PROGRAM

20th BIENNIAL

CONFERENCE ON RUMEN FUNCTION

CONGRESS HOTEL, CHICAGO, IL

November 7-9, 1989

PRE-CONFERENCE MIXER - CONGRESS HOTEL

(Lincoln Room)

Tuesday, November 7 (8:00 - 11:00 p.m.)

PODIUM PRESENTATIONS (Gold Room)

POSTER PRESENTATIONS (Francis I Room)

Wednesday and Thursday, November 8 & 9

Support for Mixer and for Coffee on Wednesday and Thursday Kindly Supplied by:

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MICROBIOLOGY PANEL - J. B. Russell, Panel Chairperson

1) 8:00 -- (Invited Paper): *Physiology of plant cell wall digestion by rumen bacteria.* C. W. Forsberg, J. Gong, L. Huang, M. McGavin, K. McDermaid, and A. Matte, University of Guelph, Ontario, Canada

2) 8:30 -- *Cloning of an endoglucanase gene from Ruminococcus flavefaciens.* B. A. White, G. T. Howard, S. Rosenzweig, and J. H. Clarke, University of Illinois, Urbana

3) 8:45 -- *Cloning and sequencing of a xylanase gene from Butyrivibrio fibrisolvens 49 and homologous DNA sequences in other strains of Butyrivibrio.* B. M. Mannarelli and S. Evans, USDA-ARS, Northern Regional Research Center, Peoria, IL

4) 9:00 -- *Outer membrane binding proteins are required for starch uptake by colonic Bacteroides.* K. L. Anderson, L. A. Bedzyk, and A. A. Salyers, University of Illinois, Urbana


6) 9:30 -- *Effects of phenylpropionic acid on cellulolysis by Ruminococcus albus in continuous culture.* M. Morrison, R. I. Mackie, and A. Kistner, University of Illinois, Urbana

7) 9:45 -- *The digestion of barley, maize and wheat by selected species of ruminal bacteria.* T. A. McAllister, L. M. Rode, K.-J. Cheng, C. W. Forsberg, and J. G. Buchanan-Smith, Agriculture Canada and University of Guelph, Canada

10:00 -- BREAK

8) 10:15 -- *Factors affecting lactate uptake by Selenomonas ruminantium HD4.* D. J. Nisbet and S. A. Martin, University of Georgia, Athens

9) 10:30 -- *Amino acid transport by a monensin-sensitive ammonia-producing ruminal bacterium.* G. Chen and J. B. Russell, Cornell University and USDA-ARS, Ithaca

10) 10:45 -- *Effect of monensin challenge on sodium and potassium concentrations in monensin-resistant and monensin-sensitive strains of Bacteroides ruminicola.* M. C. Morehead and K. A. Dawson, University of Kentucky, Lexington
11) 11:00 -- Anaerobic fungi are not always eliminated from the rumen by short-term treatment with monensin. G. Gordon and M. Phillips, CSIRO, Blacktown, NSW, Australia

12) 11:15 -- Resistant sporangia in anaerobic fungi allow survival outside rumen. D. Wubah, M. S. Fuller, and D. E. Akin, University of Georgia and USDA-ARS, Athens

13) 11:30 -- Effect of defaunation on gastrointestinal peptide hormones in sheep. P. P. Frumholtz, R. J. Wallace, and E. R. Orskov, Rowett Research Institute, Scotland

14) 11:45 -- Post-prandial pH moderation by ruminal ciliated protozoa in cattle fed a high-grain diet. T. G. Nagaraja, G. Towne, and A. A. Beharka, Kansas State University, Manhattan

15) 12:00 -- Association of a temperate bacteriophage with a ruminal cellulytic anaerobe resembling Ruminicoccus albus T. Tadesc and M. T. Yokoyama, Michigan State University, East Lansing

12:15 -- LUNCH

AGRONOMY PANEL - J. C. Burns, Panel Chairperson


17) 2:00 -- Re-evaluation of lignin's role in forage fiber digestibility. H. G. Jung and K. P. Vogel, USDA-ARS, St. Paul, MN and Lincoln, NE


2:30 -- BREAK

PHYSIOPATHOLOGY PANEL - R. H. Dunlop, Panel Chairperson

19) 2:45 -- (Invited Paper) The role of ruminants in the future. L. P. Milligan, J. Kelly, D. Taylor, and A. Vaage, Dept. of Animal and Poultry Science, University of Guelph, Ontario, Canada

20) 3:15 -- The effects of pilocarpine on ruminal and digestive characteristics of beef steers fed on a high grain diet. J. Peters, The Upjohn Company, Kalamazoo, MI

21) 3:30 -- The effects of ruminal lactic acidosis on blood $K^+$ levels of calves. E. C. Crichlow, J. S. Kim, and M. D. McMullen, Dept. Vet. Physiol. Sci., University of Saskatchewan, Saskatoon, Saskatchewan, Canada
Physiopathology panel (cont'd.)

22) 3:45 -- *Polioencephalomalacia (PEM) of calves associated with elevated rumen sulfide concentrations.* D. H. Gould, M. M. McAllister, J. C. Savage, and D. W. Hamar, Department of Pathology, Colorado State University, Fort Collins

23) 4:00 -- *Diet-related response to parathyroid hormone (PTH) in Blue Duiker antelope.* B. L. Roeder, R. F. Wideman, G. A. Varga, B. W. Hollis, and R. M. Leach, Depts. Veterinary Science, Poultry Science, Dairy and Animal Science, Penn State University, University Park, PA, and Medical University of South Carolina, Charleston

24) 4:15 -- *Toxic effect of oak tannin extract compared in sheep and goats.* H. Narjisse, M. El Honsali, and J. D. Olsen, Institute of Agronomy and Veterinary Medicine, Hassan II, Rabat, Morocco, and USDA-ARS Poisonous Plants Research Laboratory, Logan, UT


4:45 -- BUSINESS MEETING

5:00-6:00 -- *Posters in Francis I Room. Presenters should attend their own posters during this interval.*

Thursday, November 9
(Gold Room)

Nutrition panel -- J. T. Huber, Chairperson

26) 8:00 -- (Invited Paper): *Effective fiber and its role in rumen function and productivity of the dairy cow.* K. A. Beauchemin and J. G. Buchanan-Smith, Agriculture Canada, Lethbridge, Alberta, and University of Guelph, Ontario

27) 8:30 -- *Effect of specific gravity of alfalfa hay and silage on rumen stratification.* M. A. Wattiaux, L. D. Satter, and D. R. Mertens, University of Wisconsin, USDA-ARS, U.S. Forage Research Center, Madison

28) 8:45 -- *Effect of added inert rumen bulk and feeding polyethylene glycol (PEG) on intake, digestibility and rumen kinetics in the early lactation dairy cow.* T. R. Johnson and D. K. Combs, Department of Dairy Science, University of Wisconsin, Madison
NUTRITION PANEL (cont’d)

29) 9:00 -- *Sluicing through the rumen.* J. D. Garza, J. Zorrilla-Rios, and F. Owens, Department of Animal Science, Oklahoma State University, Stillwater

30) 9:15 -- *A compartmental model to describe ruminal in situ digestion.* J. van Milgen, M. R. Murphy, and L. L. Berger, Department of Animal Sciences, University of Illinois, Urbana-Champaign

31) 9:30 -- *Development of an isolated rumen epithelial cell incubation system.* R. L. Baldwin, VI, and B. W. Jesse, Department of Animal Sciences, Rutgers, The State University, New Brunswick


10:00 -- **BREAK**

33) 10:15 -- *Impact of type and level of protein or energy supplementation on in vitro digestibility of kikuyu (Pennisetum clandestinum) and pangola (Digitaria decumbens) grasses.* J. R. Carpenter, S. Y. Iha, and R. Y. Niino-Duponte, Department of Animal Science, University of Hawaii, Manoa, Honolulu

34) 10:30 -- *Microbial fermentation and site of nutrient digestion in steers fed diets varying in forage and energy source.* L. Kung, Jr., R. S. Tung, and B. R. Carmean, Department of Animal Science, University of Delaware, Newark

35) 10:45 -- *Total starch and relative starch availability of feed grains.* M. H. Poore, T. P. Eck, R. S. Swingle, and C. B. Theurer, Department of Animal Science, University of Arizona, Tucson

36) 11:00 -- *Acid or formaldehyde treatment of alfalfa silage for milk production.* S. A. Nagel and G. A. Broderick, Department of Dairy Science, and USDA-ARS, University of Wisconsin, Madison

37) 11:15 -- *Reproductive parameters of dairy cows fed urea during early lactation.* D. P. Casper, C. L. Austin, and D. J. Schingoethe, Department of Dairy Science, South Dakota State University, Brookings

38) 11:30 -- *Rumen cation and methane responses to diet additions of Na, K, and/or lasalocid.* D. E. Johnson, H. P. Phetteplace, and W. V. Rumpler, Department of Animal Science, Colorado State University, Fort Collins

39) 11:45 -- *Impact of NaCl intake on rumen digesta kinetics.* J. Zorrilla-Rios, J. D. Garza, and F. Owens, Department of Animal Science, Oklahoma State University, Stillwater
**POSTERS**
*(Francis I Room)*

**MICROBIOLOGY PANEL**

40) **Cellulose digestion and cellulase regulation and distribution in *Fibrobacter succinogenes* S45.** L. Huang and C. W. Forsberg, University of Guelph, Ontario, Canada

41) **β-Galactosidase activity of *Fibrobacter succinogenes* S85.** P. Javorsky, S. F. Lee, A. M. Gibbins, and C. W. Forsberg, University of Guelph, Ontario, Canada

42) **Heterologous expression of genes for xylanolytic enzymes from *Bacteroides* species in *Bacteroides fragilis* and *Escherichia coli*.** T. R. Whitehead and R. B. Hespell, USDA-ARS, Northern Regional Research Center, Peoria, IL

43) **The origin and properties of forms of *Ruminococcus flavefaciens* strains 007 which differ in their ability to degrade cotton fibres.** C. S. Stewart, S. H. Duncan, and H. J. Flint, Rowett Research Institute, Scotland

44) **Interaction of ruminal bacteria in the production and utilization of dextrins from soluble starch.** M. A. Cotta, USDA-ARS, Northern Regional Research Center, Peoria, IL

45) **Degradation of barley straw, ryegrass and alfalfa cell walls by *Clostridium longisporum* and *Ruminococcus albus*.** V. H. Vare 1, A. J. Richardson, and C. S. Stewart, USDA-ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE, and Rowett Research Institute, Scotland

46) **Evidence of inhibition of cellulolysis in an anaerobic rumen fungus by glucose, cellobiose, and soluble starch.** M. Morrison and R. I. Mackie, University of Illinois, Urbana

47) **Degradation of wheat straw and alkaline hydrogen peroxide treated wheat straw by *Ruminococcus flavefaciens* and *Ruminococcus albus*.** A. A. Odenyo, R. I. Mackie, and B. A. White, University of Illinois, Urbana

48) **Stimulated cellulose degradation in co-cultures containing yeast and cellulolytic rumen bacteria.** K. A. Dawson, G. A. Harrison, K. E. Newman, and S. Jenkins, University of Kentucky, Lexington

49) **Cell surface structures of ruminal cellulolytic bacteria.** J. Miron, M. T. Yokoyama, and R. Lamed, Institute of Animal Science, The Volcani Center, Israel, Michigan State University, East Lansing, and Tel-Aviv University, Israel

50) **Effects of microminerals on the growth characteristics of cellulolytic ruminal bacteria.** D. C. Sangwan, R. I. Mackie, and B. A. White, University of Illinois, Urbana
MICROBIOLOGY PANEL (cont'd.)

