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:

American Cyanamid Company

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WEDNESDAY, November 8, 1989
(Gold Room, Congress Hotel)

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. McDermid, 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

5) 9:15 -- ***Cellulose fine structure and its in vitro digestion kinetics.*** P. J. Weimer, J. N. Lopez-Guisa, and A. D. French, USDA-ARS, U.S. Dairy Forage Center, Madison, WI

6) 9:30 -- ***Effects of phenylpropionic acid on cellulolysis by Ruminicoccus 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 cellulolytic 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

16) 1:30 -- **(Invited Paper):** ***Cell wall carbohydrate digestibility in ruminants: Plant imposed limits.*** D. R. Buxton, USDA-ARS, Dept. of Agronomy, Iowa State University, Ames

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

18) 2:15 -- ***Evaluation of the CAPTEC controlled release devise for fecal output estimation.*** J. C. Burns, K. R. Pond, J. M. Luginbuhl, and D. S. Fisher, Dept. of Crops Science and Animal Science, North Carolina State University, Raleigh

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, Sasktachewan, 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

25) 4:30 -- ***Lathyrus sylvestris (flatpea) toxicity in sheep and evidence for adaptive tolerance.*** M. A. Rasmussen, J. G. Foster, and M. J. Allison, USDA-ARS, National Animal Disease Center, Ames, IA, and USDA-ARS Appalachian Soil and Water Conservation Research Laboratory, Beckley, WV

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

32) 9:45 -- ***Energy supplementation to rotationally grazed dairy cows.*** L. C. Solorzano, P. E. Naasz, J. S. Liesman, B. B. Bartlett, H. F. Bucholtz, and R. S. Emery, Department of Animal Science, Michigan State University, East Lansing

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. Zorrila-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 Ruminicoccus 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. Varel, 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 Ruminicoccus 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

64) *Photosensitivity of cattle grazing alfalfa pastures.* M. L. Schlegel, C. J. Wachenheim, M. E. Benson, J. R. Black, W. J. Moline, H. D. Ritchie, G. D. Schwab, and S. R. Rust, Dept. of Animal Science, Michigan State University, East Lansing

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.* G. 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

73) *Effect of chemical drying agent treatment of alfalfa hay on nutrient digestibility and lactational performance of mid-lactation Holstein cows.* C. J. Ziemer, A. J. Heinrichs, C. J. Canale, and G. A. Varga, Dept. Dairy and Animal Science, Pennsylvania State University, University Park

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, G. Campbell, and J. G. Buchanan-Smith, Dept. of Animal and Poultry Science, University of Guelph, Ontario, Canada

78) ***Effect of short duration ionophore rotations on feedlot cattle performance.*** M. E. Hubbert, A. B. Hohnson, and L. A. Peterson, Hoffmann-La Roche, Nutley, NJ

79) ***Evaluation of ruminal <u>in vitro</u> systems.*** A. de Jong, Inst. Animal Nutrition Bayer, Leverkusen, Federal Republic of Germany

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:	STEERING COMMITTEE:
AGRONOMY	*J. C. Burns*	*R. D. Hatfield*
MICROBIOLOGY	*J. B. Russell*	*M. T. Yokoyama*
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PHYSIOPATHOLOGY	*R. H. Dunlop*	*R. H. Dunlop*

Chairperson -- *M. J. Allison*

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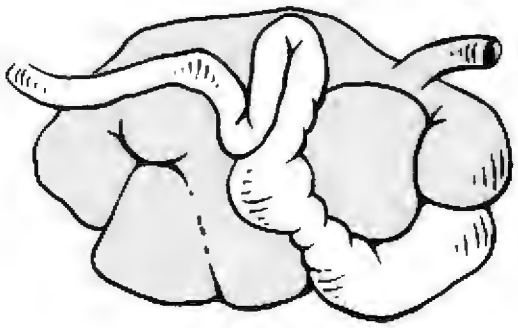
Abstracts

Conference on Rumen Function

Volume 20, 1989



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32) 9:45 -- ***Energy supplementation to rotationally grazed dairy cows.*** L. C. Solorzano, P. E. Naasz, J. S. Liesman, B. B. Bartlett, H. F. Bucholtz, and R. S. Emery, Department of Animal Science, Michigan State University, East Lansing

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. Zorrila-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 Ruminicoccus 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. Varel, 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 Ruminicoccus 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

64) *Photosensitivity of cattle grazing alfalfa pastures.* M. L. Schlegel, C. J. Wachenheim, M. E. Benson, J. R. Black, W. J. Moline, H. D. Ritchie, G. D. Schwab, and S. R. Rust, Dept. of Animal Science, Michigan State University, East Lansing

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.* G. 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

73) *Effect of chemical drying agent treatment of alfalfa hay on nutrient digestibility and lactational performance of mid-lactation Holstein cows.* C. J. Ziemer, A. J. Heinrichs, C. J. Canale, and G. A. Varga, Dept. Dairy and Animal Science, Pennsylvania State University, University Park

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

78) ***Effect of short duration ionophore rotations on feedlot cattle performance.*** M. E. Hubbert, A. B. Hohnson, and L. A. Peterson, Hoffmann-La Roche, Nutley, NJ

79) ***Evaluation of ruminal in vitro systems.*** A. de Jong, Inst. Animal Nutrition Bayer, Leverkusen, Federal Republic of Germany

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:	STEERING COMMITTEE:
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Arrangements -- *S. F. Kotarski*

1

PHYSIOLOGY OF PLANT CELL WALL DIGESTION BY RUMEN BACTERIA

C.W. Forsberg*, J. Gong, L. Huang, M. McGavin, K. McDermid and A. Matte, Department of Microbiology, University of Guelph, Guelph, Ont., N1G 2W1

Plant cell walls are a mesh of cross-linked polymers consisting primarily of cellulose and hemicellulose. Digestion requires the presence of a broad range of enzymes strategically located to sequentially cleave different types of glycosidic and ester linkages, and peptide bonds. The major fibrolytic rumen bacteria as exemplified by Fibrobacter succinogenes appear to possess many of the physiological adaptations seemingly necessary for digestion of cell walls. They bind to cellulose and secrete a broad range of cellulases and hemicellulases at the cell surface which hydrolyse plant polymers. The regulation of synthesis and secretion of these enzymes in F. succinogenes 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 cel3 gene which codes for endoglucanase 3.

2

CLONING OF AN ENDOGLUCANASE GENE FROM *Ruminococcus flavefaciens*. B. A. White*, C. T. Howard, S. Rosenzweig, and J. H. Clarke, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

An endoglucanase gene *celB* was isolated from genomic library of *Ruminococcus flavefaciens* FD-1 DNA constructed using the *Escherichia coli* bacteriophage λ vector, λZAP II. The recombinant phage progeny were screened for cellulolytic activity by plating on *E. coli* in soft agar overlay containing 1.0% (w/v) Ostazin brilliant red - hydroxyethyl cellulose (OBR-HEC). An OBR-HEC positive recombinant phage designated FD1-37 was purified and the plasmid was excised from recombinant phage using R_{exe} helperphage and rescued in *E. coli* XL-1- Blue. The plasmid designated pBAW101 harbored 2 *Eco*RI fragements of 2.65 and 3.37 kilobases (kb) from *R. flavefaciens* in the *E. coli* vector pBluescript. Southern blot analysis using ^{32}P- labelled probe hybridized with *R. flavefaciens* FD-1 DNA confirming the origin of DNA insert. The probe did not hybridize with other endoglucanase genes from *R. flavefaciens* designated *cel* A (pMEB200) and *celC*. Substrate specificity of the gene encoded by this clone as well as an extensive restriction map will be presented.

3

CLONING AND SEQUENCING OF A XYLANASE GENE FROM BUTYRIVIBRIO FIBRISOLVENS STRAIN 49 AND HOMOLOGOUS DNA SEQUENCES IN OTHER STRAINS OF BUTYRIVIBRIO. B. M. MANNARELLI AND S. EVANS, NRRC, ARS, USDA, PEORIA, IL 61604.

A gene coding for xylanase activity, xynA, from the anaerobic ruminal bacterium Butyrivibrio fibrisolvens strain 49 was cloned into Escherichia coli JM83 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. Expression of xylanase activity required parts of both EcoRI segments. In E. coli the cloned xylanase enzyme remained cell associated. The enzyme exhibited no arabinosidase, cellulase, α-glucosidase, or xylosidase activity. The isoelectric point of the cloned protein was approximately 9.8, and optimal xylanase activity was obtained at pH 5.4. The nucleotide sequence of the 1,535 base pair EcoRV-EcoRI segment from the B. fibrisolvens chromosome that includes the xynA gene was determined. An open reading frame was found that encoded for a polypeptide of 411 amino acid residues of 46,664 daltons. A putative ribosome binding site, promoter, and leader sequence were identified. Comparison of the xynA protein sequence to that of the xynA protein from alkalophilic Bacillus sp. strain C-125 revealed considerable homology with 37% identical residues or conservative changes. The cloned xylanase gene hybridized strongly to chromosomal sequences in only two of five closely related Butyrivibrio strains; the gene appeared to be absent in the other three.

4

OUTER MEMBRANE BINDING PROTEINS ARE REQUIRED FOR STARCH UPTAKE BY COLONIC BACTEROIDES. K. L. Anderson, L. A. Bedzyk, and A. A. Salyers, Dept. Microbiology, University of Illinois, Urbana, IL 61801

Most 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 deficiency indicates such binding is required for starch utilization probably as a prelude to transporting the starch molecule through the outer membrane. Additional studies demonstrated that binding involves at least two components; one with an affinity for large glucose oligomers (>G6), the second with a high affinity for maltodextrins (G4-G7). 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 numerous genes in several operons.

5

CELLULOSE FINE STRUCTURE AND ITS IN VITRO DIGESTION KINETICS. P.J. Weimer, J.M. Lopez-Guisa, and A.D. French. USDA/ARS, U.S. Dairy Forage Research Center, Madison, WI 53706. and Southern Regional Research Center, New Orleans LA 70124.

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 fit first order rate equations that incorporated discrete lag times. Kinetic parameters were compared to relative crystallinities determined by x-ray diffraction, and to estimated gross specific surface areas (GSSA) of the different types of cellulose. GSSA was determined by optical microscopy and was based on smooth cylindrical or square-prismatic models of the particles. For celluloses having similar crystallinities and simple non-aggregating particle morphologies, the fermentation rate constants displayed strong positive correlation (r^2=0.98) with GSSA; lag times displayed a strong negative correlation (r^2=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 higher concentrations of phosphoric acid resulted 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 of ruminal bacteria are predominantly surface bound rather than extracellular. The decreased rate of digestion of samples containing some cellulose II suggests that ruminal organisms do not rapidly adapt to utilization of different cellulose forms.

