study was to assess the ability of a H_2-utilizing acetogenic bacterium to interact with H_2-producing microorganisms in cellulose fermentation. Two cellulolytic bacteria, *Ruminococcus flavefaciens* 007 and *R. albus* 7, and one anaerobic fungus, *Neocallimastix frontalis* MCH3, were associated in coculture with an acetogenic strain (Ser 8) isolated from lamb rumen. Cellulose disappearance and end products of cellulose fermentation were measured after 2, 4, 6 and 8 days of incubation. In presence of strain Ser8, the rate of cellulose degradation by *N. fronlalis* and *R. albus* were greatly increased (60% vs 28% and 52% vs 10% of dry matter disappearance by day 4,respectively) whereas that of *R. flavefaciens* was not affected. In cocultures, H_2 and formate were not found and fungal lactate production was decreased (85 micromol vs 232 microlmol by day 8) as *R . flavefaciens* propionate yield (2 micromol vs 76 micromol by day 8). In contrast, acetate yield was largely increased (four- to sixfold greater by day 8) but the contribution of each associated strains was not determined. These data suggest that acetogens, as archaea methanogens, are able to shift the metabolism of H_2-producing microorganisms by hydrogen transfer.

#44 ENERGY SPILLING IN MIXED RUMINAL BACTERIA. J. S. Van Kessel and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14853 (607-255-4508).

Mixed ruminal bacteria which were grown in continuous culture (0.07 h^{-1}, pH 6.7) had 3 fold lower growth yields when glucose or starch concentrations were increased and ammonia became the growth-limiting nutrient, but ammonia deprivation had little effect on cultures which were provided with an excess of cellulose. When ammonia-excess, glucose-limited continuous cultures were given a pulse dose of glucose, the specific rate of glucose consumption increased at a faster rate than growth, and there was a 3 fold decline in yield. The increased rate of glucose consumption was associated with a 7 fold increase in intracellular ATP. Based on these results, it appears that ruminal bacteria which ferment soluble carbohydrates have the ability to spill ATP when energy is in excess and the catabolic rate is faster than the anabolic rate.

#45 ISOLATION OF A RUMINAL CLOSIRIDIUMSP. WHICH DEGRADES PURIFIED CHITIN AND SHELLFISH WASTE. M. A. Cobos. S. T,. Flegler and M. T. Yokoyama. Dept. of Animal Science, Michigan State University, East Lansing, MI 48824 (353-2299).

Using an anaerobic selective medium with chitin as the sole energy source and ruminal fluid from fistulated dairy cows as inocula, a gram-positive, motile sporeforming rod has been isolated which degrades chitin. Scanning electron micrographs of young cultures show vegetative cells 3 to 5 micrometers in length with rounded ends. However, in old cultures the bacterium may grow either as a long filament up to 30 micrometers in length or develop a terminal swollen spore. The ruminal clostridium is a strict anaerobe, which also rapidly degrades crabshell meal and shrimp carapace. This bacterium requires clarified rumen fluid for optimum grow and chitinolytic activity. It will grow in GCS-RF medium, but appears unable to degrade cellulose. Its biochemical and physiological characteristics are still under investigation. One interesting property of this bacterium is its ability to tightly bind to chitin. With shrimp carapace as a chitin source, the mechanism of attachment and degradation by this bacterium was examined using scanning electron micr oscopy. These observations show that the bacterium attaches to the inner surface of the shrimp carapace but not to the outer surface, which remains clean even at the end of the degradation process. After attachment, the clostridium starts to colonize and degrade the multilayers of chitin that comprise the shrimp carapace to finally reach the outer surface. These data suggest that the bacterium recognizes specific attachment sites on chitin. Since chitin degradation is initiated only after bacterial attachment occurs, study of the factors that influence this adhesion may result in more efficient use of chitin or shellfish waste as a feedstuff for ruminants.

#46 ISOLATION AND CHARACTERIZATION OF RUMINAL ACETOGENIC BACTERIA. P. Boccazzi, R. S. Pinder and J. A. Patterson. Department of Animal Science, Purdue University, West Lafayette, IN, 47907. 317-494-4826.