51) ATPase-dependent energy spilling by the ruminal bacterium Streptococcus bovis. J. B. Russell and H. J. Strobel, USDA-ARS and Cornell University, Ithaca, NY

52) The interaction between pH and ionophores on continuous cultures of Streptococcus bovis. J. M. Chow and J. B. Russell, Cornell University and USDA-ARS, Ithaca, NY

53) In vitro and in vivo models of acute acidosis induced by different diets. K. Barsuhn, S. T. Chester, K. A. White, J. A. Robinson, and S. F. Kotarski, The Upjohn Company, Kalamazoo, MI

54) Selenomonas ruminantium HD4 fermentation and cell yield response to limiting and non-limiting concentrations of ammonium chloride. S. C. Ricke and D. M. Schaefer, USDA-ARS and University of Wisconsin, Madison

55) Activities of ammonia-assimilatory enzymes of Ruminococcus flavefaciens FD1. P. A. Duncan and R. I. Mackie, University of Illinois, Urbana

56) Transformation systems of plasmid constructions for use in Bacteroides ruminicola. A. M. Thomson and H. J. Flint, Rowett Research Institute, Scotland

57) Isolation and regulation of genes concerned with xylan utilisation in Ruminococcus flavefaciens. H. J. Flint and C. A. McPherson, Rowett Research Institute, Scotland

58) More cloning of endoglucanase genes from Ruminococcus flavefaciens. V. K. Gupta, G. T. Howard, S. Rosenzweig, and B. A. White, University of Illinois, Urbana

59) Electroporation of Ruminococcus flavefaciens for transfer of DNA. M. Morrison and B. A. White, University of Illinois, Urbana

60) Restriction/modification systems in Ruminococcus albus 8 and Ruminococcus flavefaciens FD-1. M. Morrison and B. A. White, University of Illinois, Urbana

61) Growth characteristics of dihydroxypyridine-degrading bacteria (isolates 32-24). C. S. McSweeney, R. I. Mackie, and M. Morrison, University of Illinois, Urbana

AGRONOMY PANEL

62) Chlorogenic acid and its influence on neutral detergent fiber digestion. D. J. R. Cheney, J. A. Patterson, and J. H. Cherney, Depts. of Animal Science and Agronomy, Purdue University, West Lafayette, IN
**PHYSIOPATHOLOGY PANEL**

63) *Ruminal anaerobes and pyrrolizidine alkaloid detoxification.* A. M. Craig, L. L. Blythe, and E. D. Lassen, College of Veterinary Medicine, Oregon State University, Corvallis


**NUTRITION PANEL**

65) *Evaluation of a rapid enzyme/detergent procedure to quantify bacterial crude protein (CP) in digestive residues of forage-fed ruminants.* K. A. Mowell and L. D. Bunting, Dept. of Dairy Science, Louisiana State University, Baton Rouge


68) *Inadequacy of xylose as a rumen escape marker.* J. Zorrila-Rios, J. D. Garza, and F. Owens, Dept. of Animal Science, Oklahoma State University, Stillwater


70) *In vitro synthesis and biohydrogenation of long-chain fatty acids in diets containing megalac or animal-vegetable blend.* Zhiguo Wu and D. L. Palmquist, Dept. Dairy Science, OARDC, The Ohio State University, Wooster

71) *Microbial CP association with and NDF digestibility of untreated and ammoniated Bermudagrass hay.* D. B. Vagnoni, W. M. Craig, and R. N. Gates, Louisiana State University Agricultural Center, Baton Rouge


NUTRITION PANEL (cont’d)

74) Influence of roughage source on apparent extent of ruminal digestion of starch in 65 and 90% concentrate diets for steers.  J. R. Barcena-Gama, R. S. Swingle, M. H. Poore, and J. A. Moore, Dept. of Animal Science, University of Arizona, Tucson

75) Influence of level of feed intake on characteristics of digestion of dry-rolled versus steam-flaked corn based finishing diets.  R. A. Zinn and M. K. Song, Dept. of Animal Science, University of California, El Centro

76) Cumulative effects of dietary concentrate level on site and extent of forage fiber digestion in lambs.  D. W. Kennedy and L. D. Bunting, Dept. of Dairy Science, Louisiana State University Agriculture Center, Baton Rouge

77) The significance of chewing during eating and rumination on forage digestion in cattle.  Y. Dong, A. S. Vaage, C. Campbell, and J. G. Buchanan-Smith, Dept. of Animal and Poultry Science, University of Guelph, Ontario, Canada


80) The flow rate of non-ammonia N and α-amino N at abomasum in cross-bred calves fed urea-treated straw supplemented with by-pass protein.  V. Kumar and T. K. Walli, National Dairy Research Institute, Indian Council of Agricultural Research, Karnal, 132001 India

PANEL CHAIRPERSONS:

AGRONOMY  J. C. Burns
MICROBIOLOGY  J. B. Russell
NUTRITION  J. T. Huber
PHYSIOPATHOLOGY  R. H. Dunlop

STEERING COMMITTEE:

R. D. Hatfield
M. T. Yokoyama
F. N. Owens
R. H. Dunlop

Chairperson -- M. J. Allison
Treasurer -- J. R. Russell
Arrangements -- S. F. Kotarski
Abstracts
Conference on Rumen Function
Volume 20, 1989

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R. H. Dunlop

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61) **Growth characteristics of dihydroxypyridine-degrading bacteria (isolates 32-24)**. C. S. McSweeney, R. I. Mackie, and M. Morrison, University of Illinois, Urbana

AGRONOMY PANEL

62) **Chlorogenic acid and its influence on neutral detergent fiber digestion**. D. J. R. Cheney, J. A. Patterson, and J. H. Cherney, Depts. of Animal Science and Agronomy, Purdue University, West Lafayette, IN
### PHYSIOPATHOLOGY PANEL

63) **Ruminal anaerobes and pyrrolizidine alkaloid detoxification.** A. M. Craig, L. L. Blythe, and E. D. Lassen, College of Veterinary Medicine, Oregon State University, Corvallis


### NUTRITION PANEL

65) **Evaluation of a rapid enzyme/detergent procedure to quantify bacterial crude protein (CP) in digestive residues of forage-fed ruminants.** K. A. Mowell and L. D. Bunting, Dept. of Dairy Science, Louisiana State University, Baton Rouge

66) **Effects of energy level, feeding frequency and bacterial isolation techniques on bacterial composition and flow at the duodenum of steers.** M. J. Cecava, N. R. Merchen, L. L. Berger, and G. C. Fahey, Jr., Dept. of Animal Sciences, University of Illinois, Urbana

67) **Influence of protein degradation and diet type on fermentation in a continuous culture system.** D. J. Illg, M. D. Stern, H. R. Mansfield, and B. A. Crooker, Dept. of Animal Science, University of Minnesota, St. Paul

68) **Inadequacy of xylose as a rumen escape marker.** J. Zorrila-Rios, J. D. Garza, and F. Owens, Dept. of Animal Science, Oklahoma State University, Stillwater

69) **Effects of Cr concentration and particle size of mordanted fibers on kinetic passage and fecal output estimates.** J. R. Russell, A. M. Beck, and M. R. Brasche, Dept. of Animal Science, Iowa State University, Ames

70) **In vitro synthesis and biohydrogenation of long-chain fatty acids in diets containing megalac or animal-vegetable blend.** Zhiguo Wu and D. L. Palmquist, Dept. Dairy Science, OARDC, The Ohio State University, Wooster

71) **Microbial CP association with and NDF digestibility of untreated and ammoniated Bermudagrass hay.** D. B. Vagnoni, W. M. Craig, and R. N. Gates, Louisiana State University Agricultural Center, Baton Rouge

72) **Effect of enzyme and inoculant additives on nutrient utilization of a hay-crop silage during continuous culture.** C. A. Varga, K. Karunananda, and M. R. Stokes, Dept. Dairy & Animal Science, and Agronomy, Penn State University, University Park, and Dept. Animal and Veterinary Science, University of Maine, Orono

74) Influence of roughage source on apparent extent of ruminal digestion of starch in 65 and 90% concentrate diets for steers. J. R. Barcena-Gama, R. S. Swingle, M. H. Poore, and J. A. Moore, Dept. of Animal Science, University of Arizona, Tucson

75) Influence of level of feed intake on characteristics of digestion of dry-rolled versus steam-flaked corn based finishing diets. R. A. Zinn and M. K. Song, Dept. of Animal Science, University of California, El Centro

76) Cumulative effects of dietary concentrate level on site and extent of forage fiber digestion in lambs. D. W. Kennedy and L. D. Bunting, Dept. of Dairy Science, Louisiana State University Agriculture Center, Baton Rouge

77) The significance of chewing during eating and rumination on forage digestion in cattle. Y. Dong, A. S. Vaage, C. Campbell, and J. G. Buchanan-Smith, Dept. of Animal and Poultry Science, University of Guelph, Ontario, Canada


80) The flow rate of non-ammonia N and α-amino N at abomasum in cross-bred calves fed urea-treated straw supplemented with by-pass protein. V. Kumar and T. K. Walli, National Dairy Research Institute, Indian Council of Agricultural Research, Karnal, 132001 India

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PHYSIOLOGY OF PLANT CELL WALL DIGESTION BY RUMEN BACTERIA


Plant cell walls are a mesh of cross-linked polymers consisting primarily of cellulose and hemicellulose. Digestion of these polysaccharides by ruminal microbes is essential as a source of energy for the host animal. The identification of key structures and enzymes involved in this process is crucial for understanding the mechanism of cellulosic fermentation by ruminants.

Cryotans had been determined by x-ray diffraction, and to crystalline form by optical microscopy. The regulation of synthesis and secretion of these enzymes in E. coli has been partially characterized by growth experiments. Immunological analysis of the separate cellulase enzymes during growth of cells, and from the DNA sequence of the cell genes which codes for endoglucanase J.

CLONING AND SEQUENCING OF A XYLANASE GENE FROM BUTYRIVIBrio FIBRISOLVENS STRAIN 49 AND HOMOLOGOUS DNA SEQUENCES IN OTHER STRAINS OF BUTYRIVIBRIO. B. M. Marzorati and S. Evans. ARS, USDA, Peoria, IL 61604.

A gene coding for xylanase activity, xyIA, from the anaerobic rumen bacterium Butyribriobio fibrisolvens strain 49 was cloned into Escherichia coli JM103 using plasmid pUC19. The gene was located on a 2.3 kb DNA insert composed of two adjacent EcoRI fragments of 1.65 and 0.65 kb. The expression of xylanase activity required parts of both EcoRI segments. In E. coli the cloned xylanase enzyme remained cell-associated. The enzyme exhibited no endo- or exo-xylanase activity. The isoelectric point of the cloned protein was approximately 9.8, and optimum xylanase activity was achieved at pH 5.4. The nucleotide sequence of the 1.8 kb insert was determined. The cloned xylanase gene hybridized strongly to chromosomal sequences in only two of five closely related Butyribriobio strains; the gene appeared to be absent in the other three.


The kinetics of in vitro digestion of various pure celluloses by mixed rumen microflora were examined following extraction of residual cellulose with a modified detergent procedure. All digestions in first order rate equations that incorporated discrete lag times. Kinetic parameters were compared to relate crystallinities determined by x-ray diffraction, and to estimated specific surface area (GSSA) of the different types of celluloses. GSSA was determined by optical microscopy and was based on smooth cylindrical or square cross-sections of the particles. For celluloses having similar crystallinities and simple non-aggregating particle morphologies, the fermentation rate constants displayed strong positive correlation (r=0.98) with GSSA; lag times displayed a strong negative correlation (r=-0.93) with GSSA. Crystallinity had less effect on digestion kinetics. Swelling of microcrystalline cellulose with 72-77% phosphoric acid yielded substrates which were slightly more rapidly fermented. Treatment with other types of alkali converts cellulose in a less crystalline materials which were nevertheless fermented more slowly, apparently due to partial conversion of the crystalline lattice from the type I to the type II allomorph. The dependence of rate on GSSA supports proposals that the cellulolytic enzymes are probably more efficient in binding to the substrates rather than extracellular. The decreased rate of digestion of samples containing some cellulose I suggests that ruminal organisms do not rapidly adapt to utilization of different cellulose forms.


An endoglucanase gene celB was isolated from genomic library of Ruminococcus flavescentis PD-1 DNA constructed using the EcoR1 restriction enzyme. Recombinant plasmid pdB1 was identified using plasmid library screening. The recombinant phage probe was screened for cellulosomal activity by placing on E. coli in soft agar overlay containing 1% hardwood cellulose. pdB1 was the only plasmid containing active endoglucanase ( Orr-HEC). An Orr-HEC positive recombinant phage designated pdF1 was purified and the plasmid was excised from recombinant phage using RNA helicase and cDNA in E. coli XL-1 Blue. The plasmid designated pdA100 harbored 2 EcoRI fragments of 2.65 and 3.7 kb from R. flavescentis in the E. coli vector phir. Southern blot analysis of the labeled probe hybridized with R. flavescentis PD-1 DNA confirming the origin of the insert. The probe did not hybridize with other endauthaneous starch-degrading enzymes. The encodr of the cloned protein was determined and the DNA sequence of the gene encoded by this clone was as well as an extensive restriction map was presented.

