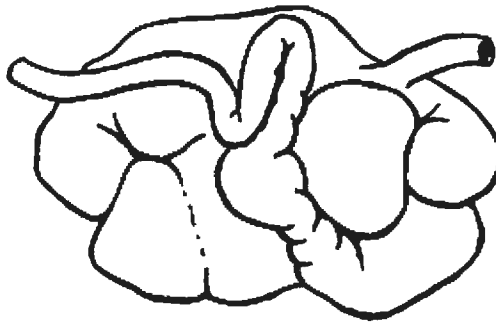


Abstracts

Conference on Rumen Function

Volume 25, 2000



49 Years of Interaction

1951-2000

25th Conference on Rumen Function

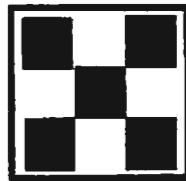
Chicago, Illinois

November 14-16, 2000

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25th
CONFERENCE ON RUMEN FUNCTION
1951-2000

Welcome to the 25th Conference on Rumen Function. The Conference has been meeting in the Congress Hotel since November 1951. Initially, the Conference focused on the problem of bloat, which continued as a central theme until 1961. The Conference then broadened its program to other factors that influence rumen fermentation and physiology. The scope of this year's Conference has been broadened further to include gastrointestinal function as well as microbiological and environmental research relating to animal wastes, food-borne pathogens and public health/safety.

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

H. W. Marston, ARC/USDA, served as Conference Chairman from 1951 until 1957 and from 1961 to 1965. N. R. Ellis, ARC/USDA, was Chairman of the 1959 meeting. C. R. Richard, CSRS/USDA, assumed the Chairmanship in 1967 and served until 1983. M. J. Allison, ARS/USDA then served as Chairman from 1985 to 1989 and J. B. Russell, ARS/USDA served as Chairman from 1991 to 1995.

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

Sincerely,



Michael S. Allen, Professor
Department of Animal Science
Michigan State University

**The XXV Conference on Rumen Function
is Dedicated to the Memory of**



Professor Marvin P. Bryant, Ph.D.

1925 - 2000

Dr. Marvin P. Bryant, age 75, also known as "Marv" to many of his friends and colleagues, died on October 16, 2000 at home in Savoy, Illinois. Born in Boise, Idaho on July 4, 1925 he was the son of Melvin Berry and Emma Louise Bucklin Bryant. Dr. Bryant graduated from Washington State University in 1949 with a Bachelor of Science degree in Bacteriology. As an undergraduate student he gained research experience working in Professor R. E. Hungate's laboratory and he continued his studies there, under the direction of Professor Hungate, obtaining his Master of Science degree in 1950 for research on rumen spirochetes. He earned his Doctor of Philosophy degree from the University of Maryland in 1955, while he was working as a Research Scientist at the Dairy Cattle Research Branch, ARS, USDA, Beltsville, Maryland. In 1955, Dr. Bryant was promoted to Sr. Research Scientist and later served as Leader, Microbiology Research until he accepted a position in the Department of Dairy Science at the University of Illinois, Urbana-Champaign (UIUC) as Associate Professor in 1964. He was promoted to Professor in the Departments of Dairy Science and Microbiology in 1966. Dr. Bryant retired in 1994 from the University of Illinois after 30 years of service. Marv's contributions to science were many and a few are mentioned below. He was recognized as the foremost rumen bacteriologist in the world for his pioneering research on the ecology, physiology and metabolism of anaerobic rumen bacteria. Many of the isolates made during these studies are still used today in research concerning rumen bacterial metabolism and taxonomy. He became interested in growing methanogenic bacteria and utilization of H₂ in anaerobic ecosystems that resulted in the development of the concept of interspecies

hydrogen transfer. This led to seminal work on the role of obligate proton reducing bacteria and their role in complete anaerobic degradation of organic matter. This research focussed on the isolation, identification and metabolism of anaerobic fatty acid and aromatic acid degrading bacteria. Marv loved bacteria and had an uncanny knack of recognizing them down the microscope. This lifelong research interest resulted in his involvement in the Bergey Trust between 1975-1988 serving on the Board of Trustees from 1975-1986. He was a co-editor of Volume 3 of Bergey's Manual of Systematic Bacteriology published in 1989 and was awarded the Bergey's Medal for Distinguished Achievement in Bacterial Taxonomy in 1996. Dr. Bryant published more than 112 peer reviewed journal articles, 24 book chapters and 19 monographs and reviews and gave many professional talks and presentations during his career. He had an active research and teaching program at UTUC and was preceptor for students completing 14 M.S., 15 Ph.D., and 21 post-doctoral programs. Dr Bryant served on editorial boards of Journal of Dairy Science, Applied and Environmental Microbiology, and the American Society of Microbiology Publications board as well as serving as editor (1969-1971) and editor in chief (1979-1980) of Applied and Environmental Microbiology. He received the Paul A Funk Award for Research, College of Agriculture, UTUC (1979); Borden Award, American Dairy Science Association (1978); Foundation for Microbiology, Lecturer, American Society of Microbiology (1975-1976); Fellow, American Academy of Microbiology (1977), Fellow, AAAS (1960); Superior Service Award, USDA, (1959). He was elected to the National Academy of Sciences in 1987. He was a founder member and regularly attended the Rumen Function Conference and served as Microbiology Panel Chair for many years. He is survived by his wife, Margaret, 5 children and 9 grandchildren.

Agenda

TUESDAY, NOVEMBER 14

GREAT HALL

8:00 - 11:00 p.m.

Poster session and mixer

WEDNESDAY, NOVEMBER 15

GREAT HALL

9:00 a.m.

Welcome and introduction, M. S. Allen, Chairperson

9:10 a.m.

#1

RUMEN EVACUATION TECHNIQUES TO DETERMINE AND COMPARE
PASSAGE RATE MARKERS.

D. I. Harvatine and J.L. Firkins, Department of Animal Sciences, The Ohio State
University

9:30 a.m.

#2

EFFECT OF DIET FERMENTABILITY ON EFFICIENCY OF MICROBIAL N
PRODUCTION.

M. Oba, and M. S. Allen. Department of Animal Science, Michigan State
University

9:50 a.m.

#3

SOLAR RADIATION'S IMPACT ON PROPORTION OF FIBER FRACTIONS
AND *IN VITRO* DIGESTION KINETICS OF TROPICAL FORAGES.

J. R. Carpenter, N. K. Ranjit and R. Y. Niino-DuPonte, Dept. of Human
Nutrition, Food and Animal Sciences, University of Hawaii at Manoa

10:10 a.m.

#4

IMPROVING THE AEROBIC STABILITY OF SILAGES WITH
LACTOBACILLUS BUCHNERI. L. Kung, Jr., N. K. Ranjit, and C. C. Taylor.

Department of Animal & Food Sciences, University of Delaware, Newark.

10:30 a.m.

#5

AMYLOLYTIC BACTERIA THAT PREVENT LACTATE ACCUMULATION
DURING *IN VITRO* RUMINAL FERMENTATIONS

¹F. Rodriguez, ²M. Rasmussen, and ¹M. Allison. ¹Department of Microbiology,
Iowa State University, ²National Animal Disease Center, USDA, Ames, IA

10:50 a.m.

Break

- 11:10 #6 PH AND RUMINAL ACID-BASE BALANCE OF LACTATING COWS FED WITH MAIZE SILAGES.
I. Fernandez¹, H. Brugère², B. Michalet-Doreau¹. ¹INRA, URH-DIM, 63122 St Genès Champanelle, FRANCE, ²Ecole Nationale Vétérinaire d'Alfort. Laboratoire de Physiologie Thérapeutique, Cedex, France
- 11:30 a.m. #7 **INVITED PRESENTATION – Richard A. Kohn, Ph.D.**
THREE CONDITIONS OF THE RUMINAL MILIEU THAT DETERMINE PH.
Contact Information: Dept. Animal and Avian Sci., University of Maryland, College Park Rkohn@wam.umd.edu, 301-405-4583
- 12:30 p.m. Lunch at your discretion
- 2:30 p.m. #8 NOVEL APPROACHES FOR INHIBITING RUMEN METHANOGENESIS.
E. M. Ungerfeld, S. R. Rust, M. K. Jain and R. Burnett, Michigan State University, East Lansing
- 2:50 p.m. #9 EFFECT OF COCONUT OIL ON TOTAL TRACT METHANOGENESIS AND RUMINAL METHANOGENS IN FAUNATED AND DEFAUNATED SHEEP.
A. Machmüller, C. R. Soliva, and M. Kreuzer, Institute of Animal Sciences, Swiss Federal Institute of Technology (ETH), Zurich
- 3:10 p.m. #10 ISOLATION AND IDENTIFICATION OF A BACTERIUM THAT INHIBITS HYPERAMMONIA PRODUCING BACTERIA.
J. L. Rychlik and J. B. Russell. Department of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY
- 3:30 p.m. - **INVITED PRESENTATION – Prof. Abigail A. Salyers, Ph.D.**
GENETIC BASIS FOR ANTIBIOTIC RESISTANCE IN BACTERIA AND ITS RELEVANCE TO AGRICULTURE
Contact Information: Dept. of Microbiology, University of Illinois, Urbana, IL 61801 abigails@life.uiuc.edu, (217) 333-7378
- 4:30 p.m. Break
- 4:50 p.m. #11 ANALYSES OF ANTIBIOTIC RESISTANCE GENES IN ANAEROBIC BACTERIA AND TOTAL DNA FROM SWINE MANURE.
T. R. Whitehead and M. A. Cotta. USDA/ARS, Natl. Cent. Agric. Utilizn. Res., Peoria, IL