6

EFFECT OF PHENYLPROPIONIC ACID ON CELLULOLYSIS BY *RUMINOCOCCUS ALBUS* IN CONTINUOUS CULTURE. M. Morrison, R.I. Mackie, and A. Kistner, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

The likelihood that phenylpropionic acid (PPA) could stimulate cellulolysis in strains of *Ruminococcus albus* other than those already reported, was confirmed in preliminary studies in which three South African strains were grown on [U-^{14}C]-cellulose. One strain, *R. albus* Ce63, was grown in a continuous culture apparatus designed for use with solid substrates such as pebble milled cellulose. Measurements were made once bacterial populations had reached steady state at a dilution rate of 0.17/hr. The extent of cellulose disappearance was 41.1, 35.7, 90.2 and 86.9% and volumetric solubilization rate was 103.0, 97.9, 215.5 and 230.4 ng/l/h for control, PAA, PPA and PAA plus PPA grown cultures, respectively. Phase contrast micrographs clearly revealed that PPA 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 PPA not only on the kinetics of cellulose hydrolysis but also on the population density of cellulolytic microorganisms. Further studies of the physiology of PPA utilization are warranted in the quest to understand and improve the existing constraints to fiber degradation.

7

The digestion of barley, maize and wheat by selected species of ruminal bacteria. T. A. McAllister*, L. M. Rode, K.-J. Cheng, [1]C. W. Forsberg, and [1]J. G. Buchanan-Smith, Agriculture Canada, Lethbridge, Alberta, and [1]University of Guelph, 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), *Ruminobacter amylophilus* 50 (Ra) and *Butyrivibrio fibrisolvens* A38 (Bf) were incubated in quadruplicate vials containing artificial media and 0.5 g of B, M or W. Triplicate vials were analyzed for starch and ammonia after 4, 8, 12, 24, 48 and 72 of anaerobic incubation. The fourth vial was used for scanning electron microscopy (SEM). The rate of starch digestion in all grain types was greater ($P<0.001$) for Sb than for Rb or Bf. Starch digestion by Sb was greater in M ($P<0.001$) than in B or W, whereas starch digestion by Ra was greater ($P<0.05$) in B than in M or W. Bf digested starch in B and M to a similar extent but was virtually unable to digest the starch in W. Ammonia production by Bf was greater ($P<0.001$) from W than from B or M. SEM revealed that starch granules were the initial colonization sites in all grains for Sb and Ra, but not for Bf. There was subsequent colonization, but only superficial digestion, of M starch granules by Bf. Variations in the endosperm structure of cereal grains contribute to the differential effectiveness with which amylolytic species can utilize cereal starch.

8

Factors Affecting Lactate Uptake by Selenomonas ruminantium HD4. D. J. Nisbet[1,*] and S. A. Martin[1,2], Dept. of Animal and Dairy Sci.[1] and Dept. of Microbiology[2], University of Georgia, Athens, GA 30602

Growth of Selenomonas ruminantium HD4 in medium that contained 2 g/l D,L-lactate was stimulated 2-fold by 10 mM aspartate, fumarate, or malate. When all three compounds were added together, growth was increased 3-fold. Lactate uptake by intact cells of S. ruminantium HD4 was stimulated by 10 mM aspartate (4-fold), fumarate (4-fold), malate (10-fold), and combination of all three compounds increased uptake 10-fold. Excess (10 mM) glucose and maltose inhibited lactate uptake 36 and 22%, respectively. No inhibition of lactate uptake occurred with excess (10 mM) sucrose or xylose. Cells grown on xylose, glucose, and sucrose were able to mediate lactate uptake. Aspergillus oryzae fermentation extract (Amaferm) and a yeast culture (YEA-SACC) stimulated lactate uptake by 9-fold and 4-fold, respectively. A filter sterilized filtrate of Amaferm also stimulated lactate uptake 4-fold. Overall, our data demonstrates that lactate uptake is stimulated in S. ruminantium HD4 by four carbon dicarboxylic acid TCA intermediates, and an alpha amino four carbon dicarboxylic acid.

9

AMINO ACID TRANSPORT BY A MONENSIN-SENSITIVE AMMONIA-PRODUCING RUMINAL BACTERIUM. G. Chen and J. B. Russell, Dept. Animal Science, Cornell University and ARS-USDA, Ithaca, NY 14853

Strain F, a Gram-positive rod, grew rapidly with glutamate or glutamine as an energy source in the presence but not the absence of Na. Monensin, a sodium/proton antiporter, completely inhibited bacterial growth and significantly reduced ammonia production (85%), but a protonophore and valinomycin had little effect. A proton ATPase inhibitor had no effect. Transport was biphasic showing unusually high rates at high substrate concentrations. At low substrate concentrations (<100 uM), the Km values for glutamate and glutamine were 4 and 11 uM, respectively. Each carrier could be driven by a chemical gradient of sodium. An artificial $\Delta\Psi$ or $Z\Delta pH$ was unable to drive transport even if sodium was present. The glutamate carrier had a single binding site for sodium with a Km of 21 mM; the glutamine carrier appeared to have 2 binding sites and the Km was 2.8 mM. Histidine and serine were also rapidly transported by sodium dependent systems, but serine alone did not allow growth even if sodium was present. Because exponentially growing cells at pH 6.7 had little $\Delta\Psi$ (-3 mV) and a slightly reversed $Z\Delta pH$ (+17 mV), it appeared that the membrane bioenergetics of strain F were solely dependent on a sodium circulation

10

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, Ky.

Bacteroides ruminicola strain 23 could initiate growth in media containing 0.625mg of monensin/L only after extended incubation, and would not initiate growth in media containing greater quantities of monensin. Adaptation of strain 23 in media containing increasing concentrations of monensin resulted in an ionophore resistant strain which initiated growth in media containing 40mg of monensin/L. Actively growing cultures of the resistant and sensitive strains of 23 in the absence of monensin challenge maintained a similar intracellular sodium content (0.069mg Na/mg protein). However, the intracellular potassium content for the sensitive strain(0.15mg K/mg protein) was greater than that measured for the resistant strain(0.10mg K/mg protein). Challenge of the sensitive strain of 23 with 10mg of monensin/L resulted in an increase in intracellular sodium content(0.079mg Na/mg protein). Both the sensitive and resistant strains had a slightly greater intracellular potassium content after challenge with monensin(.16 and .12mg K/mg protein, respectively). These results suggest that adaptation to growth in the presence of monensin results in a change in the sodium and potassium gradients maintained by the organism, and that monensin challenge has a significant effect on intracellular sodium content.

11

ANAEROBIC FUNGI ARE NOT ALWAYS ELIMINATED FROM THE RUMEN BY SHORT-TERM TREATMENT WITH MONENSIN.
G. Gordon* and M. Phillips, CSIRO Division of Animal Production, PO Box 239, Blacktown, NSW 2148, Australia

Monensin is used as a feed additive for intensively reared ruminants and it is finding increased use in Australia for the control of pasture bloat. Recently Elliott et al. (1987; J.Agric.Sci. 109:13) reported that anaerobic fungi were eliminated from the rumen of sheep by brief treatment with monensin (20 mg/d) and that the commonly observed effects of this ionophore (depressed feed intake and altered VFA profiles) were due to the absence of fungi. The elimination of anaerobic fungi from the rumen of experimental animals would allow the assessment of the contribution of these fungi to ruminal digestion. We have attempted to extend these results by including two daily doses of sodium monensin (40 and 80 mg) in different diets fed to penned sheep for 7-10 d. Anaerobic fungi were present in all samples of ruminal digesta including those obtained when feed intake was depressed and VFA profiles altered. In vitro studies with anaerobic fungi isolated form both untreated and treated sheep indicated that resistance to the ionophore had not developed. Furthermore it was shown that the daily maximum concentrations of monensin in ruminal digesta ($\leq$ 10 µg/ml) were fungistatic rather than being fungicidal. Thus we consider that the elimination of ruminal anaerobic fungi is only possible when monensin is continually present in the rumen but the levels required are so high that animal health could be severely affected.

12

RESISTANT SPORANGIA IN ANAEROBIC FUNGI ALLOW SURVIVAL OUTSIDE RUMEN. D. Wubah and M.S. Fuller, Botany Dept., University of GA, Athens, 30602, and D.E. Akin*, Russell Research Center, ARS-USDA, Athens, GA 30613

Rumen fungi that have been studied to date are obligately anaerobic, and their life cycles have been reported to consist of sporangia and zoospores. However, one study from England indicated that fungi could be isolated from stored fecal pellets and air-dried saliva. Recently, we reported the discovery of resistant sporangia, which are typical of aerobic zoosporic fungi, from a Neocallimastix isolate. In order to evaluate the survival of rumen fungi outside the host animal and to more fully characterize these organisms, an investigation was made of fungi in dung and digesta of cattle. We isolated a Neocallimastix sp., a Caecomyces sp, and an Orpinomyces sp. from the dung and rumen of the same cow. All isolates from dung grew only in anerobic medium and best at 39 C compared to 25 C, indicating that fungi originated in the rumen and were not saprophytes from the pastures. Melanized sporangia were present in the dung from which fungi were isolated. These new isolates and other cultures have been shown to produce resting sporangia under laboratory conditions, but to date we have not been able to effect germination of resting sporangia. The discovery that many types of anaerobic fungi survive outside the rumen suggests a way in which rumen fungi may inoculate other hosts and be disseminated in nature. Additional research will result in methods for easier maintenance and handling of cultures.

13

EFFECT OF DEFAUNATION ON GASTROINTESTINAL PEPTIDE HORMONES IN SHEEP.

P.F. Frumholtz, R.J. Wallace and E.R. Orskov,
Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB, UK.

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 adult castrated male sheep received 700 g of general purpose diet twice daily. Defaunation resulted in an increased protein concentration in rumen fluid, and caused ruminal peptide concentrations to remain high for a longer time after feeding. There was no significant difference between the two treatments in secretion of the three hormones measured (gastrin G17, insulin, and cholecystokinin CCK-8). These observations suggest that there may be a species difference in the response of cattle and sheep to defaunation, or that defaunation may stimulate secretion of forms of gastric other than G17.

Itabashi, H., Kobayashi, T. and Matsumoto, M. (1983). Proc. Vth World Conf.Anim. Prod. 339-340.

14

POST-PRANDIAL PH MODERATION BY RUMINAL CILIATED PROTOZOA IN CATTLE FED A HIGH-GRAIN DIET.

T.G. Nagaraja,* Gene Towne, and A.A. Beharka, Dept. 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 rumimal emptying, omasal flushing and treatment with sodium sulfosuccinate. 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, comprised of nine genera. Defaunated cattle had lower ruminal pH ($p < .01$) than faunated cattle, but there was no treatment x time interaction. Ruminal lactate and NH_3-N concentrations were similar in both groups. Total VFA concentration had treatment by time interaction ($p < .05$) and was higher in defaunated than faunated steers. Ruminal propionate proportion was higher in defaunated ($p < .01$) than faunated steers but butyrate proportion was unaffected by the treatment. Viable anaerobic bacterial counts were higher ($p < .05$) in defaunated than faunated steers. Although, lactic acid-producers were higher and lactic acid-fermenters were lower in defaunated than faunated steers, the differences were not significant ($p < .05$). It appeared that post-prandial pH moderation by ruminal ciliated protozoa was because of reduced bacterial activity.