OUTER MEMBRANE BINDING PROTEINS ARE REQUIRED FOR STARCH UPTAKE BY COLOCAL BACTERIOPHASES. R. L. Anderson, L. A. Bedey, and A. A. Salyers. A. Microbiology, University of Illinois, Urbana, IL 61801.

Many colonic and ruminal Bacteroides readily utilize starch, however the genetic manipulability of the colonic strain. B. thetaiotaomicron, enables a more detailed study of the starch-degradation mechanisms. Starch-degrading enzymes in B. thetaiotaomicron are apparently cell-associated and not excreted as extracellular enzymes. Instead, enzymatic degradation of starch depends on transporting the molecule into the periplasmic space. This involves binding starch to the outer cell surface by binding sites composed of a protein or protein complex. Transposon-generated mutants of B. thetaiotaomicron retained normal levels of enzymatic activity, yet were unable to grow on starch. These mutants were found to be deficient in starch binding. This is consistent with the idea that cell surface starch binding is required for starch utilization probably as a prelude to transporting the starch molecule through the outer membrane. Additional studies demonstrated that binding sites for starch are present on at least two components; one with a high affinity for large glucose oligomers (Q8), the second with a high affinity for maltodextrins (Q4-Q7). The mutations in these mutants appear to be within a 20 kb region on the chromosome, while mutations in other starch binding mutants are apparently outside of this region. This suggests that the starch utilization system involves several genes in several operons.

EFFECT OF PHENYLPROPIONIC ACID ON CELLOLITISY BY RUMINOCOCUS ALBIS IN CONTINUOUS CULTURES. M. Workton, R. I. Mockle and A. Kistler. Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801.

The likelihood that phenylpropionic acid (PPA) could stimulate cellulolysins in strains of Ruminococcus albis other than those already reported, was confirmed in preliminary studies in which three South African strains were grown on [U-14C]-cellulose. One strain, R. albis C645, was grown in a continuous culture apparatus designed for use with solid substrates such as pressed milled cellulose. Measurements were made once bacterial populations had reached steady state at a dilution rate of 0.17/hr. The extent of cellulose hydrolysis was 41.1, 35.7, 90.2 and 86.9% and volumetric solubilization rate was 103.0, 97.9, 215.3 and 270.4 mg/l/hr for control, PAA, PPA and PPA plus PAA growth conditions, respectively. The present data clearly revealed that PAA had a significant stimulatory effect upon the total number of adherent cells. However, the relative proportion of adherent to non-adherent cells remains to be determined. The results were indicative of an effect from PAA not only in the cellulosic but also in the population density of cellulolytic microorganisms. Further studies of the physiology of PAA utilization are warranted in the future to understand and improve the existing constraints to fiber degradation.
Guelph, Ontario.


Differences in the digestion of barley (B), maize (M) and wheat (W) by three major ruminal starch-digesting bacterial species were characterized. Streptococcus bovis 26 (SB), Ruminococcus albus strain 50 (RA) and Ruminococcus bromii strain 5A (RB) were incubated in quadruplicate via tubes containing artificial media. The digestion of barley, maize, and wheat by selected species of bacteria was measured.

The digestion of barley, maize, and wheat by selected species of bacteria was measured. The digestion of barley, maize, and wheat by selected species of bacteria was measured. The digestion of barley, maize, and wheat by selected species of bacteria was measured. The digestion of barley, maize, and wheat by selected species of bacteria was measured.
EFFECT OF DEFACEMENT ON GASTROINTESTINAL PEPTIDE HORMONES IN SHEEP.

P.F. Frumholz, R.J. Wallace and E.R. Orskov,
Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB, U.K.

The absence of ciliate protozoa has been shown to increase the blood concentrations of insulin and gastrin in steers (Itabashi 1983). A similar study was carried out to investigate the relationship between faunation of the rumen and gut hormone secretion in sheep. Four faunated and four defaunated castrated male sheep received 700 g of general purpose diet twice daily. Defaunation resulted in increased protein concentration in rumen fluid, and caused ruminal peptide concentrations to remain high for a longer period of feeding. There was no significant difference between the two treatments in secretion of the three hormones measured (gastrin, insulin, and cholecystokinin CCK-8). These observations suggest that there may be implications for the use of coccidicides in sheep to defaunation, or that defaunation may stimulate secretion of forms of gastrin other than G17.


In studies to determine the relationship between bacteria and the major ruminal cellulolytic bacteria, we have identified a temperate phage from ruminal fluid which associates with an isolated ruminal cellulolytic bacteria resembling Ruminococcus albus. The phage produces plaques on an agar lawn of the bacteria which turned turbid with increase in incubation time. Inoculation of the phage into growing cultures of the cellulolytic bacteria, resulted in significant lysis of cells by 5 h after infection, with the release of numerous phage particles (1.8 x 10^6 pfu/ml) in the culture. With longer incubation, plaques were formed and cultures grew to the preinfection optical density level. The phage has an isometric head (50 nm) and a non-contractile, flexible tail (120 nm). Phages could be induced by mitomycin C (5 μg/ml) and UV light (40-60 sec), releasing incomplete phage particles and intact phages (10^11). Spontaneous induction was also evident. Lysates of Butyviridibacter fibrisolvens D1 and 49 were sensitive to lysis of the cellulolytic bacteria, resulting in plaques containing numerous filamentous phage particles. These results suggest that the cellulolytic isolate may harbor two bacteriophages, one of which is also infectious for the rumen and gut hormone secretion.

Lignification of cell walls is the major factor lowering digestibility of forage stems as they mature. Chemical composition may determine the inhibitory potential of lignin because lignin with a small nitrobenzene oxidation products: lignin ratio and small p-coumaric acid concentration seems to be less inhibitory. In temperate species, lignin from grass stems is about 10% more inhibitory than lignin from legumes. Cell-wall carbohydrate composition in lignified stems has limited influence on cell-wall digestibility, suggesting that lignin protects the cell wall as a whole rather than selectively protecting specific carbohydrates and/or that many cell-wall carbohydrates are protected by tightly linked hydrogen bonding. In leaves with little lignin, chemical composition of cell-wall carbohydrates may exert more influence. Digestibility of glucose and xylose is much higher in leaves than in immature stems of grasses and legumes. Xylose has the poorest digestibility of neutral sugars in stems and leaves, and is particularly low in legumes. Up to 2/4 of xylose may be digestible in leaves, compared 1/3 to 1/2 of glucose and 1/3 to 1/4 in legume stems. A better understanding of these limitations will help develop cultivars with improved digestibility.

RE-EVALUATION OF LIGNIN'S ROLE IN FORAGE FIBER DIGESTIBILITY.
H. G. Jung* and K. P. Vogel, USDA-Agricultural Research Service, St. Paul, MN 55108 and Lincoln, NE 68583

Correlation of fiber digestibility with lignification was examined in switchgrass (5 cultivars) and big bluestem (4 cultivars) collected 20 June, 21 July and 12 August 1983 from 3 field replicates. Concentration and composition of core and non-core lignins were measured. In vitro 48 h neutral detergent fiber (NDF) digestibility was determined. Lignin concentration and composition, and total and non-core fractions, were measured (P<0.05) between species for both leaf and stem tissues, and cutting date. Leaf NDF digestibility was different (P<0.05) among some cultivars, but species were not different. Stem NDF digestibility was different (P<0.05) between species, but differences varied by cutting. Digestibility of NDF was negatively correlated (P<0.05) with core lignin concentration across cuttings and species. Leaf NDF digestibility was not correlated with core lignin within cuts, while stem NDF digestibility was highly correlated with core lignin concentration (P<0.10) for all cuttings. Within species and cutting, core lignin concentration was rarely correlated (P<0.15) with NDF digestibility. Composition of core and non-core lignins was correlated (P<0.15) with NDF digestibility, and NDF digestibility was not correlated with composition of core and non-core lignins. Data suggest concentration of core lignin is not the dominant chemical characteristic of forage cell walls limiting fiber digestibility.

POST-FRANIAL PH MODERATION BY RUMINAL CILIATED PROTOZOA IN CATTLE FED A HIGH-GRAIN DIET.
T.G. Naipal*, Gene Towne, and A.A. Behnke, Dep't of Animal Sci., Kansas State University, Manhattan, KS 66506

Six ruminally-cannulated steers fed a corn-based grain diet (80% grain + 20% alfalfa hay) at 12 h intervals were assigned randomly to one of two treatment groups: Defaunated or Faunated in a crossover design. Defaunation was by ruminal emptying, omasal flushing and treatment with sodium sulfonate. Two weeks following defaunation, ruminal samples were collected before and at 1, 2, 4, 6, 8, and 12 h after feeding to measure pH, analyze fermentation products and monitor changes in lactic acid-producing and fermenting bacterial groups. Faunated steers had an average protozoan population of 426,000 g/ml comprised of nine genera. Defaunated cattle had lower ruminal pH (P<0.01) than faunated cattle, but there was no treatment x time interaction. Ruminal lactate and NH3-N concentrations were similar in both groups. Total VFA concentration had treatments by time interaction (P<0.05) and was higher in defaunated than faunated steers. Ruminal propionate proportion was higher in defaunated (P<0.01) than faunated steers but butyrate proportion was unaffected by the treatment. Viable anaerobic bacterial counts were higher (P<0.05) in defaunated than faunated steers. Although, lactic acid producers were higher and lactic acid-decomposers were lower in defaunated than faunated steers, the differences were not significant (P>0.05). It appears that postprandial pH moderation by ruminal ciliated protozoa was because of reduced bacterial activity.

CELL-WALL CARBOHYDRATE DIGESTIBILITY IN Ruminants: PLANT IMPOSED LIMITS. D. E. Buxton, A. Res. Serv., USDA; Dep't of Agronomy. Iowa State University, Ames, IA 50011

Lignification of cell walls is the major factor lowering digestibility of forage stems as they mature. Chemical composition may determine the inhibitory potential of lignin because lignin with a small nitrobenzene oxidation products: lignin ratio and small p-coumaric acid concentration seems to be less inhibitory. In temperate species, lignin from grass stems is about 10% more inhibitory than lignin from legumes. Cell-wall carbohydrate composition in lignified stems has limited influence on cell-wall digestibility, suggesting that lignin protects the cell wall as a whole rather than selectively protecting specific carbohydrates and/or that many cell-wall carbohydrates are protected by tightly linked hydrogen bonding. In leaves with little lignin, chemical composition of cell-wall carbohydrates may exert more influence. Digestibility of glucose and xylose is much higher in leaves than in immature stems of grasses and legumes. Xylose has the poorest digestibility of neutral sugars in stems and leaves, and is particularly low in legumes. Up to 2/4 of xylose may be digestible in leaves, compared 1/3 to 1/2 of glucose and 1/3 to 1/4 in legume stems. A better understanding of these limitations will help develop cultivars with improved digestibility.


The reliability of the Captec controlled-release capsule containing chronic dosage capsule was evaluated in two trials. Eight ruminally cannulated steers fed either alfalfa hay or General Bermudagrass hay (CBH) ad libitum or fed a pelleted commercial sheep diet at 2.5X (P>0.5) or 1.5X (P>0.5) of body weight were dosed orally with a Captec capsule. Release rate of chromium was determined by recovering capsules from the rumen and measuring remaining chromium every 3 days. Release rates were fastest for steers fed CH and slowest for steers fed CBH with high animal variation regardless of diet. Since each steer received only one capsule, differences should not be attributed to animal or capsule. In a second trial, nine steers fed either AD, P1.5 or P2.5 were dosed with four capsules each. Release rates of chromium oxide among capsules dosed to the same animal were not different, but release rates of chromium oxide among animals on the same diet were different (P<0.05). Release rates were higher (P<0.01) for AD (4.9%) compared to both diets of pellets, which were similar (P>0.47; P>0.761). Because of the high animal-animal variation and possible variation due to diet, Captec capsules should not be utilized to estimate fecal output of individual animals without further correction.