- 5:10 p.m. #12 TETRACYCLINE RESISTANCE GENES IN GROUNDWATER IMPACTED BY SWINE LAGOON WASTE.
J. Chee-Sanford¹, R. Aminov¹, N. Garrigues¹, I. Krapac², and R. Mackie¹, Dept. of Animal Sciences¹, University of Illinois-Urbana, and Illinois State Geological Survey², Urbana, IL
- 5:30 p.m. Dedication of the XXV Conference on Rumen Function, M. Allison
- 5:45 p.m. Dinner at your discretion

THURSDAY, NOVEMBER 16 GREAT HALL

- 8:30 a.m. #13 EFFECT OF SODIUM CHLORATE ON IN VITRO RUMINAL FERMENTATIONS.
T. R. Callaway, R. C. Anderson, S. A. Buckley and D. J. Nisbet. USDA/ARS, SPARC, College Station, TX
- 8:50 a.m. #14 THE ROLE OF LIPOTEICHOIC ACIDS IN THE RESISTANCE OF STREPTOCOCCUS BOVIS TO NISIN.
H. C. Mantovani and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY
- 9:10 a.m. **INVITED PRESENTATION- Karen Nelson, Ph.D.**
INCREASING OUR UNDERSTANDING OF BIOLOGICAL SYSTEMS WITH MICROBIAL GENOME DATA
Contact Information: The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850 KENelson@tigr.org, 301-838-3565
- 10:10 a.m. Break
- 10:30 a.m. #15 PCR SCREENING OF THE RUMEN MICROBIAL META-GENOME FOR *luxS* SEQUENCE HOMOLOGUES.
D. A. Antonopoulos, K. A. D. Piggott, B. A. White, Department of Animal Sciences, University of Illinois, Urbana

- 10:50 a.m. #16 **THE RUMINOCOCCUS ALBUS 7 BACTERIOCIN: AN UNEXPECTEDLY LARGE PROTEIN WITH HOMOLOGY TO ENDOGLUCANASES.**
J. Chen, D. M. Stevenson, and P. J. Weimer, Dept. of Bacteriology, Univ. of Wisconsin-Madison, and USDA-ARS, U.S. Dairy Forage Res. Center, Madison, WI
- 11:10 a.m. #17 **DISTRIBUTION OF TETRACYCLINE RESISTANCE DETERMINANTS ENCODING RIBOSOMAL PROTECTION PROTEIN GENES IN ANIMAL PRODUCTION SYSTEMS.**
N. Garrigues, R. I. Aminov, R. I. Mackie, Dept. of Animal Science, University of Illinois, Urbana
- 11:30 a.m. **Business Meeting**
- 12:00 noon **Adjourn**

Posters

- #18 **METHOD TO MEASURE FRACTIONAL RATE OF VOLATILE FATTY ACID ABSORPTION FROM THE RUMEN.**
M. S. Allen¹, L. E. Armentano², M. N. Pereira², Y. Ying¹ and J. Xu. Michigan State University¹ and University of Wisconsin, Madison²
- #19 **THERMODYNAMICS OF RUMINAL FERMENTATION.**
R. A. Kohn, Dept. Animal and Avian Sci., University of Maryland, College Park, MD
- #20 **IS RUMINAL VISCOSITY INVOLVED IN THE MICROBIAL FIBROLYTIC ACTIVITY DECREASE WITH HIGH CEREAL DIET?**
C. Martin, I. Fernandez, Y. Rochette, B. Michalet-Doreau. INRA, URH-DIM, 63122 St Genès Champanelle, FRANCE
- #21 **SELECTED-ION-FLOW-TUBE MASS SPECTROMETRIC ANALYSIS OF RUMEN GASES.**
R.J. Dewhurst¹, R.T. Evans¹, P. Spanel² and D. Smith³, ¹Institute of Grassland and Environmental Research, Aberystwyth, ²J. Heyrovsky Institute of Physical Chemistry, Prague, Czech Republic and ³Centre for Science and Technology in Medicine, Keele University, Stoke-on-Trent
- #22 **AMMONIA PRODUCTION AND PROTEIN DEGRADABILITY AS AFFECTED BY FOOD SOURCES AND PURIFIED MONENSIN OR RUMENSIN.**
R.P. Lana, N.G.S. Barbosa, A.B. Mancio. Dept. de Zootecnia, Univ. Fed. Vicosa, 36571-000, Vicosa, MG, BRAZIL

- #23 EFFECTS OF MONENSIN AND LASALOCID ON FERMENTATION OF AMINO ACIDS BY MIXED RUMINAL BACTERIA.
R.P. Lana, J.S. Oliveira, R.G. Veloso, P.M.M. Nunes, A.C. Borges. Dept. de Zootecnia, Univ. Fed. Vicosa, 36571-000, Vicosa, MG, BRAZIL
- #24 MICROBIAL FERMENTATION IN CONTINUOUS CULTURES RECEIVING FAT BEFORE OR AFTER THE ADDITION OF AN IONOPHORE.
M. Croucher and V. Fellner, Department of Animal Science. North Carolina State University, Raleigh
- #25 DISTRIBUTIONS OF MICROBIAL MASS AND FIBROLYTIC ENZYME ACTIVITIES IN DIFFERENT SIZE OF FEED PARTICLES FROM RUMEN CONTENTS OF SHEEP
J. Pan, T. Suzuki, K. Ueda, K. Tanaka, and M. Okubo, Graduate School of Agriculture, Hokkaido University, Sapporo-shi
- #26 USE OF *IN VITRO* FERMENTATION FOR DETERMINING THE POTENTIAL CONJUGATED LINOLEIC ACID (CLA) PRODUCING CAPACITY OF RUMINANT DIETS.
E. Frantz, R. Robinson, B. Jacobson, and K. Griswold, Department of Animal Science, Food and Nutrition, Southern Illinois University, Carbondale
- #27 CONCENTRATIONS OF *TRANS*-18:1 AND CLA IN TISSUES OF LAMBS FINISHED ON DIFFERENT DIETS.
D. L. Palmquist, K. E. McClure, G. D. Lowe and D. D. Clevenger, Dept. of Animal Sciences, OARDC/OSU, Wooster
- #28 VARIATION IN THE CONCENTRATIONS OF ODD-CHAIN FATTY ACIDS IN MILK.
¹R. J. Dewhurst, ¹J.K. S. Tweed and ²G. B. Williams, ¹Institute of Grassland and Environmental Research, Aberystwyth, U.K. and ²Institute of Rural Studies, University of Wales, Aberystwyth
- #29 VARIATION IN THE CONCENTRATIONS OF ODD-CHAIN FATTY ACIDS IN RUMEN BACTERIA.
^{1,2}G. S. Bae, ²M. B. Chang, ³W. J. Maeng, ¹R. J. Dewhurst, ¹D. R. Davies and ¹R. J. Merry. ¹Institute of Grassland and Environmental Research, Aberystwyth, ²Department of Animal Science and Technology, Chung-Ang University, Ansong, Korea and ³College of Animal Husbandry, Kon-Kuk University, Seoul, Korea
- #30 EFFECTS OF FIBROLYTIC ENZYMES ON DEGRADATION OF ALFALFA HAY FIBER BY MIXED RUMEN MICROORGANISMS.
V. L. Nsereko, D. P. Morgavi, L. M. Rode, K. A. Beauchemin, T. A. McAllister, Agriculture and Agri-Food Canada, Lethbridge, AB

- #31 A TRICHODERMA FEED ENZYME PREPARATION ENHANCES ADHESION OF FIBROBACTER SUCCINOGENES TO COMPLEX SUBSTRATES BUT NOT TO PURE CELLULOSE.
D. P. Morgavi, V. L. Nsereko, L. M. Rode, K. A. Beauchemin, T. A. McAllister, Y. Wang.
Agriculture and Agri-Food Canada, Lethbridge, AB
- #32 EFFECT OF LEVELS OF WHOLE COTTONSEED ON RUMINAL VOLATILE FATTY ACIDS, PH AND AMMONIA.
O. Balbuena, C. L. Arakaki, C. D. Kucseva and G. A. Kosa. INTA Colonia Benitez, CP 3505, ARGENTINA
- #33 EFFECT OF LEVELS OF WHOLE COTTONSEED ON RUMINAL PROTOZOA.
C. L. Arakaki, O. Balbuena, C. D. Kucseva and G. A. Kosa. INTA Instituto de Patobiologia Castelar, ARGENTINA
- #34 MICROBIAL ACTIVITY IN GRASS-FED *IN VITRO* CONTINUOUS CULTURES IN RESPONSE TO INFUSION OF GRADED LEVELS OF SOLUBLE SUGARS.
M. R. F. Lee¹, J. M. Moorby¹, J. C. Macrae², M. K. Theodorou¹, R. J. Merry¹, D. R. Davies¹ and N. D. Scollan¹, ¹Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, UK ²Rowett Research Institute, Greenburn
- #35 EFFECTS OF BUFFERS ON pH AND MICROBIAL METABOLISM IN CONTINUOUS CULTURE OF RUMEN CONTENTS.
L. M. Aga, R. J. Koski and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul
- #36 INFLUENCE OF PATULIN LEVEL ON FERMENTATION BY RUMINAL MICROBES IN CONTINUOUS CULTURE.
M. O. Tapia, M. J. Murphy, R. J. Koski and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul
- #37 EFFECTS OF RUMEN DEGRADABLE AND UNDEGRADABLE PROTEIN ON FINISHING STEER PERFORMANCE.
C. J. Fu and M. S. Kerley, Department of Animal Sciences, University of Missouri, Columbia
- #38 CHOPPING FINENESS OF MAIZE SILAGE AND REDUCTION OF PARTICLE SIZE IN RUMEN.
I. Fernandez, A. Garcia-Rodriguez, B. Michalet-Doreau, INRA, URH-DIM, St Genès Champanelle, FRANCE