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ASSOCIATION OF A TEMPERATE BACTERIOPHAGE WITH A RUMINAL CELLULOLYTIC ANAEROBE RESEMBLING RUMINOCOCCUS ALBUS.

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In studies to determine the relationship between bacteriophages 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 produced 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 (1.6×10^8 pfu/ml) in the culture. With longer incubation, lysogenized cells increased, and cultures regrew to the preinfection optical density level. The phage has an icosahedral head (50 nm) and a non-contractile, flexible tail (120 nm). Phages could be induced by mitomycin C (5 ug/ml) and UV light (40-60 sec), releasing incomplete phage particles and intact phages (10:1). Spontaneous induction was also evident. Lawns of Butyrivibrio fibrisolvens D1 and 49 were sensitive to lysates 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 B. fibrisolvens D1 and 49.

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CELL-WALL CARBOHYDRATE DIGESTIBILITY IN RUMINANTS: PLANT IMPOSED LIMITS.

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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-courmaric acid concentration seems to be less inhibitory. In temperate species, lignin from grass stems is about 70% more inhibitory than legume lignin. 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 legume stems. Up to 3/4 of xylose may be digestible in leaves, about 1/2 in grass stems, and less than 1/4 in legume stems. A better understanding of these limitations will help develop cultivars with improved digestibility.

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REEVALUATION OF LIGNIN'S ROLE IN FORAGE FIBER DIGESTIBILITY.

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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, for both core and non-core fractions, were different ($P<.05$) between species for both leaf and stem tissues, and cutting date. Leaf NDF digestibility was different ($P<.05$) among some cultivars, but species were not different. Stem NDF digestibility was different ($P<.05$) between species, but differences varied by cutting. Digestibility of NDF was negatively correlated ($P<.05$) with core lignin concentration across cuttings and species. Leaf NDF digestibility was not correlated with core lignin within cuts, while stem NDF digestibility was negatively correlated ($P<.10$) for all cuttings. Within species and cutting, core lignin concentration was rarely correlated ($P<.15$) with NDF digestibility. Composition of core and non-core lignins was correlated ($P<.15$) with NDF digestibility, within species and cutting. Data suggest concentration of core lignin is not the dominant chemical characteristic of forage cell walls limiting fiber digestibility.

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EVAULATION OF THE CAPTEC CONTROLLED RELEASE DEVICE FOR FECAL OUTPUT ESTIMATION.

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The realibility of the Captec controlled release chromic oxide capsule was evaluated in two trials. Eight ruminally cannulated steers fed either alfalfa (AH) or Coastal bermudagrass hay (CBH) ad libitum or fed a pelleted commercial sheep diet at 2.5% (P2.5) or 1.5% (P1.5) 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 AH and slowest for steers fed CBH with high animal variation regardless of diet. Since each steer received only one capsule, differences could not be attributed to animal or capsule. In a second trial, nine steers fed either AH, P1.5 or P2.5 were dosed with four capsules each. Release rates of chromic oxide among capsules dosed to the same animal were similar ($P<.01$) however, release rates of chromic oxide among animals on the same diet were different ($P<.05$). Release rates were higher ($P<.01$) for AH (4.94%) compared to both levels of pellets, which were similar. (P2.5:4.47%; P1.5:4.21%). Because of the high among-animal variation and possible variation due to diet, Captec capsules should not be utilized to estimate fecal output of individual animals without further correction.

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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.

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THE EFFECTS OF PILOCARPINE ON RUMINAL AND DIGESTIVE CHARACTERISTICS OF BEEF STEERS FED A HIGH GRAIN DIET. J. P. Peters, B. Hibbard, R. Y. W. Shen, and S. T. Chester, Microbiology and Nutrition Research and Biostatistics and Research Information Systems, The Upjohn Co., Kalamazoo, MI 49001

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 (digestibilities of DM, OM, and N, N retention, gain and feed efficiency). Although voluntary feed intakes did not differ, there was a dose-dependent slowing of feed consumption rate. Also, ruminal pH was increased with increasing dose. Ruminal 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.

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THE EFFECTS OF RUMINAL LACTIC ACIDOSIS ON BLOOD K+ LEVELS OF CALVES. E.C. Critchlow*, J.S. Kim and M.D. McMullen. Dept. Vet. Physiol. Sci., Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada.

A consensus on the changes in blood K+ levels induced by ruminal lactic acidosis is, as yet, unavailable. In view of this, blood pH, Na+, K+, and plasma osmolality as well as rumen pH and reticular motility were monitored in 5 rumen fistulated calves before and for 40 hours after ruminal carbohydrate overloading. Within 8 hours of overloading there was a significant drop in rumen pH from 7.60 ± 0.19 (mean ± SE) to 4.46 ± 0.06. This pH decrease was accompanied by stasis or severe impairment of reticular motility. Following carbohydrate overloading venous blood pH decreased from 7.38 ± 0.01 to 7.16 ± 0.13 and blood Na+ levels increased, as a result of hemoconcentration, from 136.6 ± 0.81 to 145.0 ± 2.14 mmol/L. In spite of this hemoconcentration, indicated by an increase in plasma osmolality from 260.0 ± 1.22 to 290.2 ± 4.35 mmol/kg, blood K+ levels decreased from 3.37 ± 0.16 to 1.97 ± 0.13 mmol/L. From these findings we have concluded that ruminal lactic acidosis induces, in calves, a significant decrease in blood K+ levels which may, in part, account for the behavioral changes seen in this disease.

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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, Colorado 80523.

PEM in ruminants has often been associated with altered thiamin 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 thiamin in rumen fluid or blood. Addition of thiamin diphosphate had no demonstrable effect upon blood transketolase activity. The odor of H_2S in eructated rumen gas was associated with nasal discharge and transient elevations of respiration 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-affected, 515±151 uM, n=3, $p<0.05$). It is hypothesized that PEM can result from increased production of sulfide in the rumen and is a form of subacute H_2S neurotoxicity. (Supported by USDA/CSRS, 87-CRSR-2-3208)

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DIET-RELATED RESPONSE TO PARATHYROID HORMONE (PTH) IN BLUE DUIKER ANTELOPE. B.L. Roeder[1]*, R.F. Wideman[2], G.A. Varga[3], B.W. Hollis[4], R.M. Leach[2], Dept.'s of [1]Vet. Sci., [2]Poultry Sci., [3]Dairy and Animal Sci., Penn State Univ. (PSU), University Park, PA 16802, and [4]Medical Univ. of SC, Charleston, SC 29425.

Herd profiles of Blue duiker antelope (Cephalophus monticola bicolor) 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% Cl, and 0.29% S on an 89.2% DM basis. Sixteen young and older adult males (♂) were fed an experimental diet similarly formulated but adjusted to 4 levels of Ca and P (0.5%:0.4%, 0.8%:0.8%, 0.8%:0.4%, 1.2%:0.4%) for 4 months. Pre- and post-trial baseline plasma vitamin D, serial serum (S) and 24h urine (U) clearance studies post-PTH challenge were performed. Pre-trial, young ♂ had higher mean P_S and 25(OH)D levels, and lower Ca_S, Ca_U, P_U, and $1,25(OH)_2D$ values than older ♂. In response to PTH, both groups decreased Ca_S and P_S proceeded by a rebound in P_S 60 min later. Post-trial, young ♂ had higher mean Ca_S and P_S, similar 25(OH)D and significantly lower $1,25(OH)_2D$ values than older ♂. PTH response in both groups resulted in a rise in Ca_S and decrease followed by a rebound in P_S. Young ♂ had lower Ca_U and higher P_U excretion post-PTH. These repeated measurements indicated that unlike domestic ruminants, dietary Ca and P does significantly affect duikers' response to PTH.

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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 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 6%(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 (Flatpea) Toxicity in Sheep and Evidence for Adaptive Tolerance

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Previous reports have suggested that ruminants can successfully adapt to diets containing the lathyrogenic forage, flatpea. Our preliminary studies, however, indicated that 17- and 38-day stepwise adaptation periods inadequately protected sheep from flatpea intoxication. Symptoms of intoxication when flatpea consumption approached 75% of the diet included: anorexia, muscular tremors, incoordination, lethargy, excessive salivation, tetanic seizures, and death. In contrast, prolonged exposure (4 months) to 50% dietary flatpea resulted in successful adaptation and these animals subsequently consumed 100% flatpea with no ill effects. When rumen contents of alfalfa-fed and flatpea-fed sheep were exchanged, the formerly tolerant, flatpea-fed animal became susceptible, exhibiting symptoms of lathyrism within 48 hours. In contrast, the formerly naive, alfalfa-fed animal subsisted on 100% flatpea (administered intraruminally) with no signs of toxicity. These observations suggest that adaptive tolerance to flatpea 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. Wattiaux*, L.D Satter, and D.R. Mertens. 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 ($p<.05$), 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 0.97 to 1.08 in AH and 1.02 to 1.17 in AS. These values indicate that AS 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: 5.27, 11.74; 6.5, 13.83 for AH and AS diets respectively. Values for digesta from the reticulum were: 4.83, 10.74; 5.95, 13.27. In both locations, there was more DM in digesta with the AS than AH diet ($p<.0001$).

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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SLUICING THROUGH THE RUMEN. J.D.Garza, J. Zorrilla-Rios and E.Owens. Dept. of Animal Sci., Okla State U., Stillwater 74078.

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 an 80% concentrate or an all roughage diet once daily with free choice access to water. Ruminal outflow of PEG was only 19 to 40% of PEG intake indicating that 60 to 81% of the PEG (and of consumed water) was sluicing through the rumen without equilibrating with ruminal liquid. In a second study, 4 cattle were fed concentrate or roughage diets twice daily; ruminal evasion of consumed water averaged 79 and 44%, respectively though diurnal variation in ruminal marker concentrations were quite large. In a third study, CrEDTA was included in water and CoEDTA was dosed at 8 h intervals into the rumen. Ruminal evasion, calculated from relative marker concentrations in the rumen 3 d 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 ITS ROLE IN RUMEN FUNCTION AND PRODUCTIVITY OF THE DAIRY COW. K. A. Beauchemin and J. G. Buchanan-Smith, Agriculture Canada, Lethbridge, Alberta T1J 4B1 and University of Guelph, Guelph, Ontario N1G 2W1.