The parasympathetic nervous system is materially involved in the regulation of digestive secretions. The effect of pilocarpine HCl, a partial muscarinic agonist, was evaluated in growing beef steers (n = 8). Oral doses of 0, 2, or 4 mg/kg BW were administered daily immediately prior to offering an 88% grain:12% hay diet in a 3 period cross-over design. Digestive or performance characteristics were not improved (differences of DM, OM, and N retention, gain and feed efficiency). Although voluntary feed intakes did not differ, there was a dose-dependent slowing of feed consumption rates. Early post-feeding and cumulative liquid volume and fractional dilution rate were not affected. Digestive improvements reported to occur due to pilocarpine in mature, non-growing cattle consuming a high forage diet were not observed in growing steers fed a production type diet.


A consensus on the changes in blood K+ levels induced by ruminal lactic acidosis is, as yet, unavailable. In view of this, calves fed a low fiber, semipurified diet with added sulfate commonly developed signs and lesions of PEM. At no time was there a significant decrease in the concentration of cholin in rumen fluid or blood. Addition of choline to the diet had no demonstrable effect upon blood transketolase activity. The odor of H2S in eructated rumen gas was associated with nasal discharge and transient elevations of respiratory rate. These episodes preceded the onset of PEM. Sulfide concentrations in rumen fluid were measured sequentially by pre-column derivatization, reverse phase, ion-pair HPLC and spectrophotometric detection (660 nm) of the derived compound. Sulfide concentrations progressively increased in calves after initiation of the PEM-inducing diet. When neurological signs of PEM occurred the concentration of sulfide in the rumen fluid was 7-12X the concentration of sulfide in samples taken prior to feeding the PEM-inducing diet (controls, 52.2±10.3 uM sulfide, n=6; PEM-aflicted, 515±131 uM, p<0.05). It is hypothesized that PEM can result from increased production of sulfide in the rumen and is a form of subacute H2S neurotoxicity. (Supported by USDA/CSRS, 87-CSR-2-1208)


Herd profiles of Blue duiker antelope (Cephalophus monticolabicolor) housed at PSU revealed hyperphosphatemia, mild to moderate hypocalcemia, hyperkalemia, and metabolic acidosis in ~90% of the animals. Pelleted diet composition was 21.3% CP, 12.7% ADF, 24.8% NDF, 1.12% Ca, 0.59% P, 1.59% K, 0.56% Na, 0.94% CI, and 0.29% S on an 89.2% DM basis. Sixteen young and older cattle fed experimental diets similarly formulated but adjusted to 4 levels of Ca and P (0.5%, 1%, 1.5%, and 2% P, 0.8%, 0.8%, 0.8%, 1.2% P, 1.2% P, 1.2% P) for 4 months. Post-p and post-prandial baseline plasma vitamin D, serum calcium (Ca), inorganic phosphorus (P), and creatinine clearance rates were increased in response to PTH. Both groups decreased Ca and P, preceded by a rebound in P 60 min later. Post-trial, Young had higher mean P, and 25(OH)D levels, and lower Ca, Ca, P, and 1,25(OH)2 D values than older . In response to PTH, both groups decreased Ca and P, preceded by a rebound in P 60 min later. Post-trial, Young had higher mean Ca and P, similar 25(OH)D and significantly lower 1,25(OH)2 D values than older . PTH response in both groups resulted in a rise in Ca, and decrease followed by a rebound in P . Young had lower Ca, and higher response post-PTH. These findings indicated that unlike domestic ruminants, dietary Ca and P does significantly affect duikers’ response to PTH.


PEM in ruminants has often been associated with altered cholin metabolism. In this study, calves fed a low fiber, semipurified diet with added sulfate commonly developed signs and lesions of PEM. At no time was there a significant decrease in the concentration of cholin in rumen fluid or blood. Addition of choline to the diet had no demonstrable effect upon blood transketolase activity. The odor of H2S in eructated rumen gas was associated with nasal discharge and transient elevations of respiratory rate. These episodes preceded the onset of PEM. Sulfide concentrations in rumen fluid were measured sequentially by pre-column derivatization, reverse phase, ion-pair HPLC and spectrophotometric detection (660 nm) of the derived compound. Sulfide concentrations progressively increased in calves after initiation of the PEM-inducing diet. When neurological signs of PEM occurred the concentration of sulfide in the rumen fluid was 7-12X the concentration of sulfide in samples taken prior to feeding the PEM-inducing diet (controls, 52.2±10.3 uM sulfide, n=6; PEM-afllicted, 515±131 uM, p<0.05). It is hypothesized that PEM can result from increased production of sulfide in the rumen and is a form of subacute H2S neurotoxicity. (Supported by USDA/CSRS, 87-CSR-2-1208)

TOXIC EFFECT OF OAK TANNIN EXTRACT COMPARED IN SHEEP AND GOATS. H. Narjisse, M. El Hansali, and J.D. Eglen*, Institute of Agronomy and Veterinary Medicine, Hassan II, Rabat, Morocco and USDA-ARS Poisonous Plant Res. Lab., Logan, UT 84321.

Moroccan sheep and goats had a different response to intraruminal infusion of a mixture of tannins extracted from oak leaves (Quercus ilex). Feed intake, nitrogen balance, and rumen ammonia concentration were depressed in sheep, but were stimulated or not affected in goats. In vitro dry matter disappearance rate was depressed at 65%DM or greater tannin extract concentration in rumen fluid from sheep, but increased in rumen fluid from goats. Possible explanations of the differential tolerance of tannin by sheep and goats are discussed.
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Lathyrus sylvestris (Flatiepa) Toxicity in Sheep and Evidence for Adaptive Tolerance

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Previous reports have suggested that ruminala can successfully adapt to diets containing the lathyrhetic forage, flatiepa. Our preliminary studies, however, indicated that 17- and 38-day stepwise adaptation periods inadequately protected sheep from flatiepa intoxication. Symptoms of intoxication when flatiepa consumption approached 75% of the diet included anorexia, muscular tremors, diarrhea, excessive salivation, tetanic seizures, and death. In contrast, prolonged exposure (4 months) to 50% dietary flatiepa resulted in successful adaptation, and these animals subsequently consumed 100% flatiepa without ill effects. When rumen contents of alfalfa-fed and flatiepa-fed sheep were exchanged, the formerly tolerant, flatiepa-fed animal became susceptible, exhibiting symptoms of lathyrism within 48 hours. In contrast, the formerly naive, alfalfa-fed animal subsisted on 100% flatiepa (administered intraruminally) with no signs of toxicity. These observations suggest that adaptive tolerance to flatiepa is due to alterations in rumen metabolism.

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EFFECT OF SPECIFIC GRAVITY OF ALFALFA HAY AND SILAGE ON RUMEN STRATIFICATION

M. A. Wannamaker, J. D. Salt, and D. B. Metzger

University of Wisconsin, USDA-ARS, U.S. Dairy Forage Res. Ctr., Madison, WI 53706

An experiment was designed to assess the effect of gas entrapment on the specific gravity (SG) of alfalfa hay (AH) and alfalfa silage (AS) during in vitro digestion. After 9 h of digestion, the gas associated with the residues were 15.8% and 13.8% of the unit volume (volume of DM, water, and gas) for AH and AS, respectively, but only 6.2% and 0.1% after 27 h of digestion. Even though the functional SG decreased during the first 9 h of digestion, the more rapid and complete departure of gas from the AS residue resulted in a functional SG of 1.46 compared to 1.23 in AH after 27 h of digestion. Including the effect of both the gas and the water associated with the residue resulted in unit SG ranging from 1.06 in AH and 1.02 to 1.17 in AS. These values indicate that AH sinks at all incubation times, but AH remains buoyant between 3 and 15 h of digestion.

In a second experiment, in which 80% forage diets were fed to dairy cows, the difference in rumen stratification between AH and AS was determined. A 220 ml bottle was used to collect a constant volume of rumen digesta. The percent and the amount of DM (g/220 ml rumen digesta) collected in the ventral rumen were 54.2, 11.74, 6.5, 13.83 for AH and 57.8, 10.74, 5.95, 13.27 for AS. In both locations, there was more DM in digesta with the AS than AH diet (p < 0.0001). Method of preservation influences the SG of alfalfa particles, and the concentration of digesta DM in the ventral rumen and the reticulum. The flux of digesta through the reticulo-omasal orifice might differ between hay and silage.

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SLUCING THROUGH THE RUMEN

J. D. Garza, J. C. Ferriera-Rios, and F. Owens
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Traditionally, the rumen is viewed to thoroughly mix consumed feed and water with ruminal contents and to remove digesta at a first order rate. To examined this premise, intake of PEG in drinking water was compared to ruminal outflow of PEG. Outflow was calculated from dilution rate of ruminally dosed CoEDTA, ruminal volume (evacuation) and PEG concentration 3 times in each of 12 cattle fed either 0% concentrate or all concentrate for 30 days. Differences between digesta concentrations were quite large. In a second study, 4 cattle were fed concentrate or roughage diets twice daily: ruminal evasion was partially and not significantly different through diurnal variation in ruminal marker concentrations were quite large. In a third study, CoEDTA was included in water and CoEDTA was dosed at 12 h intervals into the rumen. Ruminal evasion, calculated from relative marker concentrations in the rumen 3 to 4 h after administration began, was 42%. Estimates for drinking water evading the rumen in mature cattle at 40% or more have been obtained under two independent approaches. Administration of compounds via drinking water may enhance the postruminal supply.

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EFFECTIVE FIBER AND 17K ROLE IN RUMEN FUNCTION AND PRODUCTIVITY OF THE DAIRY COW. K. A. Beauchemin and J. G. Buchanan-Smith

Agriculture Canada, Lethbridge, Alberta T1J 4G1 and University of Guelph, Guelph, Ontario N1G 2M1.

Effective fiber and its role in rumen function and productivity of the dairy cow are addressed. Effective fiber represents the combined aspects of chemically measurable fiber, such as neutral detergent fiber, and physically measurable fiber, such as particle length. Both chemical and physical aspects of fiber are necessary in dairy cow diets in order to maintain normal milk production and minimize the incidence of digestive disorders. Recent interest in the role of effective fiber for dairy cattle stems from increased use of high fiber forages by producers. Ingested grain is rapidly fermented in the rumen causing a marked decline in pH of ruminal fluid which depresses cellulolysis. This is particularly evident when concentrates are barley-based, finely processed or allocated improductively during the day. Effective fiber is needed to promote eating and ruminating, and saliva output increases during eating, thus increased time spent chewing enhances buffering capacity within the rumen which optimizes cellulolysis. Chewing is also essential for particle size reduction and passage of feeds from the reticulo-omasum. Understanding the role of effective fiber in dairy cattle diets can elucidate feeding strategies that compensate both for rapidly digested concentrates and forages that have low rumination potential.

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EFFECT OF ADDED INERT RUMP, RULES AND STANDING INCINERATING GLYCOL (PEG) ON INTAKE, DIGESTIBILITY AND RUMEN KINETICS IN THE EARLY LACTATION DAIRY COW. R. B. Johnson and D. B. Combs

Department of Dairy Science, University of Wisconsin-Madison 53706

Eight rumen cannulated multiparous cows were used in a replicated 4x4 Latin square design with a 2x2 factorial treatment arrangement. Forage = feeding 4% PEG (1000 MW) and replacing 25% of rumen volume with water filled bladders. Periods were 21 d and treatments began 3 wk postpartum. Diet were 36% alfalfa silage, 17% corn silage and 43% concentrate (25%CP; 19%ADF and 27% NDF). Digestibility %

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rumens</th>
<th>Digesta</th>
<th>Digesta</th>
<th>Digesta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vol. 1</td>
<td>OM</td>
<td>DM</td>
<td>AD</td>
</tr>
<tr>
<td>Total</td>
<td>11.0</td>
<td>97.5%</td>
<td>79.7%</td>
<td>31.4%</td>
</tr>
<tr>
<td>Digesta only</td>
<td>9.05</td>
<td>97.3%</td>
<td>79.7%</td>
<td>27.9%</td>
</tr>
<tr>
<td>Digesta DM</td>
<td>16.1</td>
<td>97.5%</td>
<td>79.7%</td>
<td>31.4%</td>
</tr>
</tbody>
</table>

Lambson, Co-EDTA and Ytterbium-cell wall served as digestibility and passage markers, respectively. PEG did not increase rumen solubility and did not affect ruminal outflow. DMI, ADF, DM and PCM from rumen PH. Total tract AD Digestibility was reduced by PEG (39.4 vs 47.3%). Bladder mass efficacies are on table.