- #39 IN SITU DISAPPEARANCE OF FIRST HARVEST ALFALFA HAY AS AFFECTED BY STAGE OF MATURITY AND CUTTING TIME.
H. S. Hussein¹, H. F. Mayland², J. P. Tanner¹, H. Tokuyama¹, and G. C. J. Fernandez³. School of Veterinary Medicine¹ and Department of Applied Economics and Statistics³, University of Nevada-Reno, Reno, and USDA- ARS², Kimberly, ID
- #40 IN SITU DISAPPEARANCE OF THIRD HARVEST ALFALFA HAY AS AFFECTED BY STAGE OF MATURITY AND CUTTING TIME.
H. S. Hussein¹, H. F. Mayland², S. L. Lake¹, H. Han¹, and G. C. J. Fernandez³. School of Veterinary Medicine¹ and Department of Applied Economics and Statistics³, University of Nevada-Reno, Reno, and USDA-ARS², Kimberly, ID
- #41 REDUCING NITROGEN LOSSES FROM STORED COW SLURRY USING ORGANIC TREATMENTS.
D. F. McCrory¹, P. J. Hobbs¹, E. Bakewell² and R. J. Merry² Institute of Grassland and Environmental Research, ¹North Wyke Research Station, Okehampton, Devon, UK, ²Aberystwyth Research Centre, Aberystwyth, UK
- #42 AMMONIA FLUXES FROM LAGOONS AND FEEDING AREAS OF A 190-COW DAIRY.
R. L. Kincaid¹, B. Rumburg², G. H. Mount², J. Havig², K. A. Johnson¹, H. Westberg², and B. Lamb², ¹Dept. of Animal Sciences, and the ²Laboratory for Atmospheric Research, Dept. of Civil and Environmental Engineering, Washington State University, Pullman
- #43 NUTRIENT MANAGEMENT OF BROILER LITTER FOR BEEF CATTLE ON PASTURE.
J. P. Fontenot, N. B. Frank, R. K. Shanklin and V. G. Allen, Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg
- #44 EFFECTS OF pH ON VIABILITY AND GROWTH OF ENTODINIUM CAUDATUM, ENTODINIUM EXIGUUM, EPIDINIUM CAUDATUM, AND OPHRYOSCOLEX PURKYNJEI IN VITRO.
Burk A. Dehority, Dept. Of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH
- #45 EFFECT OF SUPPLEMENTAL PROTEIN TYPE ON RUMINAL BACTERIA POPULATIONS IN WHITEFACE WETHERS CONSUMING MEDIUM QUALITY GRASS HAY.
S. L. LODGE, M. W. SALISBURY, T. T. ROSS, AND T. MAY, Department of Animal and Range Science, New Mexico State University, Las Cruces, NM

- #46 PROBIOTIC EFFECTS ON RUMEN FUNGI.
R. Calza, J. Chang, J. Schmidt, S. Albright, and G. Calza. Department of Animal Sciences, Washington State University, Pullman
- #47 ISOLATION, CHARACTERIZATION AND STUDY OF HYDROLYTIC ACTIVITIES OF ANAEROBIC RUMEN FUNGI FROM RIVERINE BUFFALO (*Bubalus bubalis*)
Amit Singha and S. Neelakantan, Dairy Microbiology Division, National Dairy Research Institute, Karnal, India
- #48 ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY IN PEA PROTEIN FERMENTS AND HYDROLYSATES
V. Vermeirssen, J. Van Camp and W. Verstraete, Lab. of Microbial Ecology and Technology, Lab. of Food Technology and Nutrition, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium.
- #49 HEMICELLULASE ENCODING GENES OF *F. SUCCINOGENES*
J.K. Ha, L.M. Malburg, Jr. H. Jeon, X. Wang, S. MacLellan and C. W. Forsberg, University of Guelph, Guelph, ON
- #50 ISOLATION AND CHARACTERIZATION OF LIPOPOLYSACCHARIDE-LIKE AND CAPSULAR MATERIALS FROM A PREDOMINANT CELLULOLYTIC RUMINAL BACTERIUM *FIBROBACTER SUCCINOGENES* S85.
E. E. Egbosimba¹, E. Vinogradov², M. B. Perry², J. S. Lam¹, and C. W. Forsberg¹. ¹University of Guelph, Guelph ON, & the ²Natl Research Council of Canada, Ottawa, ON, Canada
- #51 THE EXTREME ACID RESISTANCE OF *ESCHERICHIA COLI* IS REGULATED BY AMINO ACID AVAILABILITY AS WELL AS VOLATILE FATTY ACIDS
G. N. Jarvis and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, New York
- #52 CHARACTERIZATION OF ISOLATED BACTERIAL STRAINS WITH ANTAGONISTIC PROPERTIES AGAINST FOOD-BORNE PATHOGEN *LISTERIA MONOCYTOGENES*.
H. Roman, E. T. Ryser, S. Rust and M. T. Yokoyama. Department of Animal Science, Michigan State University, 2265F Anthony Hall, East Lansing
- #53 FACTORS AFFECTING ON THE BINDING OF THE CLONED CELLULOSE-BINDING DOMAIN, DERIVED FROM *FIBROBACTER SUCCINOGENES* EGF, TO CARBOXYMETHYLCELLULOSE.
Makoto Mitsumori, Hiroshi Kajikawa, and Sadahiro Ohomomo National Institute of Animal Industry, Tsukuba Norindanchi Japan

- #54 CLONING OF THE O-ACETYL SERINE LYASE GENE FROM THE RUMINAL BACTERIUM SELENOMONAS RUMINANTIIUM HD4.
J. D. Evans, S. F. Al-Khalidi, and S. A. Martin, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA
- #55 CLONING OF THE L-LACTATE DEHYDROGENASE GENE FROM THE RUMINAL BACTERIUM SELENOMONAS RUMINANTIIUM HD4.
J. D. Evans and S. A. Martin, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA
- #56 EFFECT OF ACACIA ANGUSTISSIMA ON BACTERIAL DIVERSITY IN THE RAT CECUM.
A. H. Smith and R. I. Mackie, Department of Animal Sciences, Univ. of Illinois, Urbana
- #57 COEXISTENCE OF RUMEN CELLULOLYTIC RUMINOCOCCUS , PREVOTELLA RUMINICOLA GA33 AND BUTYRIVIBRIO FIBRISOLVEN D1 GROWN IN BATCH CULTURES.
J. K. Browne-Silva and Tammy May. Dept. of Animal and Range Sciences, New Mexico State University, Las Cruces NM
- #58 STABILITY OF PORCINE FECAL BACTERIAL POPULATIONS AFTER INTRODUCTION OF LACTOBACILLUS SPP.
J. M. Simpson, V. J. McCracken, H. R. Gaskins, and R. I. Mackie, Dept. of Animal Sciences, University of Illinois, Urbana
- #59 APPLICATION OF GROUP SPECIFIC AMPLIFIED R-DNA RESTRICTION ANALYSIS TO DIFFERENTIATE AMONG SWINE FECAL AND WASTE STORAGE PIT SAMPLES.
C. J. Ziemer, M. A. Cotta, and T. R. Whitehead. NCAUR, ARS, USDA, 1815 University St., Peoria, IL

#1 RUMEN EVACUATION TECHNIQUES TO DETERMINE AND COMPARE PASSAGE RATE MARKERS. D. I. Harvatine and J. L. Firkins, Department of Animal Sciences, The Ohio State University, Columbus, OH 43210 (614-688-3089).

Rates of digestion (k_d) and passage (k_p) of NDF from the rumen were determined using six ruminally and duodenally cannulated cows in a 6 x 6 Latin square. The measurements of intake and Cr_2O_3 -derived duodenal flow of iNDF (after 120 h in vitro fermentation) were similar for respective treatment means. Assuming the k_p of iNDF can be interchanged for the k_p of digestible NDF (dNDF; total NDF – iNDF) and the former is assumed equivalent to the rate of intake (k_i) of iNDF, then rumen digestibility of NDF can be estimated using inputs into a single compartment model for dNDF with inputs from intakes and rumen evacuation (2 h before and 2 h after feeding on 2 d). There was a strong positive linear bias (independent of treatment; no mean bias) for ruminal NDF digestibility using this technique compared with estimates based on Cr_2O_3 -derived duodenal flow of NDF. After a dose of CoEDTA, the Co dilution was best fit in 30 of 35 observations with a biexponential model; no mean bias and only a slight linear bias were detected for fluid volume estimated using Co vs evacuation. Data for ruminal fluid volume data best fit using a monoexponential model (5 out of 35) of Co dilution were overestimated by an average of about 30%. A biexponential fit would increase fluid dilution rate measurement (weighted mean of the rates from both exponential terms) and could impact predicted escape of soluble materials in the rumen. These methods need to be compared further under more diverse feeding situations.