Hydrogen-limited continuous cultures were used to isolate autotrophic acetogenic bacteria from rumen contents of cattle on either a high roughage or a high concentrate diet. Twenty bacterial isolates were obtained and were presumptively identified based upon acetic acid production from H_2:CO_2 as acetogenic bacteria. All isolates were gram positive and used fructose, glucose, lactose, maltose and esculin. Acetic acid was the predominant volatile fatty acid produced by all isolates. One isolate was selected for further studies based upon its low hydrogen threshold value and that it contained plasmids. Acetogenic isolate H3HH is a strictly anaerobic, gram positive, non-spore forming coccus. Based on cell morphology, substrate utilization, FAME analysis and G + C composition strain H3HH is different from all other described acetogens.

#47 EFFECTS OF LIVE YEAST CELLS ON ZOOSPORE GERMINATION, GROWTH, AND CELLULOLYTIC ACTIVITY OF NEOCALLIMASTIX FRONTALIS MCH3.

F. Chaucheyras[(1)], G. Fonty[(1)], G. Bertin[(2)], and P. Gouet[(1)]. (1) Laboratoire de Microbiologie, INRA de Clermont-Ferrand-Theix, 63122 Saint-Genés-Champanelle (33-73624000), France; (2) OHF-Santel, 92300 Levallois-Perret (33-14793222), France.

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The aim of this work was to investigate the effects of a live yeast strain (*Saccharomyces cerevisiae*), which had been previously shown to increase cellulose degradation *in vivo* and in Rusitec, on the growth and activity of the rumen fungus *Neocallimastix frontalis* (strain MCH3). *In vitro*, addition of yeast cells (10^6 to 10^7/ml) to fungal zoospores in a vitamin-deficient culture medium stimulated their germination (from 2-fold to 27-fold) and increased:

1) the fungal biomass estimated with specific oligonucleotide probes;

2) cellulose degradation (8 to 40-fold with 10^7 yeast cells per ml);

3) the concentration of cellulose fermentation end-products (Hydrogen, Formate, Total Volatile Fatty Acids). In presence of yeast cells, fungal growth and activities were comparable to those measured in control cultures when the fungus was cultivated in the medium containing several B-vitamins. Therefore, these results can provide an explanation of the effect of yeasts observed *in vivo*, with certain diets, on the microbial population, especially cellulolytic microorganisms, and on plant cell-wall breakdown.

#48 MODIFICATION OF THE PHOSPHOKETOLASE ASSAY FOR IDENTIFICATION OF BIFIDOBACTERIA. J. I. Orban and J. A. Patterson. Department of Animal Sciences, Purdue University, West Lafayette, IN, 47907. 317-494-4826.

The phosphoketolase assay is commonly used as a definitive criterion for identification of bifidobacteria. Development of a reddish-violet color is a positive identification for bifidobacteria. A limitation of the assay is the time consuming process of cell disruption, either by use of the French Pressure Cell or by sonication. We have eliminated this step by use of the detergent hexadecyltrimethylammonium bromide (cetrimonium bromide, CTAB). A number of pure cultures of bifidobacteria and lactobacilli were tested by no pretreatment, sonication or the addition of CTAB (0.45 mg/ml). In all cases there was no color formation by cells that had no pretreatment, or by lactobacilli under any treatment. All bifidobacteria gave a similar color formation when sonicated or with CTAB addition. Addition of CTAB to disrupt cell membranes is an effective alternative cell disruption procedure and should increase the number of cultures that can be processed.

#49 CELLULOSE/XYLAN COMPOSITE STRUCTURES FOR STUDIES OF PLANT CELL WALL DIGESTION KINETICS. P.J. Weimer[1,2] J.M. Hackney,[3] C.R. Dietrich,[2] and H.G. Jung,[1,4] U.S.Dairy Forage Research Centerl, U.S. Department of Agricuture, Agricultural Research Service, Departments of Bacteriology[2] and Botany[3], University of Wisconsin, Madison, WI 53706, and USDA-Agricultural Research Service,4 St. Paul, MN 55108 (608-264-5408)