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A COMPARTMENTAL MODEL TO DESCRIBE RUMINAL IN SITU DIGESTION J. van Milgen, M. A. Murphy and L. L. Berger

Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL 61801

Ruminal in situ studies are frequently used to evaluate the nutritive value of feedstuffs, and different models have been employed to describe the results of such studies. Most of these models assume an instantaneous onset of digestion, which is probably not a common phenomenon in the rumen. A two pool system is proposed, comprised of a lag pool and a digestion pool. Only material in the lag pool is subject to first-order kinetics digestion. At inoculation, all available is in the lag pool and, thus, no digestion takes place. Flow of material from the lag pool towards the digestion pool is also envisaged to occur according to first-order kinetics. This process would be affected by factors like hydration, microbial attachment and enzyme synthesis. As digestion kinetics apply only to the potentially digestible fraction, the residue remaining during incubation in the rumen can be described by the sum of the size of the lag pool, the size of the digestion pool and the indigestible fraction. Residue is F0 = exp(-keτ) + F0 Yexp(-keτ) + F0 (1-exp(-keτ)) + F0, where F0 is the potentially digestible fraction, F0 is the indigestible fraction, t the rate constant (h-1), and k the digestion rate constant (h-1). The solution yields a sigmoidal curve from concentrations for t=0, an asymptote at F0, and an inflection point for t=ln(3)·keτ/h, (h). Parameters are estimated using nonlinear least-squares regression. An advantage of this model is that the first-order kinetics process delays digestion, rather than a time delay.
DEVELOPMENT OF AN ISOLATED RUMEN EPITHELIAL CELL INCUBATION SYSTEM. R.L. Baldwin, VI, and B.H. Jesse, Dept. of Animal Science, Rutgers-The State University, New Brunswick, NJ 08903.

A rumen epithelial cell isolation system has been developed using ruminants from Dorset ram lambs. The caudal-dorsal rumen wall was excised immediately after slaughter, thoroughly rinsed in warm tap water and transported in 37°C Krebs Ringer salts plus 25 K2HPO4, pH 7.4. Papillae were clipped (10 mm2) from the rumen wall. Cells were incubated with continuous shaking, at 37°C in a trypsin solution (2% trypsin, 0.1% hyaluronidase, 0.01 Units/ml elastase in Krebs Ringer bicarbonate buffer (KRB)). After ten minutes the trypsin solution was removed immediately after shaking, and fresh trypsin added to the remaining fragments. This procedure was repeated for a total of eight cycles. The two fractions, one with viable epithelial cells, were discarded. Subsequent fractions were quickly cooled on ice, and the cells pelleted by centrifugation at 60 x g. Cell pellets were re-suspended in KRB. Rumen cell viabilities range from 75-90%. Butyrate was converted to 6-hydroxybutyrate at high rates by these cells. This system should be useful in future investigations of rumen function.

Impact of Type and Level of Protein or Energy Supplementation on In Vitro Digestibility of Kikuyu (Pennisetum clandestinum) and Panicula (Guiera decumbens) Grasses. J.R. Carpenter, S.R. Dye, and R.J. Kline-DuPonte, Dept. of Anim. Sci. University of Hawaii at Manoa, Honolulu, HI 96822.

Both protein and energy are important for microbial synthesis. Forage fiber utilization in the rumen may be limited by nutritional availability; therefore this trial was conducted to evaluate the effect of different levels and types of energy (10 to 50% of DM as corn and/or barley) and protein sources. In the trial, corn or soy was added to raise the crude protein level of the total ration to either 0, 12, 16, or 20% of the DM on a total ration dry matter and cell wall digestibility of kikuyu and panicula grasses. The CP, ash, NFC, ADF, cellulose, and lignin content (% DM basis) was 5.2, 8.3, 77.9, 37.2, 28.5, 6.4 and, 4.4, 4.5, 76.8, 41.9, 34.7 and 7.4 for kikuyu and panicula grasses, respectively. The addition of either energy or protein altered the in vitro digestibility of the total rations incr ease d from 15.6 to 43.1% for kikuyu and 10.6 to 46.3% for panicula, but did not alter the CP (P=.06) and DMD (P>.05) of the CP (P=.05) and DMD (P>.05) of the CP (P=.05) and DMD (P>.05) or ADF (P>.05) in the rumen contents. These results show that energy and/or protein availability is not the limiting factor for microbial digestion of tropical forages, thus suggesting that rate of microbial hydration and/or ease of microbial attachment are the factors which limit rate of fiber digestion and passage.


Total starch in grains was determined by hydrolysis with amyloglucosidase (Solzyme L-2001). Samples of grain (200 mg) were autoclaved for 1 h in tubes with 2 ml of 20% CaCl2 (pH 2). Enzyme solution (5 ml, 50 Dialyzed units/tube in pH 4.2, 1M acetate buffer) was added, and tubes were incubated 14 h at 60°C. Glucose was determined with a glucose oxidase analyzer. In 9 runs, total starch, within run CV, and between run CV were 73.7, 8 and 2.0% for a standard sorghum grain, and 98.6, 8.7 and 1.0% for potato starch. The same system was used to determine relative starch availability except that samples were not autoclaved prior to incubation with enzyme. Hydrolysis was terminated at 0, 15, 30, 60, 120 and 240 min by boiling for 15 min. Values for 60, 120, and 240 min fit a model with an initial rapidly degraded fraction and a first order digestion rate for the slowly degraded fraction (Kd). The method was used to evaluate starch availability in flaked sorghum grain varying from 451 to 323 g/l. A linear regression test weight increased both R and Kd. To establish biological significance of the results, in vitro hydrolysis was regressed on in vivo total tract starch digestibility from 2 lactation trials evaluating grains differing in starch digestibility. Actual 4 h in vitro hydrolysis was more highly correlated with in vivo starch digestibility (R=.96) than either R (R=.86) or Kd (R=.91).


Mid-lactation Holsteins (n=18) averaging 28 Kg milk/d were supplemented with (1) a concentrate containing 48.5% dried, ground, shelled corn, 75% reje, and 8.0% minerals (DM, 6) DM = 4.26 Kg/d of DM and 4) DM + 4.26 Kg/d of DM. A period cross-over design. Periods were 30 d with data collected on d 22-25. Cows grazed on a daily rotation 21 paddocks (.4 ha each) of cool season pastures. Effects of energy supplementation were contrasted by A vs C and B vs A + C (curvilinear). Switching cows from TMR to pasture decreased milk production. The significant differences (P<.01) were seen between A and C in the 1975 (Kg/d) of milk (.86 vs .73.87 vs .67), milk fat (.57 vs .67), and .5% FCM (17.3 vs 19.5). There were no differences for B vs A + C (P>.05) in any of the measures measured. There were significant differences (P<.13) in the concentration of milk components, and changes in body weight or condition. Results indicate that there is an increasing response in the yield of milk, milk components, and FCM to increasing levels of energy supplementation. Although not statistically significant (perhaps due to low replication), means suggest there may be diminishing marginal and economic returns to energy supplementation above 4.56 Kg DM/d of concentrate.

MICROBIAL FERMENTATION AND SITE OF NUTRIENT DIGESTION IN COWS FED DIETS VARYING IN FORAGE AND ENERGY SOURCE. J. Lung, Jr., R.S. Tsung, and B.P. Camaroli, Dept. of Animal Sci., University of Delaware, Newark, DE 19717-1303.

Four Holstein steers, each fitted with a rumen fistula and duodenal t-cannula were used in a 4 x 4 Latin square design to measure rumen fermentation, rumen flow, and digestibility in diets varying in forage and energy source. All diets were 50:50 forage to concentrate on a DM basis and contained 10.1% of the DM as long-form hay. Forage and energy source combinations were: 1) alfalfa hay and barley; 2) alfalfa hay and corn; 3) corn silage and barley; and 4) corn silage and corn. Diets based on alfalfa and barley had greater ruminal organic matter digestion. Ruminal starch digestion was greater in barley (89%) vs corn (78%) diets and ruminal organic matter digestion was greater in alfalfa (63%) vs corn silage (47%) diets. Neither forage nor energy source affected ruminal microbial protein synthesis. Nitrogen digestion in the total tract was greater for barley (72%) than corn (65%) diets. Forage source had a greater effect on rumen fermentation than energy source. Rumen pH was higher (6.13 vs 5.94) and the molar % of acetate was greater (65.3 vs 61.3) while the molar % of propionate was lower (18.5 vs 21.2) in alfalfa vs corn silage diets. The acetate to propionate ratio was 5.2 and 3.0 for alfalfa and corn silage diets, respectively. No interactions were observed between forage and energy source.

ACID OR FORMALDEHYDE TREATMENT OF ALFALFA SILAGE FOR MILK PRODUCTION. S. A. Nadol and G. A. Broderick, Dept. of Dairy Science and USDAR, University of Wisconsin, Madison, WI 53706.

The objective of this experiment was to evaluate silage treatments which may increase rumen escape of alfalfa protein. Third cutting, mid-bloom alfalfa was harvested at 35% DM, and was untreated (Conole, C), treated with 6.4 lonic formic acid (F), or 2.9 kg/ton Grains (L) containing lomaldilid, H, and starch in polyethylene bags. Twenty-two multiparous cows were assigned to one of the treatments on d 18 of lactation, following a 2 wk period. Each treatment started with 9.8% alfalfa silage, 1.4% minerals, and vitamin A deficiency (DM basis). Cows received diets for 6 wk; production data are from the last 5 wk. Milk yield was covariate adjusted; all other values are actual means.

<table>
<thead>
<tr>
<th>Diet</th>
<th>CP</th>
<th>ADF</th>
<th>DM</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>21.4</td>
<td>32.9</td>
<td>15.3</td>
<td>29.3b</td>
<td>1.1b</td>
</tr>
<tr>
<td>F</td>
<td>30.8</td>
<td>29.8</td>
<td>18.2</td>
<td>32.6a</td>
<td>1.2a</td>
</tr>
<tr>
<td>G</td>
<td>31.1</td>
<td>31.9</td>
<td>19.7</td>
<td>32.3a</td>
<td>1.9a</td>
</tr>
</tbody>
</table>

Means in the same column with different superscripts differ (P<.05). From wk 7 to 10 of lactation, the ewe switched from one silage treatment to the next, each group continued on their original diet, and the other half received 4.8% fishmeal, fed to replace silage DM. After wk 2, cows within treatment groups switched diets. Fishmeal addition did not increase milk (P>.05) on any diet or milk protein percent on diet F (P>.05), but increased milk protein percent 1% on diets C and G (P<.01).
Feeding diets varying in the amount and ruminal degradability of protein has been reported to affect services per conception and days open. Data were summarized from 129 cows involved in previous research to evaluate diets containing soybean meal (n = 59) or urea (n = 70) in the concentrate mix. Total mixed diets were formulated to contain 15% crude protein on a dry matter basis. Addition of urea to the diet increased (P < 0.01) ruminal ammonia concentrations (13.3 and 16.1 mg/dl), but not (P > 0.22) serum urea concentrations (18.4 and 19.5 mg/dl). Days to first breeding (80.8 and 75.2), days open (146.4 and 145.2), and services per conception (2.70 and 2.58), were similar (P > 0.10) for cows fed soybean meal and urea. Regression analysis indicated that serum urea concentrations did not affect (P > 0.10) days to first breeding, services, and services per conception, but increased (r² = 0.49, P < 0.05) days open. When cows during early lactation were fed nutritionally balanced diets, the increased solubility of dietary nitrogen had no affect on reproduction.


Osmotically active compounds within the rumen have been used to alter ruminal fermentation through modification in the kinetics of the fluid fraction of the digesta. To test the influence of high intakes of NaCl on ruminal function and fate of drinking water, four adult ruminally cannulated steers were fed 80% concentrate diets with or without addition of 5% NaCl. Water intake, ruminal water evaporation (PEG), CO² diffusion rate and ruminal volume (evacuation) were measured. Added salt had no effect on DM intake. It increased water consumption by 84% but ruminal water evaporation remained largely unchanged at 60%. Although ruminal fluid tonicity (Go) remained at about 4%, ruminal volume was reduced by nearly 50% (36 vs 20 l). Dietary DM as % of daily intake present in the rumen was reduced by 20% (53 vs 67%) indicating enhancement of passage and(or) digestion rates(s). Ruminal fluid tonicity was 344 or 295 mOsM/kg with vs without added dietary salt. Addition of NaCl to the diet could increase delivery of specific nutrients to the small intestine by increasing the quantity of drinking water consumed evading the rumen. Reduced ruminal volume might explain why intakes of high salt roughage diets are low. Added dietary NaCl may enhance ruminal escape.