#2 EFFECT OF DIET FERMENTABILITY ON EFFICIENCY OF MICROBIAL N PRODUCTION. M. Oba, and M. S. Allen. Michigan State University, East Lansing, MI (517-432-1386)

Effect of diet fermentability on efficiency of microbial N production (MNE) was evaluated. Eight ruminally and duodenally cannulated Holstein cows (55 ± 15.9 DIM; mean \pm SD) were used in a duplicated 4 x 4 Latin square design with a 2 x 2 factorial arrangement of treatments. Experimental diets contained either ground high moisture corn (HM) or dry ground corn (DG) at two dietary starch contents (32 vs. 21%). All diets were formulated for 18% of CP, and the sources of dietary protein were alfalfa silage (50% of forage, DM basis), SBM, distillers grains, and blood meal. Amount of OM truly fermented in the rumen varied among treatments from 7.7 (DG at 21% dietary starch) to 11.3 kg/d (HM at 32% dietary starch). The MNE was lower for HM treatment compared to DG (39.7 vs. 48.4 g/kg of truly ruminally fermented OM), but was not affected by dietary starch content. Within the data set of cow-period means, MNE was not related to daily mean ruminal pH or minimum ruminal pH. However, MNE was positively correlated with rate of passage for OM, starch, and NDF ($r = 0.77, 0.75, \text{ and } 0.63$, respectively). Rapid passage rate may decrease microbial turnover in the rumen, resulting in increased MNE. The MNE was negatively correlated with rate of starch digestion ($r = -0.55$), which implies that the energy spilling theory explains lower MNE for HM treatment. However, energy spilling does not appear to be from lack of ammonia because there was no relationship between MNE and ruminal ammonia concentration. Maximum rate for microbial protein synthesis might limit maximum MNE independent from substrate availability.

#3 SOLAR RADIATION'S IMPACT ON PROPORTION OF FIBER FRACTIONS AND IN VITRO DIGESTION KINETICS OF TROPICAL FORAGES. J. R. Carpenter, N. K. Ranjit and R. Y. Niino-DuPonte, Dept. of Human Nutrition, Food and Animal Sciences, University of Hawaii at Manoa, Honolulu, HI 96822 (808-956-8393).

Variation in the performance of animals fed tropical forages of similar nutrient composition suggests differences in nutrient proportions and/or digestion kinetics. A retrospective study was undertaken using alfalfa (*Medicago sativa* L.), harvested over a 120-wk period at 4-, 5- and 6-wk intervals, and six tropical grasses (*Hemarthria altissima*; *Pennisetum purpureum*; *P. clandestinum*; *Digitaria decumbens*; *P. americanum* x *P. purpureum*; and *Cynodon aethiopicus*), harvested at 4, 8 and 12 wks of regrowth. Nutrient composition, 48-h IVTD and digestion kinetics (incubation times of 0, 8, 12, 24, 36, 48 and 72 h) were determined. Average monthly mean temperatures varied from 22 to 26°C for the alfalfa plots, and from 17 to 26°C for the grass plots. Solar radiation (SR) ranged from 14.0 to 24.4 MJ•m² during the growing periods for both forages. Increases (P<.01) in forage NDF, ADF and lignin, and decreases in crude protein and IVTD occurred with increasing SR and age of regrowth. Higher SR loads decreased LIG/ADF by 8-10%, increased ADF/NDF and Cell/ADF by 4-8% but did not alter Cell/NDF or Hemicell/NDF. The variation in extent of digestion at 72 h for the various grasses (37.8 to 82.7%) was greater than at 48 h (52.4 to 84.9%). The range in 48-h IVTD for alfalfa was 68.4 to 88.9%. These results suggest that microbial fermentation in the rumen be influenced not only by the forage's age of regrowth but also by the intensity of SR during its regrowth. Changes in plant tissue ultrastructure in response to SR may significantly influence hydration rate, microbial attachment and/or colonization, particle density, and rate and/or extent of digestion and passage.

#4 IMPROVING THE AEROBIC STABILITY OF SILAGES WITH *LACTOBACILLUS BUCHNERI*. L. Kung, Jr., N. K. Ranjit, and C. C. Taylor. Department of Animal & Food Sciences, University of Delaware, Newark, 19711 (302-831-2522)

Recently, *Lactobacillus buchneri*, a heterofermentative lactobacilli, has been used as an inoculant to specifically improve the aerobic stability of silages. This organism anaerobically degrades lactic acid to primarily acetic acid. Traditionally, heterolactic acid bacteria have not been developed as silage additives because of the theoretically greater loss of nutrients and because silages high in acetic acid may depress intake. In laboratory and farm-scale silos, silages treated with *L. buchneri* have had higher concentrations of acetic acid, lower numbers of yeasts, and improved aerobic stability when compared to untreated silages. In some cases, increases in 1,2 propanediol or propionic acid have been detected in treated silages. In studies from our lab, an application of *L. buchneri* to obtain a final concentration of about 5×10^5 cfu/g of fresh forage appears critical to obtain consistent responses. Although, the DM recovery from treated silages has been slightly less than from untreated silages, these reductions seem acceptable considering the prevention of potential loss in income producers could face when feeding spoiled silages to cows. Several recent studies have fed silages treated with *L. buchneri* to cows and sheep without negative effects on intake. The use of *Lactobacillus buchneri* should be considered when producers require an additive to improve the aerobic stability of silages.

**#5 AMYLOLYTIC BACTERIA THAT PREVENT LACTATE ACCUMULATION DURING
IN VITRO RUMINAL FERMENTATIONS ¹F. Rodriguez, ²M. Rasmussen, and ¹M. Allison.
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The goal of this study is to investigate the potential use of amylolytic bacteria in the prevention of rumen acidosis in high producing dairy cattle. Ruminal amylolytic bacteria were isolated from a rapid turnover (30%/hr), starch fermentor. This fermentor had been inoculated with highly diluted rumen contents from a grain-fed cow. Strains of amylolytic bacteria were screened for their potential to direct starch fermentation away from lactic acid production. Strain 25A (10^7 CFU/ml) reduced the accumulation of lactate by 90% when added to mixed rumen fermentations that contained excess soluble carbohydrates. Lactic acid accumulated to high concentrations (70mM) in control fermentations. When strain 25A was combined with *Megasphaera elsdenii*, strain Z2, lactate accumulation was reduced by 99%. An analysis of 16S rRNA sequences indicated that the amylolytic bacterium, strain 25A, a short Gram negative rod, is not phylogenetically clustered to *Prevotella*, *Bacteroides*, *Flavobacterium* or *Cytophaga*. Strain 25A used starch as well as cellobiose, pectin and xylan. Succinate and acetate were the major fermentation products when grown on starch. This bacterium possesses 12-methyltetradecanoic (*anteiso*-C15:0) acid as a major cell wall fatty acid. Other predominant acids included C16:0, and *iso* 15:0. Further characterization of this bacterium is necessary before it can be identified. We will continue our investigations to determine if practical methods can be developed to direct ruminal starch fermentation away from lactate production.

#6 PH AND RUMINAL ACID-BASE BALANCE OF LACTATING COWS FED WITH MAIZE SILAGES. I. Fernandez ¹, H. Brugère ², B. Michalet-Doreau ¹. ¹INRA, URH-DIM, 63122 St Genès Champanelle, FRANCE, ²Ecole Nationale Vétérinaire d'Alfort. Laboratoire de Physiologie Thérapeutique, 94704 Maisons-Alfort Cedex, FRANCE.

When rapidly fermentable carbohydrates are fed to ruminants, different fermentative profiles take place in the rumen. This may result in a decrease in pH and the extent of decrease depends on buffer system of the ruminal fluid. The aim of this study is to examine with a single method of titrimetry, the main components of buffer systems of the ruminal fluid of lactating cows fed with maize silages. Four maize silages different by their chopping length and their maturity stage, were distributed to 4 lactating cows fitted with a ruminal cannula, in a 4x4 Latin square. Ruminal pH was measured every two hours during 24 hours. Titration by strong acid and base of ruminal fluid was realized on samples collected before feeding, 2 and 5 h after feeding, and on two consecutive days. Buffer system was defined as the number of moles per liter of H⁺ required to involve a variation in pH. Two acid functions of bicarbonates (pK₁ = 6.25 et pK₂ = 10.25) and VFA (pK = 4.8) were the main components of the buffer systems in the ruminal fluid. Buffer system due to VFA increased after feeding and this due to bicarbonates decreased (Table 1). After feeding, VFA production marks strongly ruminal acidity and contributes to bicarbonates balance moving towards CO₂ production. Diet was not significant on pH parameters and buffer systems, but variations between cows were significant. The cow number 3 had the highest mean ruminal pH and the shortest time during pH stayed under 6.2. Concurrently, buffer system due to VFA was the lowest and this due to bicarbonates the highest. Titrimetry is a possible method to explain the important individual differences in resistance to ruminal acidification.

Table 1 : Ruminal acido-basic balance measured in the maxima of buffer zone

Time after feeding	-1h	+2h	+5h	Effect of time	
				-1h/+2h	+2h/+5h
<i>VFA</i>					
pK = 4.8	4.47	6.43	6.67	***	NS
SE	0.13	0.25	0.19		
<i>Bicarbonates</i>					
pK ₁ = 6.25	6.05	3.15	3.69	***	**
SE	0.20	0.27	0.25		
pK ₂ = 10.25	4.13	2.69	2.39	***	NS
SE	0.25	0.29	0.26		

** : significant at 1% ; *** : significant at 1‰

#7 THREE CONDITIONS OF THE RUMINAL MILIEU THAT DETERMINE PH.

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Ruminant nutritionists have tried for many years to understand the changes in ruminal pH that occur over time and which are affected by diet and management. In order to gain a greater understanding of these changes, ruminal pH can be attributed to three conditions of the ruminal milieu, and each of these conditions can be further studied and subdivided. Changes in ruminal pH result from 1) changes in strong ion difference (SID), 2) changes in volatile fatty acid (VFA) concentrations, and 3) changes in the partial pressure of CO₂ in the rumen fluid (pCO₂). Strong ion concentrations can be affected by diet, contribution from saliva, or even hot weather (ion excretion). VFA concentrations may result from different feed degradation or VFA absorption rates.