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-grain diets and ensiled forages by producers. Ingested grain is rapidly fermented in the rumen causing a marked decline in pH of rumen fluid which depresses cellulolysis. This is particularly evident when concentrates are barley-based, finely processed or allocated infrequently during the day. Effective fiber is needed to promote chewing during eating and ruminating. Saliva output increases during chewing, 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 reticulorumen. 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 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 53706

Eight rumen cannulated multiparous cows were used in a replicated 4x4 Latin square design with a 2x2 factorial treatment arrangement. Factors were feeding 4% PEG (1000 MW) and replacing 25% of prerial rumen volume with water filled bladders. Periods were 21 d and treatments began 3 wk postpartum. Diets were 36% alfalfa silage, 17% corn silage and 47% concentrate (23%CP, 19%ADF and 27% NDF). Lanthanum, Co-EDTA and Ytterbium-cell walls served as digestibility and passage markers, respectively. PEG did not increase rumen osmolarity and did not affect liquid outflow, DMI, 3.5% FCM, or rumen PH. Total tract ADF digestibility was reduced by PEG (39.4 vs 47.3%). Bladder main effects are in table.

	Bladders	
Treatment	+	-
DMI,kg	22.0	24.9*
FCM,kg	35.6	37.8*
Rumen Vol, l		
Total	[illegible]	97.3**
Digesta only	[illegible]	97.3
Digest Wt., kg	[illegible]	79.7*
Digesta DM,%	16.1	17.5
Digestibility %		
DM	58.8	60.7
ADF	43.4	43.3
Rumen Empty BW		
kg	563	574*
chg/21 d, kg	-9.6	9.5*

*P<.05 **P<.01

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A COMPARTMENTAL MODEL TO DESCRIBE RUMINAL IN SITU DIGESTION J. van Milgen*, M.R. Murphy and L.L. Berger
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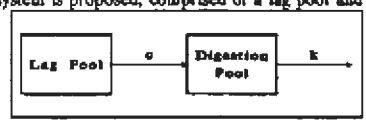
Ruminal in situ studies are frequently used to evaluate the nutritive value of feedstuffs, and different models have been employed to describe the results of such studies. Most of these models assume an instantaneous onset of digestion, which is probably not a common phenomenon in the rumen. A two pool system is proposed, comprised of a lag pool and a digestion pool. Only material in the digestion pool is subject to first-order kinetics digestion. At incubation, all material 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:



$$\text{RESIDUE} = F_d \cdot e^{-c \cdot t} + F_d \cdot c \cdot (e^{-c \cdot t} - e^{-k \cdot t})/(k-c) + F_i$$

where F_d is the potentially digestible fraction, F_i the indigestible fraction, c the lag rate constant (h^{-1}), and k the digestion rate constant (h^{-1}). The equation yields a sigmoidal curve with a maximum for $t=0$, an asymptote at F_i, and an inflection point for $t=(\ln(c)-\ln(k))/(c-k)$. Parameters are estimated using nonlinear least-squares regression. An advantage of this model is that a first-order kinetics process delays digestion, rather than a time delay.

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DEVELOPMENT OF AN ISOLATED RUMEN EPITHELIAL CELL INCUBATION SYSTEM. R.L. Baldwin, VI, and B.W. Jesse, Dept. of Animal Sciences, Rutgers, The State University, New Brunswick, NJ 08903.

A rumen epithelial cell isolation system has been developed using rumens from Dorset ram lambs. The caudal-dorsal rumen sac was removed immediately after slaughter, thoroughly rinsed in warm tap water and transported in 37°C Krebs Ringer salts plus 25 mM HEPES, pH 7.4. Papillae were clipped (10 mm^2) from the rumen wall, and 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 containing isolated cells was removed from the undigested papillary fragments, and fresh trypsin added to the remaining fragments. This procedure was repeated for a total of eight cycles. The first two fractions, containing mainly keratinized cells, were discarded. Subsequent fractions were quickly cooled on ice, and the cells pelleted by centrifugation at 60 x g. Cell pellets were resuspended in KRB. Rumen cell viabilities ranged from 75-90%. Butyrate was converted to ß-hydroxybutyrate at high rates by these cells. This system should be useful in future investigations of rumen function.

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ENERGY SUPPLEMENTATION TO ROTATIONALLY GRAZED DAIRY COWS.
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Mid-lactation Holsteins (n=18) averaging 28 Kg milk/d were supplemented every 12 h with: A) 2.5 Kg DM/d of a concentrate containing 84.6% dried, ground, shelled corn, 7% vitamins, and 8.4% minerals (GM), B) GM + 2.46 Kg DM/d of corn, or C) GM + 4.92 Kg DM/d of corn in a two period cross over design. Periods were 35 d with data collected on d 22-35. 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 by 6.1 Kg/d. There were significant differences ($P<.04$) between A and C in the yield (Kg/d) of milk (18 vs 19.9), milk fat (.59 vs .67), milk protein (.57 vs .63), and 3.5% FCM (17.3 vs 19.5). There were no differences for B vs A + C ($P>.19$) in any of the parameters measured. There were no 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 4.96 Kg DM/d of concentrate.

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Impact of Type and Level of Protein or Energy Supplementation on In Vitro Digestibility of Kikuyu (Pennisetum clandestinum) and Pangola (Digitaria decumbens) Grasses. J.K. Carpenter*, S.Y. Iha, and R.Y. Niino-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 nutrient 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 (FM, SBOM or CSM added to raise the crude protein level of the total ration to either 10, 13, 16 or 20% of the DM) on total ration dry matter and cell wall digestibility of kikuyu and pangola grasses. The CP, ash, NDF, 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 pangola grass, respectively. The addition of either energy or protein altered ($P<.05$) the in vitro digestibility of the total rations (ranged from 64.9 to 83.1% for kikuyu and 67.0 to 84.3% for pangola), but did not alter ($P>.05$) the cell wall digestibility (ranged from 63.5 to 67.5% for kikuyu and 65.3 to 72.6% for pangola). These results show that energy and/or protein availability is not the limiting factor for microbial digestion of tropical forages, thus suggesting that the efficiency of mastication, rate of hydration and/or ease of microbial attachment are the factors which limit rate of fiber digestion and passage.

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MICROBIAL FERMENTATION AND SITE OF NUTRIENT DIGESTION IN STEERS FED DIETS VARYING IN FORAGE AND ENERGY SOURCE.
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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, nutrient 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% of the DM as long alfalfa 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 greatest ruminal organic matter digestions. Ruminal starch digestion was greater in barley (89%) vs corn (78%) diets and ruminal organic matter digestion was greater in alfalfa (53%) 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 (63%) 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 3.5 and 3.0 for alfalfa and corn silage diets, respectively. No interactions were observed between forage and energy source.

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TOTAL STARCH AND RELATIVE STARCH AVAILABILITY OF FEED GRAINS.
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Total starch in grains was determined by hydrolysis with amyloglucosidase (Diazyme L-200). Samples of grain (200 mg) were autoclaved for 1 h in tubes with 2 ml of 20% $CaCl_2$ (pH 2). Enzyme solution (8 ml, 50 Diazyme 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 YSI glucose 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 98.8, .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 (R) and a first order digestion rate for the slowly degraded fraction (K_d). The method was used to evaluate starch availability in flaked sorghum grain varying from 451 to 232 g/l. Decreasing test weight increased both R and K_d. To establish biological significance of the method, in vitro hydrolysis was regressed on in vivo total tract starch digestibility from 2 lactation trials evaluating grains differing in starch availability. Actual 4 h in vitro hydrolysis was more highly correlated with in vivo digestibility (R^2=.96) than either R (R^2=.86) or K_d (R^2=.91).

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ACID OR FORMALDEHYDE TREATMENT OF ALFALFA SILAGE FOR MILK PRODUCTION. S.A. Nagel* and G.A. Broderick, Dept. of Dairy Science and USDA/ARS, 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 (Control, C), treated with 6.4 l/ton formic acid (F), or 2.9 kg/ton Grainmax® (containing formaldehyde, G), and ensiled in polyethylene bags. Twenty-two multiparous cows were assigned to one of the treatments on d 18 of lactation, following a two wk covariate period. Each treatment diet contained 98.5% alfalfa silage, 1.4% minerals and vitamins, and .1% Ketoban® (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:

Diet	CP	ADF	DMI	Milk	Fat	Protein
	(%)		(kg/day)			
C	21.4	32.9	18.3	29.2[b]	1.1[b]	.81[b]
F	20.8	29.8	18.2	32.6[a]	1.3[a]	.92[a]
G	21.1	31.9	19.7	32.5[a]	1.3[a]	.87[a,b]

[a,b]Means in the same column with different superscripts differ ($p<.05$).

From wk 7 to 10 of lactation, cows entered a switchback experiment. Half of each group continued on their original diet, and the other half received 4.8% fishmeal, fed to replace silage DM. After two wk, 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 content .1% on diets C and G ($p<.01$).

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REPRODUCTIVE PARAMETERS OF DAIRY COWS FED UREA DURING EARLY LACTATION. D. P. Casper, C. L. Austin*, and D. J. Schingoethe, Dept. of Dairy Sci., South Dakota State University, Brookings, SD 57007-0647.

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 studies evaluating diets containing soybean meal (n = 59) or urea (n = 70) in the concentrate mix. Total mixed diets were formulated to contain 16% crude protein on a dry matter basis. Addition of urea to the diet increased (P<.01) ruminal ammonia concentrations (11.3 and 16.1 mg/dl), but not (P>.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>.10) for cows fed soybean meal and urea. Regression analysis indicated that serum urea concentrations did not affect (P>.10) days to first breeding, services, and services per conception, but increased (r^2 = .049, P<.05) days open. When cows during early lactation were fed nutritionally balanced diets, the increased solubility of dietary nitrogen had no affect on reproduction.

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Rumen cation and methane responses to diet additions of Na, K and/or lasalocid. D.E. Johnson, H.P. Phetteplace and W.V. Rumpler, Dept. of Animal Sci., Colorado State Univ., Ft. Collins, CO 80523

Three 390 kg steers were fed a 71% corn diet with or without lasalocid (200 mg/hd/d), sodium or potassium (>2% as chloride salt) and lasalocid added along with a cation. Each was fed for 1½ 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 K from 37 up to 70 to 74 mM. Levels of the sum of Na + K were unchanged. Sodium chloride feeding increased ruminal Na levels by 10 to 15 mM (P<.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<.05). The ratio of acetate to propionate was decreased from 3.6 to approximately 3 by lasalocid additions (P<.05). An 11% depression in methane from lasalocid approached significance (P<.10). Cation additions also tended to depress methane 13 to 16%. The most depression of methane occurred with the combination of lasalocid + sodium or potassium (P<.05) a 23 to 29% decrease.

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IMPACT OF NaCl INTAKE ON RUMEN DIGESTA KINETICS. J. Zorrilla-Rios, J.D.Garza and F.Owens, Dept. of Animal Sci., Okla State U., Stillwater 74078.