β-Galactosidase Activity of Firmicutes Succinogenes S85

P. Javorka, S.F. Lee, A.N. Gibbins and C.W. Forsberg, Department of Microbiology and Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario.

Firmicutes succinogenes S85 exhibits low β-galactosidase activity and is unable to grow with lactose as the sole source of carbon. However, when a dense inoculum of S85 was spread on lactose plates a small colony was isolated which grew rapidly. The isolate, L2, exhibited an SDS-PAGE protein profile, and an immunoblot profile with polyclonal antibodies to whole cells of S85, which were identical to those observed for S85. The β-galactosidase was cell-associated and probably cytoplasmic. The F. succinogenes β-galactosidase had a molecular weight similar to that from F. coli, but polyclonal antibodies to the E. coli β-galactosidase did not react with it. Some fresh isolates of E. coli strains may normally express β-galactosidase. This finding may help explain the ease of inoculation of young ruminants with fibrolytic bacteria.


Three 390 kg steers were fed a 71% corn diet with or without lasalocid (200 mg/kg/d), sodium or potassium (2% as chloride salt). Lasalocid was added along with a cation. Each was fed for 14 days prior to and 2 days during 2-12 hr chamber measurements of methane production and 1 day of rumen sampling. The additions of KCl increased rumen pH from 7.7 to 7.4 and (P < 0.05) but total sodium + potassium again remained constant at 150 mm. The addition of lasalocid to the control diet elevated the K and depressed Na by 12 mm (P < 0.05). The ratio of acetate to propionate was decreased from 3.6 to approximately 3 by lasalocid additions (P < 0.05). An 11% depression in methane from lasalocid approached significance (P > 0.05). Cation additions also tended to depress methane 13 to 167. The most depression of methane occurred when the combination of lasalocid + sodium or potassium (P < 0.05) a 23 to 29% decrease.

Cellulose Digestion and Cellulase Regulation and Distribution in Firmicutes Succinogenes S85

L. Huang and C.W. Forsberg, Department of Microbiology, University of Guelph, Guelph, Ontario N1G 2V1.

Firmicutes succinogenes S85 stimulates growth on microcrystalline cellulose without a lag whether inoculated from a glucose, cellulose or cellulose culture. There is no accumulation of soluble carbohydrate during growth on cellulose. When the growth medium contains either glucose or cellulose in combination with microcrystalline cellulose, there is a lag in cellulose digestion until all of the soluble sugar had been utilized, suggesting a feedback mechanism regulating cellulose digestion. The chloride-stimulated cellulase system and periplasmic cellulolytic enzymes are produced under all tested conditions of growth. Indicating constitutive synthesis. Immuno-electron microscopy has revealed the presence of the chloride-stimulated cellulase system and an antigenically related protein on protrusions at the cell surface perhaps suggesting that these structures are involved in cellulose digestion.


In order to improve efficiency of rumen digestion, we are developing genetic systems for xylan degradation by ruminal and colonic Bacteroides species. Genetic exchange systems and shuttle vectors have been developed for colonic Bacteroides species. However, these vectors have not been successfully introduced into ruminal bacteria. To determine if colonic Bacteroides species could express a gene from a ruminal species, a cloned xylanase gene from B. ruminocola was introduced into B. fragilis and B. uniformis on a plasmid vector. Both of these non-xylanolytic organisms were able to express the xylanase gene. The specific activity of the xylanase produced was 1,400-fold higher than that observed in B. rumincola. This is the first example of heterologous expression of genes between colonic and ruminal Bacteroides. Bacteroides xylanolytic colonic species, B. ovatus, showed that xylanase, xylulidase, and arabinosidase activities were regulated in response to carbon sources used for growth. The genes for these three activities were cloned on one 1.8 kb fragment, and all three activities were expressed in E. coli.
THE ORIGIN AND PROPERTIES OF FORMS OF RUMINOCoccus
FLAECACUS STRAIN 007 WHICH DIFFER IN THEIR ABILITY TO
DEGRADE COTTON FIBERS
Colin S. Stewart, Sylvia H. Duncan and Harry J. Flint,
Rowett Research Institute, Buckburn, Aberdeen, UK, AB2 9SB

When Ruminococcus flavefaciens strain 007 was maintained by
cultivation on non-selective nutrient media containing soluble
sugars, the cotton-degrading activity diminished to about one-fifth
of the activity of the original isolate. This activity could largely be
recovered by repeated culture on media containing cotton fibers.
A comparison of the form active in cotton degradation (007C) with
the form possessing only limited ability to degrade cotton 007TS is
being made to elucidate the nature and possible importance of
factors involved in the degradation of these substrates. Cotton
form 007C was only slightly more active than 007S in
degrading filter paper, avicel, Sigmacell, barley straw and wheat
straw than was 007TS. The a-glucosidase, B-1,4-endoglucanase,
cellulase, and xylanase activity of 007S and 007C were similar,
but 007C adhered more readily than 007S to both cotton and straw.

The findings indicate that although avicel and cotton are both used
as reference substrates for assay of the ability of microorganisms
to degrade these substrates, different factors may control the
ability of growing microorganisms to degrade these substrates.
Form 007S may be essentially a "weakly adherent" mutant, and cotton
degrading ability per se does not appear to be an essential pre-
requisite for the extensive degradation of lignified plant cell walls.

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DEGRADATION OF BARLEY STRAW, RYEGRASS AND ALFALFA CELL WALLS
BY CLOSTRIDIUM LONGISPORUM AND RUMINOCoccus ALBUS.
U.S. Meat Animal Res. Ctr., Clay Center, NE 68933 and
The Rowett Research Institute, Aberdeen, AB2 9SB, UK.

The recently isolated ruminal sporeforming cellulolytic
anaerobe, Clostridium longisporum 86408, was examined for its
ability to degrade barley straw, rye grass, alfalfa cell walls (mesophyll) and epidermis and lignified cell walls (fiber) of
ryegrass, and alfalfa cell walls in comparison to strains of
Ruminococcus albus. R. albus strains degraded barley straw and
80% of the dry matter in 10 days while the clostridium degraded less than 20%. A combined inoculum of R. albus ST3 and strain 86408 was no more active than ST3
alone, and the presence of Methanobacterium smithii PS did not
increase degradative activity. In contrast, with alfalfa cell
walls as substrate, the clostridium was twice (268 wt loss) as
active as R. albus ST3 (15%). The percent DM degraded from
eric grass cell walls of mesophyll, epidermis and fiber for the
Clostridium was 58, 47 and 32, respectively, and for R. albus
ST3, 77, 73 and 63, respectively. R. albus ST3 degraded
ryegrass mesophyll cell walls most rapidly, with epidermis and
fiber cell walls being degraded at similar rates. Strain 86405 attacked the alfalfa cell wall at a rate greater than any of the ryegrass substrates. These results indicate an
unexpected degree of substrate specificity in the ability of
C. longisporum to degrade plant cell wall material.

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DEGRADATION OF WHEAT STRAW AND ALKALINE HYDROGEN PEROXIDE
TREATED WHEAT STRAW BY RUMINOCoccus FLAVEFACIENS AND
Ruminococcus albus. A. A. Osagwe, R. I. Mackie, and B. A.
White, Dept. of Animal Sci., University of Illinois,
Urbana-Champaign, IL 61801

The degradation of wheat straw (VS) and alkaline hydrogen
peroxide treated wheat straw (APW) by Ruminococcus albus 8
and Ruminococcus Flavefaciens FD-1 was determined by measuring the
growth (OD₅₀₀) of each bacterium showing a linear disappearance
( DM) of the substrate. Modified easy medium (MEM) and Defined mediums (DM) were used. Defined medium with phenyl-
propanoic acid (PPA) and phenylacetic acid (PAA) was used.
Tubes were incubated at 39°C for ten days. Both OD₂₅₀ and DM
indicated that degradative activity was very high in all the cultures
with both bacteria (FD-1, 62.5 % and strain 84.12 %) over untrreated
WS (FD-1, 17.5 % and strain 8.75 %). Most degradation occurred
between 1 and 6 days. With MEM, PAA did not have any major effect on degradation by either bacteria. R. flavefaciens FD-1 degraded 62.5% and 84.7% AHPS with PPA and PAA addition, respectively. R. albus 8 degraded 41.25% AHPS with PPA and 43.75% AHPS with PPA and PAA addition. When defined media was used, the PAA was more effective on R. albus 8. AHPS degradation of AHPS (40%) over AHPS without added PPA and PAA (2%). No effect of PAA and PAA was observed for R.
Flavefaciens FD-1. PPA and PAA were grown together. Nor was there a synergistic effect on degradation when the 2
bacteria were cultivated with either PPA or without the
substrate. DM analysis showed that R. flavefaciens FD-1 more
efficiently degraded AHPS (ca. 5.6 mg/day) than R. albus 8
(4.33 mg/day).

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INTERACTION OF RUMINAL BACTERIA IN THE PRODUCTION AND
UTILIZATION OF DEGRADATION ENZYMES.
M. A. Costa,
NRC, A.S. USDA, Pecos, IL 68704.

Mucopoly saccharides (MS) are produced during the
digestion of ruminants by complex sequences from
amylolytic ruminal bacteria. To ascertain whether these
products accumulate during growth, two strains of
starch-degrading bacteria, S. bouyeri B71 and
Bacteroides fibrisolvens 49, were grown on a variety of
starch-containing media, and the appearance of MS was
monitored. Under all growth conditions MS accumulated in
these cultures. To examine whether MS produced during
starch hydrolysis was better utilized by other ruminal
bacteria, these two species were co-cultured with
Selenomonas ruminantium HD4, a strain having a limited
capacity for starch utilization. The cultivation of S.
ruminantium with S. bouyeri resulted in little change in the
patterns of MS observed with time over that with S.
bouyeri alone. In contrast, S. ruminantium was able to compete
with B. fibrisolvens for MS. In these co-cultures, S.
ruminantium was present in high numbers (relative to B.
fibrisolvens) and MS accumulated to a much lesser degree.
These data suggest that MS produced by amylolytic ruminal
bacteria may be important intermediates in the digestion of
starch in the rumen.

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EVIDENCE OF INHIBITION OF CELLULOYSIS IN AN ANAEROBIC RUMEN
FUNGI BY GLUCOSE, CELLULOSE AND SOLUBLE STARCH.
S. Morrison
and R. E. Bickel,
Dept. of Animal Sci., University of Illinois,
Urbana-Champaign, IL 61801.

In the forage fed animal, effects associated with rumen
degradation have been attributed in part to an increased
concentration of fibrolytic, anaerobic rumen fungi (ARF).
The nutritional niche imposed by fermentation pathways
varies with the remaining members of the rumen microbiota can utilize that niche, and how fiber
degradation may be affected. A strain of anaerobic rumen
fungus was purified from goat rumen fluid and by zoospore
sporangium morphology, was considered to be Picrospora like.
After purification, the fungus was maintained on pebbled
milled cellulose, and a 4-day old culture used to inoculate media
prepared to contain either [C]-cellulose alone or a combination of cellulose and glucose, cellulobiose or starch.
The release of [C]-label was minimal for up to 24 hours in media
prepared with an additional carbohydrate and disappearance of
starch, cellulose and starch exceeded 75, 75 and 33%,
respectively. After the initial 24h, cellulose solubilization
was much more substantial when provided alone. However, this
trend was rapidly reversed by 48 hours since breakdown of
these additional carbohydrates appeared to have
reached maxims, but as long as 96 hours was required when the
fungus was grown on cellulose alone. Whilst such
observations also reflect differences in rates of biomass production the early
inhibition of cellulolysis by the carbohydrates tested
me may be analogous to what occurs in vivo. Thus, the increase in
ARA following defecation may reflect an enhanced role in
fibrolytic degradation for these microorganisms.

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STIMULATED CELLULOSE DEGRADATION IN COCULTURES CONTAINING
YEAST AND CELULLYOLYTIC RUMEN BACTERIA.
K. A. Dawson, D. A. Morrison,
K. E. Newman and S. Jenkins,
Dept. of Animal Sci. University of Kentucky,
Lexington, KY 40506.