The pCO₂ in the rumen has been shown to vary substantially for fistulated ruminants and it decreases during meals and increases by 4 hours after meals. The range in pCO₂ was .30 to .50 atm. for sheep fed a high forage diet and .50 to .70 atm. for sheep fed a high concentrate diet (Barry et al., 1977). N₂ gas from the air occupied 5 to 50% of rumen head space. An increase in pCO₂ of 0.2 atm. would decrease pH by .15 units. Even if leaky fistulas are responsible for the dilution of CO₂ in the rumen, and pCO₂ is higher in intact animals, the pCO₂ would affect pH in fistulated animals where measurements are taken. Therefore, changes in pCO₂ need to be considered when interpreting ruminal pH.

In practice, knowing the pCO₂ and pH of ruminal fluid enables calculation of the concentration of bicarbonate ion ([HCO₃⁻]) using the relationship (Kohn and Dunlap, 1998): $\text{pH} = 7.74 + \log \{ [\text{HCO}_3^-] / \text{pCO}_2 \}$. The ratio of ionic to acidic form of each VFA can also be determined from the relationship: $\text{pH} = \text{p}k_a + \log \{ [\text{A}^-] / [\text{AH}] \}$. Thus, knowing pH, pCO₂ and VFA concentrations enables direct determination of all weak ions in solution. Since all cations in solution must be balanced by anions, $\text{SID} = \text{ionic form of each VFA} + \text{HCO}_3^- - \text{H}^+$ (Stewart, 1983).

Erdman et al., (1982) reported increases in ruminal pH from using 1% NaHCO₃ or 0.8% MgO or both buffers in high concentrate dairy diets. If we assume a constant pCO₂ of 0.7 atm and use reported VFA concentrations, SID would be calculated as 0.107 or 0.109 for control or NaHCO₃ treatments but a higher 0.115 for treatments with MgO. Although both buffers increased ruminal pH, the NaHCO₃ did so by decreasing VFA by 10% while the MgO only affected SID. The question remains as to why NaHCO₃ decreased VFA concentration. Digestibility increased with NaHCO₃ and no changes were detected in passage rates. Kalscheur et al., (1997) reported an effect on pH of both proportion of forage in the ration and inclusion of buffers (NaHCO₃ and MgO together). The VFA concentrations were decreased by forage to concentrate ratio but SID was unaffected by either treatment. Krause, et al., (1999) reported lower ruminal pH for high moisture corn compared to dried corn and lower pH for fine particle size of alfalfa silage vs. course particle size. The pH ranged from 5.72 to 6.04, however the calculated SID was 0.140 for all treatments and pH effects were again explained by differences in total VFA. Tucker et al.,

(1993) reported that Na^+ and other mineral concentrations were not different 2 hours after 330 g NaHCO_3 or water were infused into the rumen.

These examples show that ruminal pH is a function of digestion and absorption, changes in strong ion concentration, and changes in pCO_2 . Although SID appears to contribute considerably to pH, and it varies in different data sets, the factors that influence SID are not well understood.

Barry T. N., A. Thompson, and D. G. Armstrong. 1977. Rumen fermentation studies on two contrasting diets. *J. Agric. Sci., Camb* 89: 183-195.

Erdman, R.A., R. W. Hemken, and L. S. Bull. 1982. Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: Effects on production, acid-base metabolism, and digestion. *J. Dairy Sci.* 65: 712-731.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997. Effect of dietary forage concentration and buffer addition on duodenal flow of trans-C18:1 fatty acids and milk fat production in dairy cows. *J. Dairy Sci.* 80: 2104-2114.

Kohn, R. A. and T. F. Dunlap. 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. *J. Anim. Sci.* 76: 1702-1709.

Krause, K. M., D. K. Coombs, and K. A. Beauchemin. 1999. Effect of corn processing and forage particle size on ruminal pH in lactating dairy cows. *J. Dairy Sci. Suppl.* 82: 43 (Abstr.).

Stewart, P. A. 1983. Modern quantitative acid-base chemistry. *Can. J. Physiol. Pharmacol.* 61: 1444

Tucker, W. B., J. F. Hogue, M. Aslam, M. Lema, P. le Ryet, I. S. Shin, M. T. van Koevering, R. K. Vernon, and G. D. Adams. 1993. Controlled ruminal infusion of sodium bicarbonate. *J. Dairy Sci.* 76: 2222-2234.

#8 NOVEL APPROACHES FOR INHIBITING RUMEN METHANOGENESIS. E. M. Ungerfeld, S. R. Rust, M. K. Jain and R. Burnett, Michigan State University, East Lansing, MI 48824 (517-432 1390)

Rumen methanogenesis is an energy loss for the ruminant and contributes to global warming. We tried three different strategies to decrease methane production *in vitro*: 1) inhibition of pyruvate decarboxylation; 2) new alternative electron sinks; and 3) methanogenic inhibitors not previously tried in rumen fermentation. We attempted to inhibit pyruvate dehydrogenases either directly or by impairing thiamine synthesis or utilization. Methanogenesis inhibition achieved with this strategy was small. Oxaloacetate, acetoacetate, beta-hydroxybutyrate, crotonate and vinylacetic acid, each at 6, 12 and 18 mM, were not effective inhibitors. However, these compounds increased fermented organic matter (FOM) between 11 and 70 % (their own fermentation not included). Propionic acid and ethyl 2-butynoate, each at 6, 12 and 18 mM, inhibited methanogenesis by a maximum of 76 and 79 %, respectively. Fermentation shifted from acetate to propionate and butyrate. FOM decreased between 16 and 36 %. The eukaryotic and archaeal DNA-polymerase inhibitor aphidicolin at 30, 60 or 150 microM did not affect methanogenesis. Lumazine at 0.3, 0.6 and 1.2 mM decreased methanogenesis by about 50 % and FOM between 12 and 17 %. The coenzyme M analog bromopropanesulfonate at 1, 10 and 50 microM did not affect methanogenesis. We speculate that combinations of methanogenic inhibitors and organic acids that improved FOM may lift the fermentation constraints caused by the former.

#9 EFFECT OF COCONUT OIL ON TOTAL TRACT METHANOGENESIS AND RUMINAL METHANOGENS IN FAUNATED AND DEFAUNATED SHEEP. A. Machmüller, C. R. Soliva, and M. Kreuzer, Institute of Animal Sciences, Swiss Federal Institute of Technology (ETH), ETH Zentrum/LFW, CH-8092 Zurich, Switzerland (41-1-632-3269)

The present study was carried out with three adult wethers in a 2 x 2 factorial Latin square arrangement, regarding the effect of dietary coconut oil (CO, 5% vs. rumen-protected fat, 5%) and defaunation (faunated vs. chemically defaunated). Diets consisted of 54% maize silage, 5% hay, and 41% concentrate (DM basis). Total digestive tract methane release was measured in open-circuit respiratory chambers. In rumen fluid, methanogens, total bacteria and ciliate protozoa were enumerated. Methanogens were quantified by *in situ* hybridization with fluorescent (CY3) labeled oligonucleotide probes (ARC915) targeting 16S rRNA sequence of Archaea. Defaunation treatment did not affect total tract methane release but increased ruminal methanogen ($P < 0.01$) and total bacteria counts ($P < 0.001$). Independent of the defaunation treatment, CO decreased ($P < 0.05$) total tract methane release by 20%. CO seems to be without general effect on ruminal methanogens but significant interactions occurred with defaunation treatment ($P < 0.05$) with the highest methanogen counts when CO was fed to defaunated sheep. Beside a differing activity of the ruminal methanogens, the incongruence between number of ruminal methanogens and total tract methane release also could have resulted from a varying proportion of hindgut fermentation in methanogenesis.

#10 ISOLATION AND IDENTIFICATION OF A BACTERIUM THAT INHIBITS HYPERAMMONIA PRODUCING BACTERIA. J. L. Rychlik and J. B. Russell. Department of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY 14852 (607-255-4508)

When ruminal fluid from a cow fed hay was serially diluted into broth containing 15 mg/ml of Trypticase and Casaminoacids, little OD (<0.25) or ammonia accumulation was observed at dilutions greater than 10^7 , and hyperammonia producing bacteria (HAB) could not be isolated. These high dilution tubes, however, contained thin rods that caused zones of clearing in *C. sticklandii*, *C. aminophilum* and *P. anaerobius* agar overlays. The thin rods stained Gram-positive and were monensin sensitive. Little growth was observed on Trypticase, but glucose, xylose and variety of other sugars were rapidly fermented. Because butyrate was the predominant end-product of carbohydrate fermentation, the thin rods were presumptively identified as butyrvibrio. The isolate (JL5) most active against *C. sticklandii* also inhibited a variety of other Gram-positive ruminal bacteria. Cell-free-supernatants from exponentially growing cultures that were autoclaved retained more than 50% activity. The activity was not sensitive to proteinase K, pronase E or trypsin, but it was reversibly inactivated by oxygen. The "butyrvibriocin" could be precipitated with 60% ammonium sulfate, dialyzed (3500 MW cutoff) and separated on a PAGE gel. Based on these results, it appears that most probable number methods cannot be used to enumerate ruminal HAB. When ruminal fluid was diluted in broth, HAB were inhibited by bacteriocin producing bacteria, and the isolation of HAB was also prevented.

#11 ANALYSES OF ANTIBIOTIC RESISTANCE GENES IN ANAEROBIC BACTERIA AND TOTAL DNA FROM SWINE MANURE. T. R. Whitehead and M. A. Cotta. USDA/ARS, Natl. Cent. Agric. Utilizn. Res., Peoria, IL (309-681-6273).