Biosynthetic and chemisynthetic composites of cellulose and xylan were prepared as model compounds for studies of the kinetics of cell wall digestion by ruminal bacteria. Biosynthetic composites were prepared by growing the cellulose-synthesizing bacterium *Acetobacter aceti* var. *xylinum* in glucose-containing media supplemented with a linear, water-soluble homoxylan isolated from tobacco stalks. The composites were purified by solvent extraction and boiling in alkali. X-ray diffraction revealed that these composites were highly crystalline (although somewhat less so than pure bacterial cellulose), and chemical analysis indicated that the composites had maximal levels of xylan incorporation of approximately 9%. Chemisynthetic composites were prepared by dissolving cellulose in organic solvents containing sulfur dioxide, then regenerating in water in the presence of an equal weight of tobacco stalk xylan. The chemisynthetic composites were essentially noncrystalline and had maximal levels of xylan incorporation of approximately 7%. Levels of xylan incorporation into the composites increased with increasing concentrations of xylan in the medium. The materials provide a series of composite structures having different compositions and crystallinities, and thus will be useful in evaluating the role of interactions between cellulose and xylan on the ruminal digestion process.

#50 CHARACTERIZATION OF AN ANTI-CELLULOLYTIC FACTOR FROM CICER MILKVETCH. T.C. Maleniak[1] and P.J. Weimer,[1,2] Department of Bacteriology,[1] University of Wisconsin-Madison; and U.S. Dairy Forage Research Center,[2] U.S. Department of Agriculture, Agricultural Research Service, Madison, WI 53706.(608-264-5408)

Previous experiments with the perennial legume cicer milkvetch (*Astragalus cicer*) revealed that this forage contains a heat-stable factor of > 30 kD molecular weight that specifically inhibits digestion of cellulose by ruminal microorganisms. The inhibitor was extracted from Wiley-milled leaves with water at 39C for 3 h. This extract was subjected to a purification scheme that included heat treatment, ether extraction, column chromatography, and preparative-scale isoelectric focusing. Active fractions (pH range 2.6-3.2) were pooled and immediately refocused to minimize activity losses. At all stages of the purification, the inhibitor was assayed in microtiter plates that contained a cellulose-amended medium inoculated with *Ruminococcus flavefaciens* FD-1. After overnight incubation, presence of the inhibitor was easily detected by lack of production of the yellow pigment associated with growth on cellulose. Partially purified active fractions contained both protein and carbohydrate, and retained activity upon freezing after the ether extraction step. Size-exclusion chromatography or ultrafiltration occasionally resulted in fragmentation of the inhibitor, and some fragments retained limited anti-cellulolytic activity.

#51 THE EVALUATION OF DIFFERENT LABELLING METHODS OF AN OLIGONUCLEOTIDE PROBE FOR THE DETECTION OF ANAEROBIC FUNGI IN THE RUMEN. L. Millet, G. Fonty, and Ph. Gouet. Laboratoire de Microbiologie, INRA de Clermont-Ferrand-Theix, 63122 Saint-Genés-Champanelle (33-73624000), France.

A nucleic acid enabling the identification, by molecular hybridization, of a genomic fragment specific to anaerobic fungi of the rumen, was labeled with either ^{32}P (radioactive) or with DIG or ECL systems (non-radioactive probes). The DIG (BOEHRINGER MANNHEIM) allows a 3' end labeling using a dideoxynucleotide coupled with digoxigenin. After membrane hybridization (slot blot), immunodetection of the probe is carrried out using alkaline phosphatase conjugated with the antidigoxigenin antibody and visualized, by autoradiography, with a chemiluminescent substrate (AMPPD). The ECL (AMERSHAM) system is based on the association of fluorescein with a deoxynucleotide. Six to eight units are attached to the probe. Detection, following membrane hybridization, is achieved by the addition of an anti-fluorescein antibody combined with peroxidase which act upon a chemiluminescent substrate (luminol).
The sensitivity and specificity of this probe was assessed, for all three types of labeling, on DNA samples taken from: (1) fungi (*Piromyces communis*) in a pure culture; (2) the rumen content of gnotobiotic lambs devoid of fungi and protozoa or habouring a known and controlled fungal flora; (3) the rumen content of an adult stag. The detection sensitivity was marginally better with the ^{32}P (0.5 mg vs. 1 mg) labeling. On the other hand, the specificity of the probe was the same irrespective of the method of labeling used and of the origin of the DNA samples. The use of non-radioactive oligonucleotide probes can therefore be envisaged for the detection of anaerobic fungi in the rumen.