Aerobic cultures and cocultures of cellulolytic rumen bacteria,
and yeast strains (50% normal and 50% commercial) from commercial
products were examined to evaluate their ability to degrade
filter paper disks under anaerobic conditions. None of the yeast
strains degraded the disks. Cocultures containing yeast strains and
Bacteroides succinogenes degraded the disk at a slower rate
(.386 mg/ml) than with anaerobic cultures alone (4.32 mg/ml).
However, the lag time before the initiation of digestion
was much shorter in cocultures than in the aerobic cultures
(6h). The net result was a 2- to 3-fold increase in cellulose
digestion during the first 96 h of incubation. The total extent of
digestion after 108 h was similar in cocultures and aerobic
44.6 and 47.9 mg of 100 mg provided respectively).
Addition of yeast extract (1 mg/ml) did not significantly
alter coculture digestion patterns. Partial solubilization of cellulose
digestion was observed in cocultures of yeast and Ruminococcus albus.
His study suggests that low concentrations of live yeast (>10⁹/ml)
can significantly influence cellulose digestion by ruminal
bacteria.

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THE ORIGIN AND PROPERTIES OF FORMS OF RUMINOCoccus
FLAVEFACIENS STRAIN 007 WHICH DIFFER IN THEIR ABILITY TO
DEGRADE COTTON FIBERS

Energy sufficient bacteria can metabolize energy sources in the absence of growth, but the mechanism of energy spilling was not understood. Heat is the ultimate end-product of energy spilling reactions, and non-growing, energy-sufficient cultures of Streptococcus bovis produced heat at a rate of 0.15 uW/g protein (0.1 nmol glucose/g protein). Since dicyclohexylcarbodiimide (DCCD), an inhibitor of proton ATPases, eliminated heat production, it appeared that a futile cycle of protons and the membrane bound proton ATPase were responsible for energy spilling. This idea was supported by the observation that protophores and monooxygenase increased the rate of heat production. Since the rate of energy spilling was 3.7 times the maintenance rate, it appears that DCCD-sensitive energy dissipation may be regulated. If S. bovis were the only bacteria in the rumen (0.8 kg protein), its maximal rate of energy spilling could account for as much as 0.9 kg of glucose fermentation per h. Whether other ruminal bacteria have the capacity to spill energy at the same rate as S. bovis has yet to be determined.


The objective of this study was to assess fermentation and cell yield response of Selectomonas ruminans HD4 through a range of physiological and non-physiological ammonia-nitrogen (NH₃-N) concentrations. Cells were grown in continuous culture with a defined ascorbate-reduced basal medium containing either 0.5, 3.0, 25.0, 50.0, 100.0, and 200.0 mM NH₄Cl and dilution rates (DR) were pooled with categorical means (hrs⁻¹) of 0.07, 0.14, 0.24, and 0.40. NH₃-N was the growth-limiting nutrient (Kₘ = 71.5 μM) when 0.5 mM NH₄Cl was provided. Glucose disappearance and acetate (A) and propionate (P) concentrations formed were lower at 0.5 mM versus the higher NH₄Cl concentrations (P < 0.05). Lactate (L) was higher at 0.5 and 5.0 mM NH₄Cl, regardless if NH₄Cl was used at 0.5, 2.5, and 25.0 mM versus 0.5 mM NH₄Cl and twice as much NH₃-N was used at 50.0, 100.0 and 200.0 mM NH₃-N than at 5.0 and 25.0 mM NH₄Cl (P < 0.05). Glucose disappearance and product carbon formation rates were lower at 0.5 mM NH₄Cl versus the higher NH₄Cl concentrations (P < 0.05). L increased fivefold at the fastest DR for 5.0 mM NH₄Cl while A and P decreased under these conditions whereas L remained low and A and P remained high for all DR when NH₄Cl concentrations were 25.0 mM and above. Cell yield, expressed as YGurate and Y ATP were nearly doubled when NHCl was increased from 0.5 mM NH₄Cl (25.1 g cells per mole glucose disappeared) and 13.9 g cells per mole ATP produced, respectively, to the higher NH₄Cl concentrations and were higher at 25.0 mM NH₄Cl (48.2 and 23.3, respectively) (P < 0.05). YGurate was highest at the lowest NH₄Cl concentration. Apparently, maximal fermentation rate and maximal bacterial yield do not occur at the same NH₄Cl concentration for this organism.
ACTIVITIES OF AMMONIA-ASSIMILATORY ENZYMES OF Ruminococcus flavefaciens FD-1. E. A. Duncan and R. A. Maskie, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

Ruminococcus flavefaciens FD-1 was grown in batch cultures under conditions of carbon (5mm NH₄Cl, 10mm cellulose) and nitrogen (1mm NH₄Cl, 20mm cellulose) limitation. Cells were harvested in mid-exponential and stationary phase by centrifugation. The resulting cell paste was pressed through a French pressure cell, and cell free extracts were assayed for the presence of glutamate dehydrogenase (GDH), glutamine synthetase (GS), aspartagine synthetase (AGS) and glutamate synthase (GOGAT) activities. NADPH-linked GDH activity was only detected under conditions of nitrogen limitation (12 vs 150 nmol/min/mg protein, respectively). In contrast, activities of GS and AS measured using the forward assay were only detected under conditions of nitrogen limitation (12 and 17 nmol/min/mg protein, respectively). GOGAT activity was also higher under N-limiting conditions. These data will serve as references for further research on the efficiency of N-utilization in this important cellulolytic bacterial species.

ISOLATION AND REGULATION OF GENES CONCERNED WITH XYLAN UTILISATION IN RUMINOCOCCUS FLAVEFACIENS. H. J. Flint and C. A. McPherson, Nutrition Division, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.

Ruminococcus flavefaciens FD-1 is a recently isolated strain able to utilise xylan as well as cellulose or cellobiose for growth. Xylanase and β xylosidase activities show evidence of regulation, being higher in xylan grown than cellobiose grown cells. Evidence has been obtained for the existence of at least 4 distinct xylanase genes in this strain, based on DNA hybridisation, following the isolation of lambda bacteriophage clones expressing xylanase activity. Two of these genes, and an associated mixed linkage β glucanase gene, have been subcloned in plasmid vectors. Enhanced transcription from regions of DNA carrying certain of these cloned genes was demonstrated in hybridisation studies for R. flavefaciens cells grown on xylan compared with cellobiose.

TRANSFORMATION SYSTEMS AND PLASMID CONSTRUCTIONS FOR USE IN BACTERIOIDES RUMINICOLA. A.M. Thomson and R.J. Flint, Nutrition Division, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB.

Transformation of Bacteroides ruminicola F101, a derivative of strain R4, by the naturally occurring tetracycline resistance plasmid pBR322 (8.5 kbp) was achieved using electroporation at frequencies up to 10⁶ V/cm. A series of plasmides gave transformation of B. uniformis 100, but not B. ruminicola F101, by the E. coli/Bacteroides shuttle vector pBR322. A potential shuttle plasmid for use in B. ruminicola has been constructed from a cryptic B. ruminicola plasmid (pBR12), an E. coli plasmid carrying a multiple cloning site and a Bacteroides drug resistance marker.


A genomic library of Ruminococcus flavefaciens FD-1 DNA was constructed using the Escherichia coli bacteriophage λ vector. E. colilambda phage progeny were screened for cellulolytic activity by placing with appropriate host E. coli in soft agar (0.7%) overlay containing 1.0% (w/v) Dextran brilliant red - hydroxethyl cellulose (OBR-HEC). An OBR-HEC positive recombinant phage designated FDl-71 was plaque purified to greater than 99% and then the insert DNA in the plasmid plasmid was excised from FDl-71 using λ helper phage and rescued in E. coli XL-1-Blue. Of the 16 E. coli clones with rescued plasmid plasmid, one clone, designated FDl-71A, gave positive carboxymethyl cellulose hydrolysis when screened using the congo-red staining method. The presence of a DNA insert (2.2 kb) in the plasmid was confirmed by endonuclease restriction and site estimation by agarose gel electrophoresis. By-labelled probe was generated using the 2.2 kb DNA insert from this plasmid (pBAM201) for hybridization with with R. flavefaciens FD-1 chromosomal DNA confirming the origin of DNA insert. Furthermore, no hybridization of the probe with other endoglucanase genes cloned from this strain, celA (pME8200) and celD (pME8201), was detected. This indicated its unique identity as celC. Substrate specificity shows that the gene encodes an enzyme that degrades CMC, and xylan to a lesser extent. Further work on the characterization of celC is in progress.

ELECTROPORATION OF Ruminococcus flavefaciens FOR TRANSFER OF DNA. M. Morrison* and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801.

Leucenea iucuocophala is a tropical leguminous shrub with considerable potential as a nitrogen supplement for increasing animal production. However, application is limited by the occurrence of minor toxicities, such as a free phenolic, 1,2-pyridone-4 (1,4-DHP) in the rumen. Recently, two ruminal bacteria (78-1 and 32-24) have been isolated by M.J. Allison which are able to degrade 1,2-DHP and to form the commercially available isomer 2,3-DHP. Isolate 32-24, a short gram positive strain, was grown on a semi-defined medium of 15% clarified rumen fluid, minerals, casitone, arginine, B vitamins and 2,3-DHP. Growth, as measured by optical density, was directly proportional to the concentration of DHP in the medium and the generation time for growth 13-18h when DHP was not limiting. In contrast, medium with arginine but no 2,3-DHP showed little growth. Therefore, DHP was used as an energy source for growth of this organism. Plasmids have not been detected in mini-preps of strain 32-24 and the mechanism of transfer of resistance remains to be determined.

RUMINAL BACTERIA AND PYRROLIZIDINE ALKALOID DETOXIFICATION
A.H. Craig*, L.L. Bythue and E.D. Lassan, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331
Degradation of toxic molecules by anaerobic microorganisms has come to the fore of scientific investigation in the late 1980's. The rumen of the sheep contains such organisms that, through a series of experiments in our laboratory, has been shown to degrade the pyrrolizidine alkaloids by two different routes (Senecio jacobi). This mechanism is proposed as the primary reason why sheep are resistant to pyrrolizidine alkaloid toxicity and cattle and horses are not. A series of experiments were conducted to support this hypothesis.

First, physiological quantities of crystalline pyrrolizidine alkaloid extracted from tansy ragwort were chronically infused into the livers of sheep via the portal vein. Classic hepato-pathy was seen to occur without hepatogenous photosenstivity.

Secondly, ruminal fluid taken from sheep had a rapid, i.e., less than 24 h, degradation of the pyrrolizidine alkaloids when incubated in an artificial rumen. Comparable treatment of bovine ruminal fluid evidenced only minor detoxification of the pyrrolizidine alkaloids, i.e., 26% in 48 h. Finally, differential contribution experiments evidenced that the primary microorganisms responsible for the detoxification were one or more of the small bacteria. In addition, it has been found that these microorganisms can detoxify a number of primary pollutants.

PHOTOSENSITIVITY OF CATTLE GRAZING ALFALFA PASTURES
A trial was conducted to characterize photosensitivity in cattle grazing a predominately alfalfa pasture. Ninety-six Holstein steers (48 per diet) were placed on an alfalfa grazing trial in July, 1972.

Fifteen days after the initiation of the trial, the first steer showed signs of hair loss and skin lesions. Twenty-one steers experiencing photosensitivity plus 11 non-affected steers were removed from the pasture. This event was observed to be the onset of the sheep dehydrogenase (SDH), a liver enzyme. Steers were weighed at each bleeding date. The normal range of SDH levels is 24-42 IU/L. During the photosensitive period, steers had high levels of SDH. After the steers were taken off pasture, SDH levels dropped to normal levels except group 1 (P<0.05).

Steers were divided into groups: (P>0.05) control steers, group 9, had greater ADG than group 1 steers during the recovery period (P<0.05). Steers with the greatest hair loss did not have greater levels of SDH in the serum. The increase levels of SDH in all steers indicate hepatoenous photosensitivity.
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INFLUENCE OF PROTEIN DEGRADATION AND DIET TYPE ON FERMENTATION IN A CONTINUOUS CULTURE SYSTEM. D. I. Hig* M. D. Smith, H. R. Mesfin and B. A. Gopher

Protein degradation and diet type were evaluated in a 2 X 4 factorial design using a continuous culture system. Raw or extruded soybeans (SB) were the sole source of supplemental protein. Diet types ranged from alfalfa hay (AH) to corn silage as the major forage source. Diets contained 17% crude protein and 21% acid detergent fiber. Results of the fermentations (listed below) indicate that changing the forage source and concentration of soybean meal had no effect on microbial fiber degradation or nitrogen flow, but did increase the number of lipolytic microorganisms.