Recent reports have suggested feeding of antibiotics to domestic animals may result in increased microbial resistance to antibiotics, which can have an impact on human health. We have initiated an investigation of antibiotic resistant (AR) anaerobic bacteria present in both pig feces and manure storage pits. Samples were collected from a local swine facility. AR bacteria were enumerated on complex media with and without tetracycline (Tc), erythromycin (Em), or tylosin (Ty). AR bacteria were found in all samples, and the level of resistance ranged from 4% Em resistance to 32% Ty resistance. Several Em/Ty resistant strains were also Tc resistant. Em/Ty resistant bacteria were subjected to PCR tests for the presence of a different class of erm genes. At least two strains that were ermB positive were also Tc resistant and contained a tetM gene by PCR testing and sequencing. One Em resistant strain was found to contain a 4.1-kb plasmid. This plasmid contained an ermT gene and was capable of replicating and providing Em resistance in *B. subtilis*, *S. gordonii*, and *E. coli*. Total DNA preparations from each sample were tested with Em and Tc gene PCR primers and the products randomly cloned and sequenced for similarity analyses with known AR genes. The finding of a high number of identified and unidentified AR eubacteria in swine feces and manure storage pits suggests that these ecosystems may serve as reservoirs of antibiotic resistance genes.

#12 TETRACYCLINE RESISTANCE GENES IN GROUNDWATER IMPACTED BY SWINE LAGOON WASTE. J. Chee-Sanford¹, R. Aminov¹, N. Garrigues¹, I. Krapac², and R. Mackie^{1*}, Dept. of Animal Sciences¹, University of Illinois-Urbana, and Illinois State Geological Survey², Urbana, IL 61801 (217-244-2526*)

The widespread use of antibiotics in agriculture has raised several issues of concern related to human and animal health. These concerns include the increasing emergence of antibiotic resistance in bacteria and whether the genes conferring resistance can be widely disseminated through the food chain and the environment. In this study, we used PCR-based methods to assess the presence of several tetracycline (tet) resistance genes in the waste lagoons and groundwater underlying two swine farms. Groundwater was impacted by seepage from the lagoons, as indicated by the presence of fecal bacteria and chemical determinants. PCR screening of DNA from the lagoons indicated the presence of all eight tet resistance determinants used in this study. Nearly all the groundwater samples contained multiple tet resistance genes. Tet-resistant enterococci isolated from the lagoons, as well as several typical environmental isolates from groundwater, harbored the tet(M) gene, which was not predominant in the environmental samples. Most of the isolates had multiple drug resistance phenotypes. Denaturing gradient gel electrophoresis (DGGE) demonstrated the conservation of the tet(M) gene in environmental samples and in the isolates. This study demonstrated the occurrence of tet resistance genes in the environment, likely as a direct impact of agriculture, and will contribute to the increasing interest in the molecular ecology of antibiotic resistance.

#13 EFFECT OF SODIUM CHLORATE ON IN VITRO RUMINAL FERMENTATIONS. T. R. Callaway, R. C. Anderson, S. A. Buckley and D. J. Nisbet. USDA/ARS, SPARC, College Station, TX 77845 (979-260-9397)

Almost 1/3 of all cattle in the U.S. contain *Escherichia coli* O157:H7, a food-borne pathogen of concern to the beef industry. *E. coli* is capable of anaerobic respiration using nitrate as an electron acceptor. In addition to reducing nitrate to nitrite, nitrate reductase co-metabolically reduces chlorate to chlorite, which accumulates inside the cell, killing the bacterium. Bacteria lacking nitrate reductase are chlorate insensitive. Addition of sodium chlorate (0, 1.25, 2.5, 5, 10, and 20 mM) to in vitro ruminal fluid incubations of starch and ground alfalfa reduced ($P < 0.05$) *E. coli* O157:H7 populations. However, chlorate treatment did not impact total anaerobic bacterial populations, final pH, acetate:propionate, methane or total VFA. 10 μ M monensin had no effect on *E. coli* O157:H7 and no interactions between chlorate and monensin were observed. Continuous culture studies with mixed ruminal microbial bacteria indicated that 10 mM chlorate reduced *E. coli* O157:H7 but did not alter the VFA pattern or total anaerobic bacterial populations. When cattle were given 100 mM chlorate as drinking water, the VFA profile and ruminal pH were unchanged. Total ruminal wild-type *E. coli* and coliforms were decreased non-significantly by chlorate treatment, but fecal *E. coli* were significantly ($P < 0.05$) decreased from 10⁴ to less than 10 cells/ml. Chlorate treatment kills *E. coli*, including O157:H7, but did not alter the ruminal fermentation in vitro or in vivo.

#14 THE ROLE OF LIPOTEICHOIC ACIDS IN THE RESISTANCE OF STREPTOCOCCUS BOVIS TO NISIN. H. C. Mantovani and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Previous work indicated that nisin and monensin had similar effects on ruminal fermentation in vitro, but it was unclear if ruminal bacteria could become nisin-resistant. *Streptococcus bovis* JB1 was initially inhibited by nisin (1 microM), and viability decreased 3 logs. However, some of the cells survived, and these nisin-resistant cells grew as rapidly as untreated ones. Nisin-resistant cultures remained nisin-resistant even if nisin was not present, but competition studies indicated that nisin-sensitive cells could displace the resistant ones if nisin was not present. Fresh isolates were initially as nisin sensitive as the wild type *S. bovis* JB1, but they also developed nisin resistance. Nisin-sensitive, glucose-energized cells lost virtually all of their intracellular potassium if 1 microM nisin was added, but resistant cells retained potassium even after 10 microM nisin was added. Nisin-resistant cells were less hydrophobic and more lysozyme-resistant than nisin-sensitive cells. Because the nisin-resistant cells bound less cytochrome c, it appeared that nisin was being excluded by a net positive (less negative) charge. Nisin-resistant cells had more lipoteichoic acid than nisin-sensitive cells and de-esterified lipoteichoic acids from nisin-resistant cells migrated more slowly through a polyacrylamide gel than those from nisin-sensitive cells. These results indicated that lipoteichoic acids could be modified to increase the resistance of *S. bovis* to nisin. *S. bovis* JB1 cultures were still sensitive to monensin, tetracycline, vancomycin and bacitracin, but ampicillin resistance was 1000-fold greater.

#15 PCR SCREENING OF THE RUMEN MICROBIAL META-GENOME FOR *luxS* SEQUENCE HOMOLOGUES. D. A. Antonopoulos, K. A. D. Piggott, B. A. White, Department of Animal Sciences, University of Illinois, Urbana, IL 61801 (217-244 – 4305)

Our laboratory has focused on delineating cellulase sequences from various ruminal microbial strains to understand cellulase diversity and function. Regulation of cellulase function at the molecular level, however, has not been addressed adequately. One intriguing aspect of ruminal organisms is the extensive lag phase associated with strains grown as pure cultures in defined medium containing cellulose. The luciferase system described in *Vibrio* has provided a model for quorum sensing. We hypothesize that an analogous quorum sensing system may exist in relation to cellulose degradation in the rumen. Primers specific to conserved regions of the regulatory molecule, *luxS*, of the luciferase system were designed and used to PCR-survey DNA samples extracted from whole rumen contents of eight steers. Four steers were fed a diet of medium-quality grass-legume hay at maintenance intake. The other four steers were fed a diet of 20% hay, 52% corn, 5% corn steep liquor, 3% miscellaneous, and 20% of either cornstarch, corn gluten feed, modified corn fiber, or distillers dried grains. PCR amplification yielded single bands of ~200 bp (predicted size based on consensus sequences used to design the primers) in three of the four DNA samples from steers fed grass-legume hay while no PCR products were detected in the samples from grain-fed animals. These bands are currently being cloned for sequence determination.

- #16 THE RUMINOCOCCUS ALBUS 7 BACTERIOCCIN: AN UNEXPECTEDLY LARGE PROTEIN WITH HOMOLOGY TO ENDOGLUCANASES. J. Chen, D. M. Stevenson, and P. J. Weimer, Dept. of Bacteriology, Univ. of Wisconsin-Madison, and USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI 53706 (608-264-5408).**

Inhibition of growth of *R. flavefaciens* strains FD-1 and B34b by *R. albus* 7 is due to a heat-labile, protease sensitive agent, suggesting that the inhibitor is a bacteriocin. The agent was purified from culture supernatants of *R. albus* 7 by ammonium sulfate precipitation, ultrafiltration, gel filtration, and anion-exchange chromatography. The active fraction from the last step contained two proteins (MW ~36 kDa and ~45kDa). The 36kDa protein contained the inhibitory activity, and was purified by electroblotting and subjected to N-terminal and internal sequencing. BLAST sequence analysis revealed no homology to small, cationic bacteriocins, but weak homology to some *Clostridium* and *Ruminococcus* endoglucanases. Oligonucleotide probes, designed from the N-terminal amino acid sequence, hybridized to a 4 kb HindIII fragment of *R. albus* 7 DNA. Fragments in this size range were ligated into pGEM-3Z and electro-transformed into *E. coli* JM109. Transformants were screened by colony hybridization, and one was found to contain a cloned ~1.4 kb HindIII fragment that may contain the gene for the bacteriocin.