Nutrition-Agronomy Panel

#52 EFFECT OF LEVEL OF MONOCALCIUM PHOSPHATE IN HIGH FAT DIETS ON RUMEN pH, ACETATE, PROPIONATE, BUTYRATE PRODUCTION AND DIGESTIBILITY OF NUTRIENTS.
C.T. Kadzere,[1,2] E. Masama[1] and J. Munowenyu.[1] [1,2]Department of Livestock and Pasture Science, University of Fort Hare, PB X 1314, Alice 5700, Republic of South Africa, Tel: 0404-32011 Ext2025. [1] Department of Animal Science, University of Zimbabwe, PB MP 168, Mt Pleasant, Harare, Zimbabwe.

In a digestibility and a rumen function trial, the effect of varying levels of dietary (MCP) in high fat complete diets was assessed. For the digestibility test, fifteen mature whether sheep were randomly allotted to three groups of five animals each and put in single metabolism cages and fed a high lipid diet (16% of DM) in a 28 day trial. Three other mature rumen fistulated sheep were put into single pens and each fed one of the corresponding diets for the digestibility test. About 1/2 litre rumen contents were collected an hour after the morning feeding on days 1,7, 14, 21 and 28 of the experiment and pH and the concentration of acetate, propionate and butyrate was measured. The concentration of acetate, propionate and butyrate (VFAs) in rumen contents decreased as the amount of dietary MCP increased and as the trial progressed. The apparent digestibility of dry matter, organic matter, crude fat, crude protein and gross energy improved with increasing level of dietary MCP. Digestibility of the NFE fraction was raised ($P<0.05$) from 44.1% in group 1 to 51.5% in group 3. Conversely, the apparent digestibility of crude fibre dropped ($P<0.05$) from 54,7% in group 1 to 37,5% in group 3. High levels of MCP in high fat diets raised the rumen pH and lowered the production of volatile fatty acids with a resultant infuence on the digestibility of nutrients.

#53 EFFECTS OF DIETARY CALCIUM LEVELS ON DIGESTIVE FUNCTION IN HOLSTEIN STEERS FED A HIGH-FAT FINISHING DIET. R.A. Zinn. Department of Animal Science, Imperial Valley Agricultural Center, University of California, El Centro 92243 (619-352- 0111)

Ten Holstein steers (348 kg) with "T" cannulas in the rumen and proximal duodenum were used in a crossover design experiment to evaluate the influence of .7 and .9% dietary calcium (1.18 vs 1.80% limestone, DM basis) on digestive function. There were no treatment effects on DM intake. However, increasing calcium level from .7 to .9% decreased ruminal digestion of OM (5%, $P < .05$), ADF (37%, $P < .10$), and feed N (18%, $P < .05$), and increased ruminal microbial efficiency (12%, $P < .10$) and ruminal N efficiency (14%, $P < .01$). Increasing calcium level to .9% also increased postruminal digestibility of OM (4%, $P < .05$) and N (4%, $P < .01$). There were no treatment effects ($P > .10$) on total tract digestion of OM, ADF, starch or N. The net effect of increasing calcium level from .7 to .9% was greater postruminal absorption, as a percentage of intake, of OM (12%, $P < .01$), N (19%, $P < .01$) and lipid (10%, $P < .10$). Post ruminal calcium disappearance was greater (142%, $P < .10$) with .9% dietary calcium. Increasing the level of calcium did not influence ($P > .10$) either ruminal pH or soluble calcium. Ruminal soluble calcium (Ca_S, mM) was closely associated with ruminal pH ($R^2 = .93$). There were not treatment effects of ruminal VFA molar proportions.

#54 THE EFFECT OF MOLASSES UREA BLOCKS ON SOME RUMEN PARAMETERS OF BULLS GRAZING NON IRRIGATED PASTURES DURING THE DRY SEASON A.J. Ayala, R. Pinto and I.R. Armendariz, Dept. Nutricion, FMVZ- UADY, PO Box 116-4, Merida, Yuc., 97100, MEXICO, (99) 47-15-43