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 evasion (PEG), CoEDTA dilution rate and ruminal volume (evacuation) were measured. Added salt had no effect on DM intake. It increased water consumption by 84% but ruminal water evasion remained largely unchanged at 60%. Although ruminal liquid dilution rate (Co) remained stable at about 4.3%, ruminal volume was reduced by nearly 50% (38 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.

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CELLULOSE DIGESTION AND CELLULASE REGULATION AND DISTRIBUTION IN FIBROBACTER SUCCINOGENES S85

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Fibrobacter succinogenes S85 initiates growth on microcrystalline cellulose without a lag whether inoculated from a glucose, cellobiose or cellulose culture. There is no accumulation of soluble carbohydrate during growth on cellulose. When the growth medium contains either glucose or cellobiose 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 cellobiosidase and periplasmic cellodextrinase are produced under all tested conditions of growth, indicating constitutive synthesis. Immunoelectron microscopy has revealed the presence of the chloride-stimulated cellobiosidase or an antigenically related protein on protrusions at the cell surface perhaps suggesting that these structures are involved in cellulose digestion.

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β-GALACTOSIDASE ACTIVITY OF FIBROBACTER SUCCINOGENES S85

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Fibrobacter 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 variant 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 E. coli, but polyclonal antibodies to the E. coli β-galactosidase did not react with it. Some fresh isolates of F. succinogenes may normally express β-galactosidase. This finding may help explain the ease of inoculation of young ruminants with fibrolytic bacteria.

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Heterologous Expression of Genes for Xylanolytic Enzymes from Bacteroides Species in Bacteroides fragilis and Escherichia coli. T.R. Whitehead and R.B. Hespell, NRRC, ARS/USDA, Peoria, IL 61604.

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. ruminicola 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. ruminicola. This is the first example of heterologous expression of genes between colonic and ruminal Bacteroides. Studies with the xylanolytic colonic species, B. ovatus, showed that xylanase, xylosidase, and arabinosidase activities were regulated in response to carbon source used for growth. The genes for these three activities were cloned on one 3.8-kb EcoRI fragment, and all three activities were expressed in E. coli.

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THE ORIGIN AND PROPERTIES OF FORMS OF *RUMINOCOCCUS FLAVEFACIENS* STRAIN 007 WHICH DIFFER IN THEIR ABILITY TO DEGRADE COTTON FIBRES

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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 fibres. A comparison of the form active in cotton degradation (007C) with the form possessing only limited ability to degrade cotton (007S) is being made to elucidate the nature and possible importance of factors involved in the degradation of highly ordered celluloses such as cotton. Form 007C was only slightly more active than 007S in degrading filter paper, avicel, Sigmacell, barley straw and wheat straw than was 007S. The β-glucosidase, β-1-4 endoglucanase, cellobiosidase 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 highly ordered celluloses, 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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INTERACTION OF RUMINAL BACTERIA IN THE PRODUCTION AND UTILIZATION OF DEXTRINS FROM SOLUBLE STARCH. M. A. Cotta, NRRC, ARS, USDA, Peoria, IL 61604.

Maltooligosaccharides (MS) are produced during the hydrolysis of soluble starch by crude enzyme preparations from amylolytic ruminal bacteria. To ascertain whether these products accumulate during growth, two strains of starch-degrading bacteria, Streptococcus bovis JB1 and Butyrivibrio fibrisolvens 49, were grown in 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 would be available for crossfeeding to 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. bovis resulted in little change in the patterns of MS observed with time over that with S. bovis 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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DEGRADATION OF BARLEY STRAW, RYEGRASS AND ALFALFA CELL WALLS BY CLOSTRIDIUM LONGISPORUM AND RUMINOCOCCUS ALBUS. V. H. Varel*[1], A. J. Richardson[2] and C. S. Stewart[2], USDA/ARS U.S. Meat Animal Res. Ctr., Clay Center, NE 68933 and The Rowett Research Institute, Aberdeen, AB2 9SB UK[2]

The recently isolated ruminal sporeforming cellulolytic anaerobe, Clostridium longisporum B6405, was examined for its ability to degrade barley straw, non-lignified 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 between 20 and 28% of the dry matter (DM) in barley straw in 10 days while the clostridium degraded less than 2%. A combined inoculum of R. albus SY3 and strain B6405 was no more active than SY3 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 (28% wt loss) as active as R. albus SY3 (15%). The percent DM degraded from ryegrass cell walls of mesophyll, epidermis and fiber for the clostridium was 50, 47 and 32, respectively, and for R. albus SY3, 77, 73 and 63, respectively. R. albus SY3 degraded ryegrass mesophyll cell walls most rapidly, with epidermis and fiber cell walls being degraded at similar rates. Strain B6405 attacked the alfalfa cell walls 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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EVIDENCE OF INHIBITION OF CELLWOLYSIS IN AN ANAEROBIC RUMEN FUNGUS BY GLUCOSE, CELLOBIOSE AND SOLUBLE STARCH. M. Morrison and R. I. Mackie, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

In the forage fed animal, effects associated with rumen defaunation have been attributed in part to an increased concentration of fibrolytic, anaerobic rumen fungi (ARF). The nutritional niche exposed by defaunation procedures warrants investigation to assess how 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 and sporangium morphology, was considered to be *Piromonas* like. After purification, the fungus was maintained on pebble milled cellulose, and a 4-day old culture used to inoculate media prepared to contain either [U-^{14}C]-cellulose alone or a combination of cellulose and glucose, cellobiose or starch. The release of ^{14}C-label was minimal for up to 24 hours in media prepared with an additional carbohydrate and disappearance of glucose, cellulose and starch exceeded 75, 75 and 33%, respectively. Over the initial 24h, cellulose solubilization was much more substantial when provided alone. However, this trend was rapidly reversed by 48 hours since breakdown of cellulose plus additional carbohydrates appeared to have reached maxima, 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 may be analogous to what occurs in vivo. Thus, the increase in ARF after defaunation need not reflect an enhanced role in fiber degradation for these microbes.

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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. Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

The degradation of wheat straw (WS) and alkaline hydrogen peroxide treated wheat straw (AHPWS) by *Ruminococcus albus* 8 and *Ruminococcus flavefaciens* FD-1 was determined by measuring the growth (OD_{600}) of each bacterium and determining dry matter disappearance (DM) of the substrate. Modified easy medium (MEM) and defined medium with or without the addition of phenyl-propanoic acid (PPA) and phenylacetic acid (PAA) was used. Tubes were incubated at 39°C for ten days. Both OD_{600} and DM indicated that AHPWS was degraded to much greater extent by both bacteria (FD-1,62.5% and strain 8,41.25%) over untreated WS (FD-1,17.5% and strain 8 7.5%). Most degradation occurred between day 1 and day 4. With MEM, addition of PPA and PAA did not have any major effect on degradation by either bacteria. *R. flavefaciens* FD-1 degraded 62.5% AHPWS and 58.75% AHPWS with PPA and PAA addition, and *R. albus* 8 degraded 41.25% AHPWS and 43.75% AHPWS with PPA and PAA addition. When defined media was used, the addition of PPA and PAA enhanced *R. albus* 8 degradation of AHPWS (40%) over AHPWS without added PPA and PAA (25%). No effect of PPA and PAA was observed for *R. flavefaciens* FD-1 or when the two bacteria were grown together. Nor was there a synergistic effect on degradation when the 2 bacteria were cocultured with either WS or AHPWS as the substrate. DM analysis showed that *R. flavefaciens* FD-1 more efficiently degraded AHPWS (ca. 5.8 mg/day) than *R. albus* 8 (4.37 mg/day).

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STIMULATED CELLULOSE DEGRADATION IN COCULTURES CONTAINING YEAST AND CELLULOLYTIC RUMEN BACTERIA. K. A. Dawson*, G. A. Harrison, K. E. Newman and S. Jenkins, Dept. of Animal Sci. University of Kentucky, Lexington, KY 40546.

Axenic cultures and cocultures of cellulolytic rumen bacteria, and yeast strains (Saccharomyces cerevisiae) 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 disks at a slower rate (.386 mg/hl than did axenic cultures of B. succinogenes (.424 ag/h). However, the lag time before the initiation of digestion was much shorter in cocultures (45 hl than in the axenic cultures (61 h). 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 168 h was similar in cocultures and axenic cultures (46.6 and 47.9 mg of 100 mg provided, respectively). Addition of yeast extract (1 mg/ml) did not significantly alter degradation patterns. Similar stimulation of cellulose digestion was observed in cocultures of yeast and Ruminococcus albus. This study suggests that low concentrations of live yeast ($<10^5$ /ml) can significantly influence cellulose digestion by ruminal bacteria.

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CELL SURFACE STRUCTURES OF RUMINAL CELLULOLYTIC BACTERIA J. Miron, M.T. Yokoyama and R. Lamed, Instit. of Animal Sci., The Volcani Center, Bet Dagan 50250, Israel; Dept. of Animal Sci., Michigan State Univ., East Lansing, MI 48824; and Dept. of Biotechnol., Tel-Aviv. Univ., Ramat Aviv, Israel.

Previous research has demonstrated the occurrence of discrete cell surface structures (cellulosomes) considered to be responsible for efficient cellulolysis and adhesion of cellulolytic bacteria. In this study, the cationized ferritin-scanning electron microscopy procedure (CF-SEM) was used to determine if similar cell surface structures could be shown for ruminal cellulolytic bacteria. Bacteroides (Fibrobacter) succinogenes S85, Ruminococcus flavefaciens FD1 and Ruminococcus albus 7 were grown on either lucerne cell walls (0.4%) or cellobiose (0.2%). Strains adapted to these substrates were examined by CF-SEM. When grown on cell walls, all strains showed the presence of amorphous protuberant structures on their cell surface, with an extensive network of these structures bridging cell to cell and attaching cells to the substrate. In contrast, when grown on cellobiose, the strains showed considerably less or none of these protuberances on their cell surfaces. Cultures adapted to cell walls showed a higher cellulose adhesion than cultures adapted to cellobiose. These results suggest that the presence of these protuberant structures on the cell surface of ruminal cellulolytic stains is induced by growth on lucerne cell walls, and inhibited by growth on cellobiose.