- #17 DISTRIBUTION OF TETRACYCLINE RESISTANCE DETERMINANTS ENCODING RIBOSOMAL PROTECTION PROTEIN GENES IN ANIMAL PRODUCTION SYSTEMS. N. Garrigues, R. I. Aminov, R. I. Mackie, Dept. of Animal Science, University of Illinois, Urbana, IL 61801 (217-333-8809)**

The emergence of antibiotic resistance is a serious problem and we have focused on the development of molecular tools for detection and identification of tetracycline resistance genes originating from production animals. We designed a set of PCR primers for detection of eight classes of ribosomal protection proteins (RPPs) for retrieval and sequence analysis of the corresponding gene fragments from a variety of bacterial and environmental sources. These primers were used to detect the circulation of these genes in the rumen of cows, in swine feed and feces, and in swine fecal streptococci. Classes O and W were found in the intestinal content of both animals, while the presence of Tet M was confined to pigs and Tet Q to the rumen. The *tet(O)* and *tet(W)* genes circulating in the microbiota of the rumen and gastrointestinal tract of pigs were identical despite the differences in animal hosts and antibiotic use regimens. All swine fecal streptococci possess *tet(O)* and 22% of them additionally carry *tet(M)*. This population could be considered as one of the main reservoir of these resistance genes in the pig gastrointestinal tract. All classes of RPPs, with the exception of Tet T and TetB P, were found in the different components of swine feed.

#18 METHOD TO MEASURE FRACTIONAL RATE OF VOLATILE FATTY ACID ABSORPTION FROM THE RUMEN. M. S. Allen¹, L. E. Armentano², M. N. Pereira², Y. Ying¹ and J. Xu¹, Michigan State University¹ and University of Wisconsin, Madison², (517-432-1386)

A method was developed to measure rate of absorption of valeric acid from the rumen, as an index of fractional rate of volatile fatty acid absorption. Valeric acid was chosen because it is a short-chain fatty acid with low ruminal background concentration and metabolism of valerate by mixed ruminal microbes was not detected for incubations up to 20 h in vitro. Solutions (634 ml, pH 6.0) containing valeric acid (1.84 moles) and Co-EDTA (5 g) were pulse-dosed into the rumen (5 sites) and ruminal fluid was sampled over time (5 sites). Primary assumptions are as follows: valerate is removed from the rumen by absorption and passage, cobalt is removed from the rumen by passage only, liquid pool size in the rumen is constant, and that dilution or concentration (by water ingestion, saliva flow, flux of water across ruminal wall) affect valerate and cobalt equally. Rate of concentration decline of valerate and cobalt from the rumen over time were estimated by nonlinear regression. Rate of absorption of valerate was estimated by subtracting rate of concentration decline over time determined for cobalt from that determined for valerate. Preliminary work with non-lactating, non-pregnant, mature Holstein cows resulted in fractional rates of valerate absorption ranging from 0.16 to 0.25 h⁻¹ following a 2 week adaptation period for diets of low or high fermentability.

#19 THERMODYNAMICS OF RUMINAL FERMENTATION. R. A. Kohn, Dept. Animal and Avian Sci., University of Maryland, College Park, MD 20742 (301-405-4583)

Chemical reactions are either controlled thermodynamically or kinetically. With kinetic control, the profile of products formed depends on substrate concentrations and enzyme activities that control the rates of synthesis for competing pathways. Thermodynamic control occurs when reactants are sufficiently limited relative to products that the reactions cannot proceed according to the Second Law of Thermodynamics. Under these circumstances, thermodynamics controls which pathway branches are available and the final concentration of products. Complex biological pathways have built in inefficiencies so that it is not feasible for the reactions to proceed all the way to equilibrium. The free energy efficiency of ATP synthesis can be determined from the concentrations of reactants and products. For example, the efficiency of acetate and propionate synthesis was estimated to be approximately .55 but that of butyrate and lactate synthesis was estimated to be .43. Efficiency of methanogenesis was .65 and that of reductive acetogenesis was slightly greater than 1. Reductive acetogenesis would only be feasible to a small extent under normal ruminal conditions. The observed efficiencies for methanogenesis, and acetate and propionate synthesis may approach the maximal efficiencies that are feasible allowing for the necessary losses of heat from fermentation. Thus, their concentration would be limited by thermodynamics.

#20 IS RUMINAL VISCOSITY INVOLVED IN THE MICROBIAL FIBROLYTIC ACTIVITY DECREASE WITH HIGH CEREAL DIET? C. Martin, I. Fernandez, Y. Rochette, B. Michalet-Doreau. INRA, URH-DIM, 63122 St Genès Champanelle, FRANCE.

The supplementation of forage diets with cereals is known to depress ruminal fiber digestion by decreasing microbial fibrolytic activity of the solid-associated micro-organisms (SAM) through a decrease in ruminal pH. But an increase in the ruminal viscosity with cereal rich diets may also be involved in the inhibition of the microbial fibrolytic activity. The aim of this study was also to assess the ruminal pH, viscosity and microbial fibrolytic activity in the rumen in response to barley supplementation. Four sheep fitted with a rumen cannula were fed twice daily on a diet consisted of 100% alfalfa hay (H) or supplemented with 60% barley (HB). Ruminal contents were withdrawn on 2 consecutive days at 1h before feeding and 3h after feeding. Samples were filtrated (100 µm) and 2 ml of fluid were maintained at 39°C for measurements of pH and viscosity with a shear-stress Carri-med CSL 100 Rheometer. SAM enzymes were extracted from the solid phase of the rumen content by sonication. Fibrolytic specific activity (avicelase) was measured by the amount of reduced sugar released from purified substrate. Means of the two sampling times on two consecutive days are presented. As expected, avicelase activity of the SAM was lower with HB compared to H (Table 1). Addition of barley in the diet involved a decrease in pH, but also an increase in the viscosity of the ruminal fluid. Compared to diet H, the abundance of rapidly digested barley starch in the rumen with HB involved an accelerated production of organic acids and bacterial mucopolysaccharides (slime) that might explain the increase in the ruminal fluid viscosity. Ruminal viscosities variations induced by barley supplementation might be involved in the decrease of microbial fibrolytic activity through a limitation of microbial enzymes diffusion and/or an accumulation of inhibitor substances of enzyme activity. Future studies required to assess the specific effect of the ruminal viscosity on these microbial events.

Effect of barley supplementation on the ruminal pH, viscosity (mPa.s) and specific activity of avicelase (µmol reduced sugar/mg protein/h)

	H	HB	SE	Diet effect ¹
Avicelase	1.36	0.32	0.23	**
pH	6.54	6.00	0.19	*
Viscosity	3.59	9.02	1.14	**

¹*P < 0.10; **P < 0.01

- #21 SELECTED-ION-FLOW-TUBE MASS SPECTROMETRIC ANALYSIS OF RUMEN GASES.** R.J. Dewhurst¹, R.T. Evans¹, P. Spanel² and D. Smith³, ¹Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK (+44 1970 823072), ²J. Heyrovsky Institute of Physical Chemistry, Prague 8, Czech Republic and ³Centre for Science and Technology in Medicine, Keele University, Stoke-on-Trent ST4 7QB, UK

Selected-ion-flow-tube mass spectrometry provides precise on-line analysis of most gases present in a sample. This study investigated its use as a potential alternative to stomach tubing or rumenocentesis. Samples of rumen headspace gas and corresponding samples of rumen liquor were taken from 3 fistulated cows at intervals (1, 3 and 7 hours) after morning feed (grass silage and concentrates). The cows consumed 16.8 kg/d of DM and were producing 28 kg/d of milk. Results were analyzed according to interval after feeding. There were small changes in pH (6.40, 6.50, 6.67; SED=0.076) and VFA concentrations (mmol/L: 94, 81, 79; SED=6.9) and a larger decline in ammonia-N concentrations (mg/L: 114, 57, 44; SED=33.7), though these were not statistically significant. Sulfides predominated in rumen gas and their concentrations (ppm) changed markedly after feeding: hydrogen sulfide: 204, 108, 30; SED=16.6; P<0.001; methyl sulfide: 16.6, 4.0, 0.8; SED=3.29; P<0.05; and dimethyl sulfide: 11.7, 2.0, 0.5; SED=2.83; P<0.05. These changes mirrored ammonia concentrations in liquor and may reflect their common dependence on the fermentation of (sulfur) amino acids. Ammonia concentrations in rumen gas varied in the opposite direction to concentrations in liquor (r=-0.59; P<0.1) and likely depend more on pH. Ammonia concentrations in rumen gas could provide a diagnostic for sub-acute ruminal acidosis. Low levels of alcohols and VFA were detected in rumen gas (20-500 ppb). The molar proportions of VFA were similar in gas and liquor samples, with gas containing slightly less acetic acid and disproportionately more valeric and caproic acids.

- #22 AMMONIA PRODUCTION AND PROTEIN DEGRADABILITY AS AFFECTED BY FOOD SOURCES AND PURIFIED MONENSIN OR RUMENSIN.** R.P. Lana, N.G.S. Barbosa, A.B. Mancio. Dept. de Zootecnia, Univ. Fed. Vicosa, 36571-000, Vicosa, MG, BRAZIL (021 31 899 3288)

Ruminal feed protein degradation was assessed with a newly developed in vitro technique. Three energetic (corn meal, CM; wheat meaddlings, WM; and sorghum, SO) and 3 proteic sources (soybean meal, SM; corn gluten meal, CG; and urea, UR), alone or with 5 microM monensin or Rumensin, were incubated in duplicate. The feed (100 mg) was added to 9.8 ml of culture (pellets from rumen fluid resuspended in equal volume with Mc Dougall's media) and .2 ml of ethanol with or w/o the ionophores. Among the proteic sources, NH₃ production was greater (P<.01) for UR, then SM and CG, and decreased by the ionophores, except for SM. Soluble protein was similar for all three feeds, but increased with the ionophores, except for UR. Microbial protein decreased with UR and SM and increased with CG. Among the energetic sources, NH₃ was lower for SO, then CM and WM. There was no treatment effect on soluble protein, but microbial protein decreased by ionophore when SO was used (P<.05). Protein degradability (((N-NH₃ + N-soluble protein + N-micr)/N-food)*100) was better estimated for the proteic sources; the observed values in 48 hours incubation were 60% for SM and 35% for CG.