The aim of this work was to evaluate the molasses urea (10%) blocks (MUB) on rumen DM degradability (RDMD) of the forage and to characterize the NH_3, pH and rumen pool size of bulls grazing non irrigated pastures. Four bulls with rumen canulae and an average LW of 322 kg were used in a cross over design. The animals grazed a paddock of 3.4 ha from 17:00 to 06:00 h, during the day, they were penned in groups of two animals with or without MUB. A sample of the pasture grazed (Pl) and of rumen content (RC) was taken to evaluate its RDMD compared with a hay of high quality (P2). The pasture availability, Crude Protein (CP) and Neutral Detergent Fiber (NDF) of Pl were 742 kgDM/ani, 4.0% and 75.2% respectively. The contents of CP and NDF of P2 and RC were 10.3%, 64.1%, 8.3% and 72.7% respectively. The intake of MUB was 236 g/ani/day. The MUB increased ($p<0.05$) NH_3 in rumen liquor, being 12.21 for MUB and 9.96 mg/100 ml without MUB. The rumen pH with or without MUB was 6.99. The potential and rate of RDMD were not effect ($p>0.05$) by MUB being for Pl, 53.8 and 3.6 and 52.3 and 4.9 with or without blocks respectively, for P2 and RC the means for potential and for rate were 82.5 and 7.4 and 48.5 and 4.04 respectively however, there were high differences ($p<0.001$) between forages. The MUB had no effect on the rumen pool size, being the mean of the total rumen volume 42.9 kg and its DM 13.9%. It is suggested that the high DM availability on field and the presence of shrubs at grazing could explain the poor effect of MUB supplementation on the rumen parameters evaluated.

#55 THE EFFECT OF INCREASING AVAILABLE PROTEIN TO FERMENTABLE CARBOHYDRATE RATIO ON MICROBIAL GROWTH. G.E. Catlett and M.S. Kerley. Department of Animal Science, University of Missouri, Columbia, MO 65211 (3 14-882-3876).

A batch culture experiment was performed to determine the ratio of hydrophilic peptide to fermentable carbohydrate that maximized fiber digestion and growth of ruminal microbes. Big bluestem (*Andropogon gerardii*) hay (8% CP; 77% NDF) was used as the forage source and isolated soy protein as the hydrophilic peptide source. Five treatments were compared; no hydrophilic peptide (0), and fermentable carbohydrate to hydrophilic peptide ratios of 0.75 (.75), 1.5 (1.5), 3 (3), and 6 (6). Batch culture fermentations were conducted over two runs with duplicate flasks (100 ml) prepared to stop fermentation at 0, 6, 12, 18, 24, 30, 36, and 48 hours to measure microbial growth, fiber digestion, carboxymethylcellulase activity (CMCase), and ammonia and peptide concentration. No significant difference ($P>.05$) was observed for NDF disappearance during the first 18 hours. However, by 48 hours there was an increase ($P<.05$) in the percent NDF remaining for .75 compared to 1.5, 3, and 6 treatments. After 12 hours of fermentation, NH3 concentration for the .75 and 1.5 treatments increased over time while treatments 3, 6, and 0 reached a plateau. There was an increase in microbial growth up to 18 hours for .75 and 1.5 treatments. Growth of bacteria after 18 hours occurred for the high protein (.75) treatment. As the concentration of protein increased peptide concentration increased ($P<.05$). No differences ($P>.05$) in CMCase occurred among treatments. In conclusion, protein (peptides) improved the efficiency of bacterial growth by stimulating growth of the non-fibrolytic bacteria.

#56 EPIPHYTIC TOTAL VIABLE COUNT AND LACTIC ACID PRODUCING BACTERIA IN THE STANDING ALFALFA CROP AND ALFALFA FORAGE CHOPPED PRIOR TO ENSILING AT DIFFERENT TIMES OF THE DAY AND TIME OF REGROWTH. A.A. Rodriguez, S.R. Rust, and M.T. Yokoyama. Michigan State University, East Lansing, MI 48824 (517-353-8401)