<table>
<thead>
<tr>
<th>Soy Type</th>
<th>Diet Type</th>
<th>Raw Ext</th>
<th>10SB</th>
<th>15SB</th>
<th>20SB</th>
<th>25SB</th>
<th>30AH</th>
<th>40AH</th>
<th>50AH</th>
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<td>ADP digestion, %</td>
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<td>60.3</td>
<td>55.9</td>
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<td>Nitrogen flow, g/d</td>
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<td>Bacterial N</td>
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<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.3</td>
<td></td>
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</table>

Effects of Cr concentration and particle size of 

Fiber, extracted from ground (2.5 X 7.5 cm) alfalfa-hay hay, was mordanted with 2 or 5% Cr (1.55 and 3.08 bound Cr), dried and either re-ground through a 1 mm screen (fine) or not re-ground (coarse). Four steers, fed ground (2.5 X 7.5 cm) alfalfa-hay hay, were re-fed with dietary 10 g of the mordanted fibers on day 10 in each period of a 4 x 4 Latin Square digestion trial with 10 d adjustment and 2 d fecal collection periods. Passage kinetic parameters and fecal outputs were estimated from the fecal Cr concentrations using age-dependent 1- or 2-pool models. In both the 1- and 2-pool models, initial Cr concentrations and passage rates were higher (P<.01) for the fine mordanted fibers. Fiber particle size did not affect fecal output rates. Mordanted fiber was more resistant to hydrolysis than the coarse mordanted fibers. Fecal output estimates (P<.01) and deviations from true fecal outputs (P<.10) were lower when calculated from the passage kinetics of 1.55% Cr- than 3.08% Cr-mordanted fibers.

Microbial CP association with and NDF digestibility of Untreated, and Ammoniated Bermudagrass Hay.

Six ruminally cannulated Holstein steers were used in a 5x4 incomplete Latin square design of animals and periods to study the effects of NPN and ammoniation (M) in situ forage diets. In situ forage diets were: untreated bermudagrass hay (H), ammoniated (3% of DM) bermudagrass hay (AH), H+M, AH+M, H plus ura (HUV) and H+HUV. Steers were fed a concentrate supplement containing the required M (200 mg/d) and/or U (100 g/d). Both H and AH were incubated in situ in each steer for 12, 24, 48 and 96 h. Microbial CP association with residual in situ DM (M) and apparent (A-CPD) and corrected (C-CPD) CP digestibilities were determined. Averaged across time, MCP was greater (P<.05) for AH (43 mg/g) than H (34 mg/g). Due to MCP, A-CPD was lower (.51.9% to 46.3%) than C-CPD (51.3% to 77.6%). Monensin reduced MCP of AH but not H (M x forage, P<.05) and as a result A-CPD was greater for AH than H. C-CPD was not affected (P>.1) by forage type. Extent of NDF-bound CP digestibility was not affected by M in steers receiving U or AH but was decreased by M in steers receiving no NPN (M x NPN, P<.05).

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Urinary excretion of orally dosed xylose, a non-metabolized sugar, has been used to estimate intestinal absorption of sugars in both non-ruminants (man, horse, dog) and calves, sheep and cows. Because xylose is catabolized in the rumen or if absorbed, it is eliminated in urine, xylose urinary excretion was tested as a sensitive marker of true ileal escape. Variable excretion patterns (0% to 7% of dose) following administration of xylose to adult steers in drinking water led us to examine its urinary excretion during a 48 h period following administration in the feed, the duodenum or intravenously. Of orally fed xylose, from 1 to 12% of dose was recovered. Of duodenally dosed xylose, recoveries range from 13 to 32%, while after intravenous administrations, to 61% was detected in urine. Although blood concentrations of xylose may serve as a qualitative index of absorptive function in non-ruminants, absorbed xylose was not quantitatively excreted, presumably due either to recycling to the gastrointestinal tract and fermentation by microorganisms or to greater tissue metabolism in ruminants than non-ruminants. The search for a ruminally metabolized, absorbed but quantitatively excreted marker to estimate ileal escape under various feeding, environmental and animal conditions continues.

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IN VITRO SYNTHESIS AND BIOHYDROGENATION OF LONG-CHAIN FATTY ACIDS IN DIETS CONTAINING MEGALAC OR ANIMAL-VEGETABLE BLEND. Zheng Wu and D. L. Paulsont, Department of Dairy Science, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691.

Both increases and decreases of long-chain fatty acids (LCFA) in ruminant content have been reported from studies with predominantly ruminant cows. An in vivo trial with 9 data and 7 treatments of levels of fresh and ensiled grass was black designed to record changes of LCFA during incubation. Diets were ground alfalfa hay containing 0, 3, 6, 9, and 12% Megalac® (M) or animal-vegetable blend fat (AV). Following the procedure of Goering and Van Soest, 3 g diets were added to 40 mL medium solution in each tube were associated with 10 mL rumen fluid and incubated for 24 h. LCFA in diets, fermented products, and inoculums were analyzed using a one-step GLC procedure. Changes of FA in fermented products compared to initial diets were computed after correction for contribution from inoculation. Synthesis of FA during incubation was determined by regression as 7.2 mg/g substrate. The FA species increased most were 14:0 and 14:1. Added fats did not affect FA synthesis under the present high roughage diet conditions. Basal bacteria of unsaturated FA were 42 and 69% (P<.01) in M and AV diets, respectively, and corresponding sat FA in M and AV fat obtained by correction for basal diet were 44 and 75% (P<.01). Though the BA values were lower than obtained in a previous in vivo trial, they were consistent in trend. The results of the trial supported the idea that Megalac is more stable than AV in the rumen as suggested by its lower BA, and that rumen microbes are capable of synthesizing LCFA. Any disappearance of LCFA noted in two experiments may be related to chain-shortening and absorption in the rumen and variable digestibility of maskers. (Supported in part by Church and Dwight, Inc., Princeton, NJ 08540).

Key Words: Fatty acid synthesis, Biodegradation.
The effect of chemical drying agents on nutrient digestibility and lactational performance was studied. First-cut alfalfa hay (late bud) was harvested with treatments applied at mowing (7.35 kg/ha). Treatments consisted of untreated control (0), commercial drying agent (1), and high concentration of NaCl, MgSO4, and CaCO3 (2). Six multistem Holstein cows, 120-150 kg of postpartum, were fed diets twice daily; diets consisted of 55% alfalfa hay and 45% concentrate (DM basis). There were no differences in milk yield (kg/d), fat %, or protein (g/d) for treatments 0 (27.2, 2.5, 3.46; 27.3, 3.48, 2.7; 27.7, 3.37, 3.28) for treatments 0, 1, and 2 respectively. However, contrast of treatment 0 vs 1 showed a significant (P<0.05) decrease in milk protein %, dry matter intake (kg/d) did not differ for treatments 0 and 1 (23.2, 24.6 kg/d). Digestible OM (DM) 61.0, 60.4, 61.3 (P<0.01) was affected by drying treatments. Blood electrolytes, Na, K, and Cl (mmol/l) were not different between treatments (means 154.2, 54.7, 103.1). No treatment differences were observed for blood pH, hemoglobin (%), or HCO3- (mmol/l) means 37.6, 35.4, 36.5. Treatment of nitrogen drying agent did not alter nutrient digestibility, milk production, or selected blood measurements in mid-lactation cows.


Four Holstein steers (208 kg) with T-cannulas in the rumen and proximal duodenum were used to determine the influence of level of feeding on corn processing on characteristics of ruminal and total tract digestion. The basal diet contained (DM basis) 6% alfalfa hay, 6% sudangrass hay, 75% corn, 3% casein, 2% yeast meal and 5% minerals and supplements. The corn portion of the diet was provided as either DR (DR = 54 kg/steer) or SF (SF = 36 kg/steer). Feed intake was restricted to allow for 0.64 kg/d of hay and 0.18 kg/d of corn. Dry matter and neutral detergent fiber (NDF) intakes were greater (P<0.01) for steers fed DR than SF. Ruminal digestion of OM, starch and NDF were not influenced (P>0.10) by feed intake. Post-ruminal digestion of OM and NDF was greater (P<0.05) for DR than SF. Post-ruminal digestion of ADF was greater (P<0.05) for DR than SF. The effect of feed intake on passage rate and fluid passage rate was not determined. Time constant of passage rate (hr) from the small intestine to the cecum was greater (P<0.05) for DR than SF. The passage rate of digesta was greater (P<0.05) for DR than SF. Passage rate and fluid passage rate were not affected (P>0.10) by time spent in the rumen.


Twelve rumenally and abomasally-cannulated wether lambs (26 kg) were blocked by weight and randomly assigned to 14.3% CP experimental diets consisting of bromegrass hay (BR) and a semi-purified concentrate mixture in ratios of 90:10, 70:30 and 50:50. Diets were fed at the rate of 2 kg DM/100 kg BW in equal portions at 12-h intervals. A 14-d dietary adjustment preceded 6-d period of sample collection. All dietary nutrient flow, particulate passage rate and fluid passage rate were not different for treatments 0, 1, and 2 respectively. Ruminal pH and digestion of NDF, ADF and hemicellulose (HC) declined linearly (P<0.05) with increasing concentrate level. Rumenal pH (hours after meal) was affected (P<0.05) by time spent in the rumen. Correlations of pH hours with ruminal NDF (r = -0.78; P<0.003), ADF (r = -0.72; P<0.009) and HC (r = -0.66; P<0.02) were observed. A 24-h feeding cycle. Total ruminal VFA concentrations were correlated with ruminal digestibilities of NDF (r = -0.90; P<0.0001). Contributes (r = -0.86; P<0.0001).


Eight trials were conducted to evaluate the effect of a daily (D) and weekly (W) alternation of lasilodac (L) and monensin sodium (MT) compared to continuous L or MT. The studies were conducted in 1987 and 1988 at five locations with a total of 121 pens of cattle. Pen was used as the experimental unit in all statistical analysis. Each trial was individually analyzed prior to pooling and a weighted analysis of variance was conducted on the combined data set.

Eight Trial Summary

<table>
<thead>
<tr>
<th>Item</th>
<th>MT</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Item (Pen)</td>
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<td>32</td>
<td>25</td>
<td>32</td>
<td></td>
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<tr>
<td>DM Intake (lb)</td>
<td>22.2d</td>
<td>21.7b</td>
<td>22.1c</td>
<td>21.9b,c</td>
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</tr>
<tr>
<td>Gain, Daily (lb)</td>
<td>3.62b</td>
<td>3.64a</td>
<td>3.80b</td>
<td>3.66b</td>
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<td></td>
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<tr>
<td>Feed Gain</td>
<td>6.19</td>
<td>6.10a</td>
<td>6.06b</td>
<td>5.97c</td>
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</tbody>
</table>

a,b,c Means in same row with different subscripts are significantly different (P<0.05).

This data suggests that alternating L and MT on a daily basis improves feed efficiency and average daily gain over L or MT fed continuously.
To evaluate ruminal in vitro models, the effects of some antimicrobials of different classes were compared on VFA concentrations in two models based on mixed rumen microorganisms as well as in vivo. In the first trial using batch cultures 1, 5, 10 and 25 µg/ml monensin, avoparcin and penicillin were tested. Monensin dose-dependently enhanced propionate production (by 10 to 26 % compared with the control), whereas avoparcin stimulated propionate only slightly (1 to 13 %) - but significantly - penicillin substantially reduced propionate as well as total VFA levels. A clear dose-effect relationship was absent after avoparcin. In the second trial the compounds were tested in rumen-fistulated sheep. Whereas 15, 30, 60 and 120 ppm monensin and 30, 60 and 120 ppm avoparcin dose-dependently enhanced propionate proportions (from 19 to 39 and 32 %, resp.), penicillin (1 mg/kg) transiently reduced and thereafter increased propionate as compared to the initial values. Finally, a continuous rumen model (Rusitec) was used. Whilst 1 to 25 mg monensin and avoparcin improved the fermentation in Rusitec dose-dependently, penicillin adversely affected the fermentation comparable to in vivo. In conclusion, whilst batch cultures reliably predict the efficacy of ionophores, they may not do so for avoparcin and penicillin. Rusitec is a promising method to overcome such shortcomings.