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EFFECTS OF MICROMINERALS ON THE GROWTH CHARACTERISTICS OF CELLULOLYTIC RUMINAL BACTERIA. D. C. Sanevan,* R. I. Mackie, and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

Pure cultures of cellulolytic ruminal bacteria (*Ruminococcus flavefaciens* FD-1, *Ruminococcus albus* 8, and *Bacteroides succinogenes* S-85) were used to study the influence of molybdenum (Mo), manganese (Mn), zinc (Zn), cobalt (Co) and iron (Fe) on growth rate of these strains inoculated in 0.1% cellobiose broth media. Nominal levels of Zn, Co, Fe in media were 0, 2.5, 5.0, 7.5, 10.0, 15.0 and 25.0 mg/L, while Mo was at 0, 25, 50, 75, 100, 150 and 250 mg/L and Mn was at 0, 10, 20, 40, 60, 80, and 100 mg/L in the broth. Cell growth ($O.D._{600}$) was recorded every hour until stationary phase. The results indicated that FD-1 and S-85 responded well up to 75 mg/L Mo concentration in the medium, then growth rate declined as Mo level in media increased. Strain 8 was found to be more sensitive to Mo levels and showed retarded growth beyond 25 mg/L. Mn stimulated growth of all bacterial strains when its concentration increased from 10 to 40 mg/L in the media. However, it had an inhibitory effect on bacterial growth beyond 60 mg for strain 8 and 80 mg for FD-1, while no such effect was observed for S-85. The concentration of Zn up to 7.5 mg/L in media stimulated growth for FD-1 and strain 8. Beyond this level it retarded the growth rate in these strains. However, S-85 continued to grow well even at 25 mg/L of Zn. Cobalt at 0 mg/L and greater concentration in the media had an inhibitory effect on FD-1 and strain 8, while 15 mg/L cobalt slowed down cell growth in S-85. Iron increased the bacterial growth rate of FD-1 and strain 8 up to 10 mg/L in media and then a decreasing trend was observed beyond this level. However, no deleterious effect of Fe supplementation was observed on S-85 even at 25 mg/L level. Thus, optimal levels of microminerals for growth of three cellulolytic strains were different, with S-85 showing higher requirements for the trace elements tested.

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ATPase-DEPENDENT ENERGY SPILLING BY THE RUMINAL BACTERIUM STREPTOCOCCUS BOVIS. J. B. Russell and H. J. Strobel*. ARS-USDA and Dept. Animal Science, Cornell University, Ithaca, NY 14853

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/ug protein (6.1 mmol glucose/h/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 protonophores and monensin 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 bacterium 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.

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THE INTERACTION BETWEEN pH AND IONOPHORES ON CONTINUOUS CULTURES OF STREPTOCOCCUS BOVIS. J. M. Chow and J. B. Russell. Dept. of Animal Science, Cornell University and ARS/USDA, Ithaca, NY 14853.

The effects of ionophores on pure cultures of ruminal bacteria have usually been measured in batch culture and at near neutral pH. When Streptococcus bovis JB1 was grown in continuous culture at pH 6.7 with a dilution rate of 0.1 per h, monensin concentrations as high as 0.2 uM had little effect on yield. As monensin was increased further, yield declined, but some growth was observed even if the concentration was as great as 20 uM. Since less than 30% of the decline in yield was explained by a switch to lactate, it appeared that a majority of the growth inhibition was caused by a futile cycle of ions through the cell membrane. When pH was decreased from 6.7 to 5.7, the amount of monensin needed to decrease yield by 50% was 10 fold lower (0.14 versus 1.43 uM). Lasalocid was 6 fold more potent than monensin at 6.7, and it was only 2.5 fold more inhibitory if pH was decreased to 5.7. Because unadapted cells and continuous cultures did not grow in batch culture if monensin was greater than 10 uM, the concept of mimimum inhibitory concentration (MIC) is questionable.

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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, Microbiology and Nutrition Research and Biostatistics and Research Information Systems, The Upjohn Co., Kalamazoo, Michigan 49001.

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Selenomonas ruminantium, HD_4 Fermentation and Cell Yield Response to Limiting and Non-Limiting Concentrations of Ammonium Chloride - S.C. Ricke[1] and D.M. Schaefer[2], ARS-USDA and Microbiology Dept., North Carolina State Univ., Raleigh, NC[1], and Meat and Animal Science Dept., Univ. of Wisc., Madison, WI.[2]

The objective of this study was to assess fermentation and cell yield response of *Selenomonas ruminantium* HD_4 through a range of physiological and non-physiological ammonia-nitrogen (NH_3-N) concentrations. Cells were grown in continuous culture with a defined ascorbate - reduced basal medium containing either 0.5, 5.0, 25.0, 50.0, 100.0, and 200.0 mM NH_4Cl and dilution rates (DR) were pooled with categorical means (hrs^{-1}) of 0.07, 0.14, 0.24 and 0.40. NH_3-N was the growth-limiting nutrient (K_s = 71.5 uM) when 0.5 mM NH_4Cl was provided. Glucose disappeared and acetate (A) and propionate (PROP) concentrations formed were lower at 0.5 mM versus the higher NH_4Cl concentrations ($P < 0.05$). Lactate (L) was higher at 0.5 and 5.0 mM NH_4Cl ($P < 0.05$). Five-fold more NH_3-N was used at 5.0 and 25.0 mM versus 0.5 mM NH_4Cl and twice as much NH_3-N was used at 50.0, 100.0 and 200.0 mM NH_4Cl than at 5.0 and 25.0 mM NH_4Cl ($P < 0.05$). Glucose disappearance and product-carbon formation rates were higher at 0.5 mM NH_4Cl versus the higher NH_4Cl concentrations ($P < 0.05$). L increased five-fold at the fastest DR for 5.0 mM NH_4Cl while A and PROP decreased under these conditions whereas L remained low and A and PROP remained high for all DR when NH_4Cl concentrations were 25.0 mM and above. Cell yield, expressed as $Y_{glucose}$ and Y_{ATP} were nearly doubled when NH_4Cl was increased from 0.5 mM NH_4Cl (25.1 g cells per mole glucose disappeared and 13.9 g cells per mole ATP produced, respectively) to the higher NH_4Cl concentrations and were highest at 25.0 mM NH_4Cl (48.2 and 23.2, respectively) ($P < 0.05$). $Y_{ammonia}$ was highest at the lowest NH_4Cl concentration. Apparently, maximal fermentation rate and maximal bacterial yield do not occur at the same NH_3-N concentration for this organism.

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ACTIVITIES OF AMMONIA-ASSIMILATORY ENZYMES OF *Ruminococcus flavefaciens* FD-1. P.A. Duncan and R.I. Mackie, 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 NH_4Cl, 10mM cellobiose) and nitrogen (1mM NH_4Cl, 20mM cellobiose) limitation. Cells were harvested in mid-expotential 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), asparagine synthetase (AS) and glutamate synthase (GOGAT) activities. NADPH-linked GDH activity was higher under conditions of carbon limitation than N-limitation (182 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.

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TRANSFORMATION SYSTEMS AND PLASMID CONSTRUCTIONS FOR USE IN *BACTEROIDES RUMINICOLA*
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Transformation of *Bacteroides ruminicola* F101, a derivative of strain B_14, by the naturally occurring tetracycline resistance plasmid pRR14 (19.5 kbp) was achieved using electroporation at frequencies up to 10^8/µg DNA. Similar procedures gave transformation of *B. uniformis* 1100, but not *B. ruminicola* F101, by the *E. coli/Bacteroides* shuttle vector pDP1. A potential shuttle plasmid for use in *B. ruminicola* has been constructed from a cryptic *B. ruminicola* plasmid (pRR12), an *E. coli* plasmid carrying a multiple cloning site and a *Bacteroides* drug resistance marker.

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ISOLATION AND REGULATION OF GENES CONCERNED WITH XYLAN UTILISATION IN *RUMINOCOCCUS FLAVEFACIENS*.
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Ruminococcus flavefaciens 17 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.

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MORE CLONING OF ENDOGLUCANASE GENES FROM *Ruminococcus flavefaciens*. V. K. Gupta*, G. T. Howard, S. Rosenzweig, and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

A genomic library of *Ruminococcus flavefaciens* FD-1 DNA was constructed using the *Escherichia coli* bacteriophage λ vector, λZAP II. Recombinant phage progeny were screened for cellulolytic activity by plating with appropriate host *E. coli* in soft agar (0.7%) overlays containing 1.0% (w/v) Ostazin brilliant red - hydroxyethyl cellulose (OBR-HEC). An OBR-HEC positive recombinant phage designated FD1-71 was plaque purified to greater than 80% and then the insert DNA in the plasmid pBluescript was excised from FD1-71 using R_{408} helperphage and rescued in *E. coli* XL-1-Blue. Of the 16 *E. coli* clones with rescued pBluescript plasmid, one clone, designated FD1-71.4 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 size estimation by agarose gel electrophoresis. ^{32}P-labelled probe was generated using the 2.2 kb DNA insert from this plasmid (pBAW201) for Southern blotting. This insert hybridized 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* (pMEB200) and *celB* (pBAW101), 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.

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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

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RESTRICTION/MODIFICATION SYSTEMS IN *Ruminococcus albus* 8 AND *Ruminococcus flavefaciens* FD-1. M. Morrison* and B. A. White, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

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GROWTH CHARACTERISTICS OF DIHYDROXYPRIDINE DEGRADING BACTERIA (ISOLATES 32-24) C.S. McSweeney, R.I. Mackie and H. Morrison, Dept. of Animal Sci., University of Illinois, Urbana-Champaign, IL 61801

Leucaena leucocephala is a tropical leguminous shrub with considerable potential as a nitrogen supplement for increasing animal production. However, application is limited by the occurrence of mimosine, a free potent goitrogen 3-hydroxy-4-1(H)-pyridone (3,4-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 3,4-DHP and its commercially available isomer 2,3-DHP. Isolate 32-24, a short gram positive straining rod 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 as 13-15h 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.

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CHLOROGENIC ACID AND ITS INFLUENCE ON NEUTRAL DETERGENT FIBER DIGESTION. D.J.R. Cherney*, J.A. Patterson, and J.H. Cherney, Dept. of Animal Sciences and Dept. of Agronomy, Purdue University, West Lafayette, IN 47907

Low molecular weight phenolic acids may decrease utilization of forage fiber by limiting microbial digestion of bound structural carbohydrates. Caffeic acid was previously identified as a major alkali-labile component of limpograss [*Hemarthria altissima* (Poir.) Staph. & Hubbard]. Chlorogenic acid was identified as the caffeic acid ester producing the relatively large amounts of caffeic acid in limpograss after base hydrolysis. In a series of experiments, chlorogenic acid in limpograss was characterized and its influence on fiber digestibility investigated. Chlorogenic acid concentrations varied with harvest date, canopy level, and morphological component (1 to 12 g kg^{-1} DM). Chlorogenic acid was released almost immediately after ground limpograss tissue was wetted with water or methanol [$Y = 12.2 + 0.55\ X$, $r^2 = 0.78$, where Y = g kg^{-1} DM and X = time (h)]. In another study, digestibility of alfalfa and orchardgrass was reduced 1-2% when a water extract of limpograss was added to incubating samples. Studies are currently being conducted to evaluate the influence of chlorogenic acid on fiber digestion by pure cultures of ruminal bacteria.