An experiment was conducted to evaluate the effects of time of day and time for regrowth on epiphytic total viable count (TVCB) and lactic acid producing bacteria (LAPB) in the standing alfalfa crop (whole plant, leaves, and stems), and alfalfa forage chopped prior to ensiling. Alfalfa plants were aseptically taken from a well established plot in the morning (0800, T1) and afternoon (1400, T2) at 21 (R1), 42 (R2) and 63 (R3) days of regrowth. Four botanical groups were enumerated for TVCB (Plate count agar) and LAPB (MRS) at each time of the day and regrowth period. Botanical groups included; whole plant (W), leaves (L), stems (S) and forage chopped prior to ensiling (CH). Epiphytic TVCB and LAPB were higher ($P<0.05$) in the CH forage as compared to the standing crop. Whole alfalfa plant had greater ($P < 0.05$) TVCB and LAPB as compared to L and S. Leaves had lower ($P<0.05$) TVCB than S, but LAPB were similar. Epiphytic populations decreased ($P<0.05$) during T2 as compared to T1. At 42 days of regrowth both groups of bacteria increased ($P<0.05$) as compared to R1, but were similar to R3. Lactic acid producing bacteria decreased ($P<0.05$) during T2 in the four botanical groups as compared to T1 . Epiphytic TVCB were lower ($P < 0.05$) in W and S during T2, but CH and S had similar populations regardless of time of day. Except for TVCB on CH, stems, W and CH had lower ($P < 0.05$) TVCB and LAPB at R1, but populations were similar at R2 and R3. Epiphytic TVCB and LAPB in L increased ($P<0.05$) with length of time for regrowth. In summary, the chopping process increased the number of epiphytic microorganisms in alfalfa. Numbers of TVCB and LAPB on the standing alfalfa crop and forage alfalfa prior to ensiling were higher during early hours of the day and increased as length of time for regrowth increased.

#57 APPLICATION OF DUAL POOL LOGISTIC EQUATIONS TO MODEL GAS PRODUCED FROM THE IN VITRO FERMENTATION OF SEVERAL COMMON FEEDSTUFFS. W.C. Stone, P.Schofield, A.N. Pell, Dept. of Animal Science, Cornell University, Ithaca, NY 14853 (607-255-2876).

Two dual pool logistic equations, containing either single or double lag terms, were used to model gas produced from the in vitro fermentation of several common feedstuffs (alfalfa haylage, grass hay, corn silage, corn, and soybean hulls).The shapes of the gas curves were quite varied and could not all be adequately modeled with conventional exponential equations. In contrast, both dual pool logistic models fit the data extremely well ($r^2 > .99$). The best fits with the smallest standard errors were most often obtained with the logistic equation utilizing a single lag term. Lag of the slow digesting fraction ranged from approximately 2 hours in grass hay to 8 hours in corn. The fast digesting fraction produced 75% of the total gas in corn, 50% in grass hay, and 12% in soybean hulls. Specific rates varied from .08 (corn silage) to .28 (soybean hulls) $hour^{-1}$ for the fast digesting fraction and from .025 (corn) to .05 (soybean hulls) $hour^{-1}$ for the slow digesting fraction.

#58 DEGRADATION OF FORAGE CRUDE PROTEIN SOLUBILITY FRACTIONS BY RUMEN EXTRACT. R. A. Kohn and M. S. Allen. Dept. of Animal Science, Michigan State University, East Lansing, 48823 (215-444-5800).

Isolation of crude protein (CP) fractions that degrade in the rumen at uniform rates across forages would enable the routine prediction of total CP degradation of a forage from the size of its CP fractions. The objective was to determine degradation rates and uniformity of 6 independent CP solubility fractions in 8 forages. Dried forages were: alfalfa (tenth bloom and late bud), bromegrass (seeded), and canarygrass (early anthesis). Ensiled forages were alfalfa (full bloom) bromegrass (boot stage), canarygrass (early anthesis) and whole plant corn (black layer formation). Each forage was incubated (38 C) in a crude enzyme extract from rumen contents for 0, 2, 6 and 24 h (n=2). The residue from the degradation was fractionated by sequential extraction into 6 parts based on solubility in: trichloroacetic acid (TCA), buffered media, acetone. detergent solution at pH 7, detergent solution in 1 N H_2SO_4 and insoluble. Solubility in TCA and acetone increased during degradation, while solubility in buffer, acid detergent solution and insoluble decreased during degradation, and solubility in pH-7 detergent did not change. Buffer soluble CP was completely and rapidly degraded across all forages, but other fractions that were degraded were less uniform within and across forages.

#59 GROWTH AND RUMEN FERMENTATION IN STEERS FED WHOLE-CROP FODDER BEET SILAGE. A.P. Moloney, P. O'Kiely, Teagasc, Grange Research Centre, Co. Meath, Ireland (+353 4125214) and X.B. Chen, Rowett Research Institute, Aberdeen, Scotland.

Whole-crop fodder beet (*Beta vulgaris*) was ensiled without (FBSN) or with (FBSA) the addition of dry sugar beet pulp (as an effluent absorbent at 159 g/kg), and had mean pH, dry matter (DM), organic matter (OM) digestibility and crude protein (CP) values of 4.06 and 3.68 (s.d. 0.070), 154 and 224 (s.d. 9.2) g/kg, 785 and 838 (s.d. 9.8) g/kg and 137 and 118 (s.d. 7.1) g/kg DM, respectively. The silages were offered *ad libitum* to steers (n=l 0/treatment; bodyweight (BW) 555 kg) as follows: FBSN + 3 kg concentrate (140 g CP/kg DM) and FBSA with O or 3 kg concentrates containing 140 (FBSAL), 163 (high degradability; FBSAH) or 160 (low degradability; FBSAU) g CP/kg DM. A sixth group was offered a barley-based concentrate *ad libitum* (CON). For FBSN, FBSA, FBSAL, FBSAH, FBSAU and CON, daily BW gain was 1062, 1219, 1222, 1349, 1141 and 1327 (s.e.d. 96) g, respectively. Ruminally fistulated steers (n=4/treatment; BW 584 kg) were fed the silage-based diets at 18 g DM/kg BW daily and at the same forage to concentrate ratio as in the growth study. For FBSN, FBSA, FBSAL, FBSAH and FBSAU, respectively, mean rumen fluid pH was 6.48, 6.53, 6.49, 6.48 and 6.48 (s.e.d. 0.198); concentrations (mmol/l) of ammonia and volatile fatty acids (VFA) and the acetate to propionate ratio in rumen fluid were 201, 103, 96, 100 and 125 (s.e.d. 32.9), 101, 85, 96, 85 and 87 (s.e.d. 10.5) and 3.1, 2.7, 2.7, 2.7 and 2.9 (s.e.d. 0.26); daily purine excretion (mmol) was 64, 88, 63, 56 and 83 (s.e.d. 17.7); rumen liquid and OM pools were 69, 75, 66, 81 and 74 (s.e.d. 18.0) ml/kg BW and 6.8, 9.1, 7.0, 7.4 and 7.1 (s.e.d. 1.49) g/kg BW. It is concluded that l) FBSA had a higher nutritive value and a different pattern of rumen fermentation than FBSN, 2) the level and form of supplementary protein did not alter growth or rumen metabolism in cattle fed FBSA.

#60 PROFILE OF RUMEN BACTERIA IN RUMEN LIQUOR UNDER DIFFERENT DIETARY REGIMES IN BUFFALO CALVES(*Bubalus bubalis*) Gill, S.S. [1]; Singh, R[2]; and Singh S[3]. [1] Veterinary Officer, Department of Animal Husbandary, Govt. of Punjab; [2] Associate Professor, Department of Veterinary Physiology, Punjab Agril. University, Ludhiana, Punjab; [3] Research Scholar, Department of Surgery & Radiology, Punjab Agril. University, Ludhiana, Punjab.

Profile of rumen bacteria in rumen liquor under different dietary regimes was undertaken on four rumen fistulated buffalo calves. The animals were varying in their age from 14 to 16 months and in weight from 100-125 kg. They were subjected to four different kinds of diet. Diet I consisted of wheat straw plus green fodder plus concentrate plus mineral mixture. Diet 11 comprised of wheat straw plus subabul concentrate plus green fodder. Diet 111 contained wheat straw treated with urea and molasses. Diet IV consisted of wheat straw exclusively. The animals were kept on each diet for a period of 21 days and fed twice daily at 8.00 hrs. and 17.00 hrs. Water was provided *ad libitum* after feeding. After 21 of adaptation period rumen liquor samples were collected before feeding (0 hr.) and subsequently at 0.5, 1.0, 2.0, 4.0 and 6.0 hrs. post-feeding for the study of rumen microbes. The results indicated that total and viable bacterial count in rumen liquor declined immediately after feeding, followed by a rising trend and attained peak at 4 hr. post-feeding under all four dietary regimes. Population of total and viable bacteria was higher under diets I and II as compared to diets III and IV. Cellulolytic, proteolytic, amylolytic and lipolytic bacterial count also showed similar trend as that of viable count for all the diets. However, highest count of cellulytic bacteria was observed on diet III while proteolytic, amylolytic and lipolytic bacteria were maximum under diet II. Lowest bacterial number was found on diet IV. Total protozoal count, Holotrichs and Entodiniomorphs were significantly higher on diets I and II as compared to diets III and IV.