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RUMINAL ANAEROBES AND PYRROLIZIDINE ALKALOID DETOXIFICATION A.M. Craig*, L.L. Blythe and E.D. Lassen, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331

Degradation of toxic molecules by anaerobic microorganisms has come to the forefront 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 found in tansy ragwort (Senecio jacobaea). This mechanism is proposed as the primary reason why sheep are resistant to pyrrolizidine alkaloid toxicosis and cattle and horses are not. A series of experiments were conducted that 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 hepatopathy as is seen in clinical and experimental cases of tansy toxicosis was the result as determined by clinical progression, alterations in serum enzymes and histopathology. Secondly, ruminal fluid taken from sheep had a rapid, i.e., less than 24 hours, 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 hours. Finally, differential centrifugation 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.

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PHOTOSENSITIVITY OF CATTLE GRAZING ALFALFA PASTURES M.L. Schlegel, C.J. Wachenheim, M.E. Benson, J.R. Black, W.J. Moline, H.D. Ritchie, G.D. Schwab, S.R. Rust, Dept. of Animal Sci., Michigan State Univ., East Lansing, MI 48824.

A trial was conducted to characterize photosensitivity in cattle grazing a predominantly alfalfa pasture. Ninety-six Holstein steers (480 lbs) were placed on an alfalfa grazing trial on May 20, 1989. 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 alfalfa pasture. The steers were taken to the Beef Cattle Research Center, fed a non-alfalfa diet and housed in pens with access to shade. A hair loss scale was devised to separate steers into 4 groups. Two blood samples were drawn from each steer 43 days apart to determine extent of liver damage by evaluating the level of sorbitol 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 ($P<.05$). Steers had similar ADG during the photosensitive period ($P>.05$) but control steers, group 0, had greater ADG than group 1 steers during the recovery period ($P<.05$). 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 hepatogenous photosensitivity.

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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 Sci., Louisiana State Univ. Agric. Center, Baton Rouge, LA 70808

Two experiments were conducted to evaluate the effectiveness of an enzyme-modified NDF procedure (MNDF) for quantifying bacterial CP (BCP) in ruminally-incubated forages and intestinal digesta. The NDF modification involved amylase treatment prior to and protease treatment after boiling of samples in neutral detergent solution. BCP was estimated by sample N loss. In Exp. 1, MNDF was compared to a ^{15}N bacterial marker for estimating BCP contamination of alfalfa (AH) or bermudagrass (BH) hays ruminally incubated for 4, 8 or 12 h. MNDF overestimated BCP in AH hay at all incubation times and in BH at 4 and 8 h. Data indicated that NDF-bound CP in legumes may be partially susceptible to MDNF treatment resulting in overestimation of BCP. Further, data from Exp. 1 suggested that MDNF may have marginal utility for ruminal incubation times less than 12 h in moderately to poorly digestible forages, and may not be at all suitable for legumes and other highly digestible forages. In Exp. 2, BCP flow to the abomasum of lambs fed tall fescue hay diets was estimated using either MNDF or a purine-N BCP marker. Flow of BCP was similar ($P>.05$) for the two methods suggesting that most non-NDF-bound CP from tall fescue hay had been digested ruminally in this experiment. Data from Exp. 2 suggested that MNDF may be used to estimate BCP synthesis in ruminants consuming some forage diets.

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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, Univ. of Illinois, Urbana 61801

Four steers (430 kg) were fed diets containing two energy levels at two feeding frequencies (FF) in a 4 X 4 Latin square design. Energy levels were 2.24 (high forage; HF) or 2.92 (low forage; LF) Mcal ME/kg DM provided by alfalfa hay/corn silage or ground corn/corn silage diets. Diets were fed twice (2X) or 12 (12X) times daily. Dry matter intakes were 2.0% BW in each period. Ruminal contents were collected at four time intervals over 4 d. Whole contents were blended with saline and mixed bacteria were isolated from strained fluid immediately (fresh bacterial isolate; FSBT) or after compositing of fluid by animal and freezing (frozen bacterial isolate; FZBT). Also, fluid- (FAB) and particle- (PAB) associated bacteria were isolated. Energy level and FF had little effect ($P > .05$) on composition of isolated fractions. Sampling time did not affect ($P > .05$) composition of bacteria isolated from steers fed 12X but had linear ($P < .05$) and quadratic ($P < .05$) effects on N:purine ratios of bacteria isolated from steers fed 2X. Nitrogen:purine ratios and estimated bacterial N flows using respective ratios are given in the table. Means within columns and items having different superscripts differ ($P < .05$). Results suggest that freezing ruminal samples before bacterial isolation will not affect N:purine ratios. Composition may be affected by fraction of ruminal contents sampled and time for animals fed infrequently.

Item	HF	LF	2X	12X
N:purine				
FSBT	$.77^{a,b}$	$.77^{a}$	$.76^{a,b}$	$.77^{a}$
FZBT	$.87^{a}$	$.78^{a}$	$.85^{a}$	$.79^{a}$
PAB	$.72^{b,c}$	$.75^{a}$	$.72^{b}$	$.74^{a}$
FAB	$.64^{c}$	$.64^{b}$	$.61^{c}$	$.66^{b}$
Duodenal N flows, g/d	236.7	234.8	213.8	247.6
Bacterial N flows, % total				
FSBT	54.5^{a}	45.2^{a}	51.1^{a}	48.7^{a}
FZBT	49.8^{a}	44.4^{a}	46.6^{a}	47.6^{a}
PAB	$47.2^{a,b}$	$42.9^{a,b}$	$44.6^{a,b}$	$45.8^{a,b}$
FAB	41.6^{b}	36.7^{b}	37.9^{b}	40.3^{b}

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INFLUENCE OF PROTEIN DEGRADATION AND DIET TYPE ON FERMENTATION IN A CONTINUOUS CULTURE SYSTEM.

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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 soybeans had no effect on fiber digestion or nitrogen flow, but did increase the number of lipolytic microorganisms.

	Soy Type		Diet Type			
Item	Raw	Ext	10SB 50AH	15SB 40AH	20SB 30AH	25SB 20AH
NDF digestion, %	55.1	54.6	52.8	52.9	58.8	55.1
ADF digestion, %	54.9	55.0	50.3	53.2	60.3	55.9
Nitrogen flow, g/d						
Non-ammonia N	1.9	1.8	2.0	1.9	1.8	1.8
Bacterial N	1.2	1.2	1.5	1.1	1.3	1.0
Microbial counts, $\log_{10}$						
Lipolytic	7.2	7.4	7.1[a]	7.2[a]	7.2[a]	7.7[b]
Cellulolytic	8.0	8.0	8.0	8.0	8.0	7.9
Proteolytic	8.3	8.4	8.4	8.4	8.5	8.2

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INADEQUACY OF XYLOSE AS A RUMEN ESCAPE MARKER. J.Zorrilla-Rios, J.D.Garza and F.Owens. Dept. of Animal Sci., Okla State U., Stillwater 74078.

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 quantitative index of ruminal 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 33%, while after intravenous administrations, 44 to 61% was detected in urine. Although blood concentrations of xylose may serve as a qualitative index of absorptive function in 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 ruminal escape under various feeding, environmental and animal conditions continues.

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EFFECTS OF Cr CONCENTRATION AND PARTICLE SIZE OF MORDANTED FIBERS ON KINETIC PASSAGE AND FECAL OUTPUT ESTIMATES. J. R. Russell*, A. M. Beck and N. R. Brasche, Dept. of Animal Sci., Iowa State University, Ames, IA 50011

Fiber, extracted from ground (2.5 x 7.5 cm) alfalfa-bromegrass hay, was mordanted with 2 or 5% Cr (1.55 and 3.08% bound Cr), dried and either reground through a 1 mm screen (fine) or not reground (coarse). Four steers, fed ground (2.5 x 7.5 cm) alfalfa-bromegrass hay, were pulsed-dosed with 30 gm of the mordanted fibers on day 10 in each period of a 4 x 4 Latin Square digestion trial with 10 d adjustment and 5 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 greater (P<.01) for the reground than for the coarse mordanted fibers. Fiber particle size did not affect fecal output estimates. Mordanted fiber Cr concentration did not affect Cr passage rates. 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.

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IN VITRO SYNTHESIS AND BIOHYDROGENATION OF LONG-CHAIN FATTY ACIDS IN DIETS CONTAINING MEGALAC OR ANIMAL-VEGETABLE BLEND

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Both increases and decreases of long-chain fatty acids (LCFA) in rumen content have been reported from studies with duodenally cannulated cows. An *in vitro* trial with 9 diets and 7 replicates of 2 each was conducted in a randomized block design to observe 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, .5 g diets in 40 ml medium solution in each flask were inoculated with 10 ml rumen fluid and incubated for 24 h. LCFA in diets, fermented products, and inoculum 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 inoculum. 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 condition. Biohydrogenation of unsaturated C_{18} FA were 42 and 69% (P<.01) in M and AV diets, respectively, and corresponding net BH in M and AV fat obtained by correction for basal diet were 44 and 73% (P<.01). Though the BH 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 BH, and that rumen microbes are capable of synthesizing LCFA. Any disappearance of LCFA noted *in vivo* experiments may be related to chain-shortening and absorption in the rumen and variability of digestion markers.
(Supported in part by Church and Dwight, Inc., Princeton, NJ 08540).
Key Words: Fatty acid synthesis, Biohydrogenation

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Microbial CP Association with and NDF Digestibility of Untreated, and Ammoniated Bermudagrass Hay.

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Six ruminally cannulated Holstein steers were used in a 6x4 incomplete Latin square design of animals and periods to study the effects of NPN and monensin (M) on in situ forage digestibility. Diets were: untreated bermudagrass hay (H), ammoniated (3% of DM) bermudagrass hay (AH), H+M, AH+M, H plus urea (H+U) and H+U+M. 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 (MCP) 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 46.1%) than C-CPD (50.3% 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 M or 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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EFFECT OF ENZYME AND INOCULANT ADDITIVES ON NUTRIENT UTILIZATION OF A HAY-CROP SILAGE DURING CONTINUOUS CULTURE.

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Second-crop mixed grass-legume forage (timothy, white clover, red clover, alfalfa) was ensiled at 30% DM in four 80 ton bunker silos either untreated (C) or after treatment at the forage harvester with a commercial enzyme mixture silage additive (E) containing cellulase and xylanase (300 ml/ton chopped forage), a multi-species bacterial inoculant (I) (6 g/ton chopped forage) or with both treatments combined (B). Forages were sampled from bunker silos at the University of Maine, dried at 50° C and ground through a 4 mm screen in a Wiley Mill. Total mixed diets were prepared in a 50:50 forage:concentrate ratio on a DM basis were in a completely randomized design with three replications. Diets contained 15.6% CP, 32% NDF and 21% ADF on a DM basis. Fermentations were conducted at a liquid dilution rate of 16%/hr and solids retention time of 24 hr. Fermenters were fed 19 g DM 4x/d. After five days of adaptation, samples were collected every day for three days and composited. Ammonia N concentrations were 8.3, 4.2, 5.3 and 9.3 for C, E, I and B (E vs, C, I, B, P<.05). Acetate molar percentage was lowest while propionate highest for E vs C, I and B (P<.05). Digestibilities of NDF and ADF were highest (P<.05) for E compared to C, I and B and were 49.4, 50.9; 46.2, 46.2; 46.8, 46.6; 37.1, 44.6, respectively. Enzyme treatment (E) resulted in altered fermentation end products and greatest digestion of fiber components.