#61 EFFECT OF MONENSIN ON RUMEN FERMENTATION, FIBER DIGESTION, AND NUMBERS OF PROTAZOA AND FUNGI IN THE RUMEN FLUID OF SHEEP FED OATEN CHAFF DIET. G.Habib., NWFP. Agricultural University Peshawar, Pakistan.

The objective of the study was to examine the effect of including high levels of monensin in forage-based diets on rumen functions and feed intake. Twelve rumen fistulated wethers given a diet of oaten chaff, urea and mineral mixture were divided into three equal groups; A, B, and C on the basis of number of protozoa in the rumen fluid. Mean protozoal numbers were 3.9, 4.2 and 4.4 x 1Os/ml rumen fluid in groups A, B and C, respectively. The basal diet of groups A, B and C was supplemented with 0, 50 and 75 mg monensin (active ingredient 910 mg/g)/kg DM, respectively. The animals were individually fed *ad libitum* once a day for 16 weeks, preceded by an adaptation period of 2 weeks.

The results are summarized in Table 1. Total dry matter intake during weeks 1 to 10 reduced ($P<0.05$) from 911 g/d on control diet to 673 and 647 g/d with 50 and 75 ppm monensin, respectively. However, feed intake during weeks 11-16 remained the same on the three diets. The molar proportions of acetate and butyrate in the rumen fluid reduced ($P<0.001$) and that of propionate increased ($P<0.001$) with feeding of monensin. Total VFA concentrations, 3h after feeding, tended to be lower in monensin-fed sheep but the difference among the three treatments were statistically not significant. Monensin in 75 ppm concentration increased ($P<0.05$) the 48h in sacco DM disappearance of crystalline cellulose (cotton wool) but did not affect that of oaten chaff. Although the number of fungal zoospores colonies cultured in the rumen fluid, tended to decrease ($P=10$) in response to monensin, the proportion of mycelial type colonies, which are more fibrolytic, remained higher ($P=0.06$) in the rumen fluid of sheep receiving monensin.

Samples of rumen fluid collected 3h after feeding throughout the experiment, showed a consistent depression ($P<0.05$) in protozoal population due to inclusion of monensin in the diet. The protozoal numbers were not different in sheep fed either 50 or 75 ppm monensin. Protozoal population in all the animals was dominated by small entodinia (30-50 micro). Changing the feeding pattern from once a day to hourly did not affect the difference in protozoal numbers between control and monensin groups.The pH of rumen fluid did not change due to diets and ranged from 6.30 to 6.96. The kinetics of rurer. liquid also did not respond to monensin feeding.

These results show that on forage based diets, 75 ppm monensin may be helpful in increasing fiber digestibility and supplying a better balance of nutrients in animals due to increase in glucogenic VFA and microbial protein flow to intestine with lower protozoal numbers in the rumen.

Table 1. Effect of monensin on rumen parameter in sheep fed a basal diet of oaten chaff and urea-minerals.

Variable	Levels of Monensin (ppm) 0	50	75	Significance
In sacco DM digestibility(%)				
Oaten chaff	63.7	63.5	64.5	NS
Cotton wool	53.1	58.9	76.3	P<0.05
Zoospores colonies(x103/ml rumen fluid)				
Total No.	23.0	8.0	10.0	P=10
Mycelial type	2.5	5.0	8.0	P=0.06
Protozoal numbers (x105/ml rumen fluid)				
Total	6.7a	1.6b	1.5b	P<.05
Small Entodinium	6.1	1.6	1.5	P<0.05
Large Entodinium	0.53a	0.05b	0.04b	P<0.09
Continuous feeding	18.8a	7.5b	6.5b	P<0.01
Volatile Fatty Acids proportions				
Total Conc.(mmol/l)	84.1	81.8	79.9	NS
Acetate	70.0a	60. 9b	57 . 4c	p<0.001
Propionate	21.6a	32.6b	36.5C	P<0.001
Butyrate	6.4a	5.4b	4.5b	p<0.05
Others	0.7	0.7	0.6	NS
*G/E ratio	0.29a	0.42b	0.46b	P<0.05

Mean with in the rows with different superscripts are statistically different. * G/E ratio= Propionate + (Propionate + 0.6 acetate + 1.4 butyrate). NS= Non significant

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