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EFFECT OF CHEMICAL DRYING AGENT TREATMENT OF ALFALFA HAY ON NUTRIENT DIGESTIBILITY AND LACTATIONAL PERFORMANCE OF MID-LACTATION HOLSTEIN COWS. C. J. Ziemer*, A. J. Heinrichs, C. J. Canale, and G. A. Varga. Department of Dairy and Animal Science. The Pennsylvania State University, University Park, PA 16802.

The effect of chemical drying agents on nutrient digestibility and lactational performance was studied. First cutting alfalfa hay (late bud) was harvested with treatments applied at mowing (7.55 kg/ha). Treatments consisted of untreated control (0), commercial drying agent (1), and 50% K_2CO_3-50% Na_2CO_3 mixture (2). Six multiparous Holstein cows, 120-150 d postpartum, were fed diets twice daily; diets consisted of 55% chopped alfalfa and 45% concentrate (DM basis). There were no differences in milk yield (kg/d), fat %, or protein % (27.2, 3.42, 3.34; 27.2, 3.48, 3.24; 27.7, 3.37, 3.28) for treatments 0, 1, and 2 respectively. However, contrast of treatment 0 vs 1 showed a significant (P<.08) decrease in milk protein %. Dry matter intake (kg/d) did not differ for treatments 0, 1, and 2 (25.2, 24.7, 25.2). Digestibilities (%) of DM (61.1, 60.4, 61.3), CP (61.8, 61.6, 60.6), ADF (27.3, 27.2, 27.6), NDF (40.4, 39.2, 41.0), and organic matter (61.3, 60.2, 61.1) were not different between treatments 0, 1, and 2, respectively. Blood electrolytes, Na, K, and Cl (mmole/l), were not different between treatments (means 145.2, 5.47, 105.3). No treatment differences were observed for blood pH, hematocrit (%), or HCO_3 (mmole/l) (means 7.40, 31.5, 26.65). Treatment of alfalfa hay with a chemical drying agent did not alter nutrient digestibility, milk production, or selected blood measurements in mid-lactation cows.

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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 Sciences, University of Arizona, Tucson 85721.

Steers were fed 65 or 90% concentrate diets based on steam-flaked milo, with chopped alfalfa hay (AH) as the roughage source in control diets. Chopped wheat straw (WS), chopped bermuda straw (BS), or cottonseed hulls (CSH) replaced the AH in 90% concentrate diets, and in 65% concentrate diets half the AH was replaced. Four separate 4x4 Latin squares were used to determine influence of roughage source on rates of passage (intact steers) and rates of digestion (rumen-cannulated steers). Apparent extent of ruminal digestion (AED) of starch was calculated from competing rates of passage and digestion. At 65% concentrate, starch AED and ruminal digestion as a percentage of total tract starch digestion were lower (P<.05) for the AH diet than for low quality roughage diets (AED = 64 for AH diet as compared to 69, 71, and 68% for WS, BS, and CSH diets, respectively; SEM = 2.0). At 90% concentrate, results were reversed; starch AED and ruminal digestion as a percentage of total tract digestion were higher (P<.05) for the AH diet (AED = 78, 68, 70, and 69% for AH, WS, BS, and CSH diets, respectively; SEM = 2.8). Total tract starch digestion averaged 97% for both concentrate levels. Results indicate that roughage source influences site of starch digestion in mixed diets for cattle.

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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 Sci., University of California, El Centro, CA 92243.

Four Holstein steers (208 kg) with "T" cannulas in the rumen and proximal duodenum were used to evaluate the influence of level of feed intake and 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% cane molasses, 2% yellow grease and 8% protein-mineral supplement. The corn portion of the diet was provided as either DR (density = .54 kg/liter) or SF (density = .36 kg/liter). Feed intake was restricted to allow for .64 versus 1.28 kg/d weight gain. Level of feeding and corn processing treatments were superimposed in a 2 X 2 factorial arrangement. Increasing feed intake from 3.4 to 4.9 kg/d enhanced (P<.01) flow of dietary components and microbial N (MN) to the small intestine. However, ruminal digestion of OM, starch and feed N were not influenced (P>.10) by feed intake. Post ruminal digestion of OM and N, and total tract digestibility of OM and DE decreased (P<.05) as feed intake was increased. Level of intake did not influence site and extent of starch digestion (P>.10). Steam flaking corn increased (P<.05) ruminal, post-ruminal and total tract digestibility of OM and starch. Post ruminal and total tract digestibility of N and DE were also increased (P<.05) by steam flaking the corn. Increasing feed intake and steam flaking corn decreased (P<.05) ruminal pH, and ruminal molar proportion of acetate and methane production, but increased (P<.10) molar proportions of propionate. Results imply that corn processing rather than feed intake level is the primary factor influencing site and extent of starch digestion. Major benefits from increasing feed intake within the range of the present study are decreased methane energy loss and enhanced microbial efficiency. The decrease in total tract digestibility of OM and DE with increasing level of feed intake is not attributable to changes in starch digestibility.

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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 Sci., Louisiana State Univ. Agric. Center, Baton Rouge, LA 70808

Twelve ruminally- and abomasally-cannulated wether lambs (26 kg) were blocked by weight and randomly assigned to 14.5% CP experimental diets consisting of bermudagrass hay (BH) and a semi-purified concentrate mixture in ratios of 90:10, 70:30 and 30:70. 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 of sample collection. Abomasal nutrient flow, particulate passage rate and fluid passage rate were determined using chromic oxide, Yb-labelled BH and Co-EDTA as markers, respectively. Ruminal pH and digestion of NDF, ADF and hemicellulose (HC) declined linearly (P<.05) with increasing concentrate level. Ruminal pH·hours (area below pH 6.7 integrated through 24 h), total VFA concentrations and bacterial CP synthesis increased linearly (P<.05) with increasing concentrate level. Postruminal digestion of cell wall components and liquid passage rate were not affected (P>.05) by treatment. Correlations of pH·hours with ruminal NDF (r=-.78; P=.003), ADF (r=-.72; P=.009) and HC (r=-.66; P=.020) disappearance were attained in a 24-hour feeding cycle. Total ruminal VFA concentrations were correlated with ruminal digestibilities of NDF (r=-.90; P=.0001), ADF (r=-.86;

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THE SIGNIFICANCE OF CHEWING DURING EATING AND RUMINATION ON FORAGE DIGESTION IN CATTLE. Y. Dong, A.S. Waage, C. Campbell and J.G. Buchanan-Smith, Dept. of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada. N1G 2W1.

Seven esophageally and ruminally fistulated steers were each fed once daily either early-cut alfalfa or late-cut bromegrass with each forage fed either short or long chopped. The purpose was to determine effectiveness of chewing during eating and rumination on particle size reduction (measured as % LP reduced to less than 2.36 mm), forage functional specific gravity (FSG) and ruminal digestion. Extent of LP reduction was greater (p<0.05) for bromegrass (50.2%) than alfalfa (24.7%) with no effect of chop length. Particle size reduction during rumination was unaffected by forage type or chop length but was greater (p<0.05) during the last 12h compared to the first 9h of the rumination period (88 vs 69%). Change in FSG during eating was greater for alfalfa (0.546 units) than bromegrass (0.478 units); however, FSG of masticated forage was less (P,0.05) for alfalfa (1.048) than bromegrass (1.084). Chop length affected change in FSG during eating for alfalfa but not for bromegrass. Rumination had a minimal effect on digesta FSG. No association between change in FSG during eating or rumination and particle size reduction was observed. Using a model based on the Mitscherlich equation, chewing during eating was shown to enhance the immediately soluble fraction of forage without any effect on digestion rate. These data suggest that chewing is a significant facilitator of forage digestion in the rumen.

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EFFECT OF SHORT DURATION IONOPHORE ROTATIONS ON FEEDLOT CATTLE PERFORMANCE.
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Eight trials were conducted to evaluate the effect of a daily (D) and weekly (W) alternation of lasalocid (L) and monensin-tylosin (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
Least Square Means

Item	L	MT	D	W
n (Pen)	32	32	25	32
DM Intake (lb)	22.2[a]	21.7[b]	22.1[a,c]	21.8[b,c]
Gain, Daily (lb)	3.66[a]	3.66[a]	3.80[b]	3.66[a]
Feed/Gain	6.11[a]	6.01[b]	5.88[c]	5.97[b,c]

[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.

EVALUATION OF RUMINAL IN VITRO SYSTEMS.

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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), without affecting total VFA. 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 molar 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 adversily 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.

THE FLOW RATE OF NAN AND α-AMINO N AT ABOMASUM IN CROSSBRED CALVES FED UREA TREATED STRAW SUPPLEMENTED WITH BYPASS PROTEIN

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A 4 x 4 latin square design experiment was conducted on 4 crossbred calves, each fitted with a rumen and an abomasal cannula. The animals were fed 4 treatment combinations, viz. T_1) untreated wheat straw and concentrate with 30% GNcake (USUC), T_2) untreated straw and concentrate having HCHO treated GNC (USTC), T_3) urea treated straw with concentrate having untreated cake (TSUC), and T_4) treated straw and concentrate having treated cake (TSTC), maintaining the R:C ratio of 65:35 in all the combinations. The average TVFA and ruminal ammonia concentrations were highest on TSUC and lowest on USTC diets. The variation among the treatments was significant ($P < 0.01$). The flow rates of NAN, NANUN (non-ammonia non-urea N), NDAN (net digestible and absorbable N, derived by subtracting ADFN from NANUN) and α-amino N, at abomasum, as g/d and as g/kg apparently digested DM, showed significant variation among the treatments ($P < 0.01$), with the highest values on TSTC and the lowest values on USUC diet. The values for NAN flow rates (as g/d) were 40.21 ± 1.19, 45.96 ± 1.48, 75.83 ± 2.33 and 81.34 ± 3.11 and for α-amino N the corresponding values were 31.28 ± 0.48, 37.49 ± 0.46, 54.39 ± 2.10 and 63.91 ± 3.09 g for the treatments T_1 to T_4 respectively. α-amino N accounted for 96.86 per cent of NDAN flowing at abomasum. The results reveal that the feeding of urea treated straw along with HCHO treated groundnut cake resulted in maximum availability of α-amino N at abomasum in crossbred calves.