#23 EFFECTS OF MONENSIN AND LASALOCID ON FERMENTATION OF AMINO ACIDS BY MIXED RUMINAL BACTERIA. R.P. Lana, J.S. Oliveira, R.G. Veloso, P.M.M. Nunes, A.C. Borges. Dept. de Zootecnia, Univ. Fed. Vicosa, 36571-000, Vicosa, MG, BRAZIL (021 31 899 3288)

The effect of the ionophores, monensin and lasalocid, was studied on ruminal fermentation of amino acids. Ruminal fluid from a steer fed elephant-grass supplemented with hydrolyzate of casein was used without (C - control) or with monensin (M) or lasalocid (L). Inocula were transferred daily to fresh tubes during 10 days and, from 11th to 20th day, each of the 3 treatments gave 3 new ones (CC, CM, CL, MC, MM, ML, LC, LM, LL, where the first letter refer to the 1st phase and the 2nd letter the 2nd phase). From 1st to 10th day of incubations with ionophores, ammonia production did not increase compared to controls. From 11th to 12th day, the ionophores were more efficient decreasing ammonia when they were absent in the 1st phase; and the lasalocid was still able to decrease ammonia and microbial protein when monensin was used in the first phase. From 16th to 20th day, independent of the 1st phase, the ionophores decreased ammonia. However, the ionophores decreased microbial protein in the 2nd phase when absent in 1st phase and increased when present in 1st phase. When the ionophores were removed (from 1st to 2nd phase), a strong increase in ammonia was detected, but this effect was not observed in the 11th to 12th days, probably due to the residual effect of the ionophores.

#24 MICROBIAL FERMENTATION IN CONTINUOUS CULTURES RECEIVING FAT BEFORE OR AFTER THE ADDITION OF AN IONOPHORE. M. Croucher and V. Fellner, Department of Animal Science. North Carolina State University, Raleigh, NC 27695. (919-515-4018).

Rumen inoculum was obtained from a lactating dairy cow, filtered and incubated in dual-flow continuous culture fermentors. The nominal volume of the vessels was 700 ml and the fractional liquid dilution rate was maintained at 6.3%/h. Alfalfa pellets (15 g/d) were added to the fermentors twice daily. The experiment consisted of two treatments assigned to four fermentors (n=2) over a period of 6 d following 3 d of adaptation to diet and fermentor. Treatments were: 1) Monensin (30 ppm; 450 µg/d) administered for 2 d followed by supplemental linoleic acid (.450g/d), and 2) Linoleic acid (.450 g/d) added for 2 d followed by monensin (450 µg/d). Data were analyzed as repeated measures. Treatment 1 lowered molar proportion of ruminal acetate by 11 % and increased that of propionate by 29 %. Treatment 2 decreased acetate in ruminal cultures by 8 % and increased propionate by less than 10 %. Methane output declined by 34 % in treatment 1. Treatment 2 reduced methane production (22%) but subsequent inclusion of monensin increased methane output. The decrease in ammonia-N concentration was greater in cultures that received treatment 1 (27 %) compared with those that received treatment 2 (9 %). Both linoleic acid and monensin affected fermentation in continuous cultures however, the ionophore induced response was much less when included after the addition of supplemental unsaturated fat.

#25 DISTRIBUTIONS OF MICROBIAL MASS AND FIBROLYTIC ENZYME ACTIVITIES IN DIFFERENT SIZE OF FEED PARTICLES FROM RUMEN CONTENTS OF SHEEP
J. Pan, T. Suzuki, K. Ueda, K. Tanaka, and M. Okubo, Graduate School of Agriculture, Hokkaido University, Sapporo-shi 060-8589 panjun@anim.agr.hokudai.ac.jp

Three ruminally cannulated wethers fed orchardgrass hay once daily were used to investigate the diurnal changes of CMCase and xylanase activities released from strained rumen solid, large particles (LP > 2390_μm), and smaller particles (2390_μm > SP > 150_μm). The bacteria and protozoa associated with those fractions were separated, and total mass of bacteria associated with particle (PAB) was also determined using DAPA (2-6-diaminopimelic acid) as a marker. The total PAB and the separated mass of protozoa associated with particle (PAP) were tended to increase from 0 h to 2 h after feeding. However, the both total and specific activities of CMCase and xylanase released from those three fractions of rumen digesta decreased up to 6 h, and then recovering to the levels at just before feeding (P<0.05). The specific activities of CMCase and xylanase were significant higher in SP than LP at initial stage. However, this difference tended to be smaller at later stage after feeding. The total activity of xylanase were higher in LP than SP at 24 h after feeding (P<0.05), although no significantly difference was observed at other time points. The PAP mass separated from LP was higher than that separated from SP at 2 h and 6 h after feeding. However, there was no significant difference in total PAB between LP and SP, although higher PAB mass was separated from LP than that from SP at 24 h after feeding. The potential NDF (Neutral detergent fiber) degradability in LP were significantly higher than those in SP at all time points, but this difference tended to be decline at later stage after feeding. These results suggested that distributions of fibrolytic enzyme activities in rumen digesta were affected by size and retention time of feed particles.

#26 USE OF *IN VITRO* FERMENTATION FOR DETERMINING THE POTENTIAL CONJUGATED LINOLEIC ACID (CLA) PRODUCING CAPACITY OF RUMINANT DIETS. E. Frantz, R. Robinson, B. Jacobson, and K. Griswold, Department of Animal Science, Food and Nutrition, Southern Illinois University, Carbondale, IL 62901 (618-453-1766)

This study was initiated to develop an *in vitro* method for the prediction of ruminal CLA production. Using *in vitro* procedures, beef finishing diets varying in either level of forage or linoleic acid were fermented, and NDF digestibility and production of CLA were determined. Corn silage and soy oil were the forage and linoleic acid sources, respectively. Two 2x3 factorial randomized complete blocks were performed with level of forage (20 vs. 40% of diet DM) and level of soy oil (0, 4, or 8% of diet DM) as the factorials. Results indicated that NDF digestion was reduced when forage level was increased from 20 to 40% of diet DM (P<0.05). Addition of 8% soy oil reduced NDF digestion when compared with 0 or 4% added soy oil (P<0.05). CLA production varied among treatments, however, CLA production increased above baseline levels with increasing linoleic acid level in the diet. The cis-10, trans-12 isomer was initially found in higher levels than the cis-9, trans-11 isomer. *In vitro* fermentation may provide a means of screening diets for ruminal CLA-producing potential via ruminal metabolism.

#27 CONCENTRATIONS OF TRANS-18:1 AND CLA IN TISSUES OF LAMBS FINISHED ON DIFFERENT DIETS. D. L. Palmquist, K. E. McClure, G. D. Lowe and D. D. Clevenger, Dept. of Animal Sciences, OARDC/OSU, Wooster, 44691. (330-263-3908)

CLA can be increased in tissues by feeding unsaturated oils. Lambs (32) were allotted and balanced by sex and weight (2 per pen) and fed four diets in a randomized block design. Diets (DM) were: 1) 2/3 corn silage:1/3 high oil whole shelled corn (HOWSC); 2) 2/3 alfalfa silage (AS):1/3 HOWSC; 3) 1/3 AS:2/3 whole shelled corn; 4) 1/3AS:2/3 HOWSC. Diets 1-4 contained 4.41, 3.64, 3.38 and 5.82% total fatty acids, and 2.06, 1.25, 1.60 and 2.66% linoleic acid. Days on test were 103.0^a, 98.7^a, 78.5^b, and 75.7^b. Lamb performance for diets 1 to 4 were: 0.98^c, 1.10^b, 1.29^a, and 1.23^a DMI (kg/d); and 0.19^b, 0.19^b, 0.27^a, and 0.27^a ADG (kg) (all $P < 0.05$). Tissue fatty acids were measured by GLC, which did not separate *trans*18:1 isomers or CLA isomers. *Trans*-18:1 and CLA in all tissues were in the order of diet 4>1>3>2, and subcutaneous fat > mesenteric fat > loin lean, except that *trans*-18:1 was higher in mesenteric than subcutaneous fat. Extent of desaturation, measured by regressing CLA on (CLA + *trans*-18:1) across diets in each tissue was lower than generally observed in mammary tissue: loin > subcutaneous > mesenteric, similar to differences in the ratio of oleic/stearic acids and reflecting tissue desaturase activity. Total CLA content of tissues was increased by feeding increased amounts of linoleic acid. Concentrations of dietary linoleic acid and tissue CLA were highly correlated ($r = 0.9$).

#28 VARIATION IN THE CONCENTRATIONS OF ODD-CHAIN FATTY ACIDS IN MILK. ¹R. J. Dewhurst, ¹J. K.S. Tweed and ²G. B. Williams, ¹Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK. (+44 1970 823072) and ²Institute of Rural Studies, University of Wales, Aberystwyth SY23 3AL, UK.

Microbial lipids contain a high proportion of odd-chain fatty acids (FA), some of which are distinctive to particular groups of micro-organisms. Some of these FA can be detected in milk and could form the basis of a new diagnostic test of rumen function. This work investigated levels of these FA in bulk milk samples from Holstein-Friesian herds producing from 4500 to 9000 litres per annum, to see if there is a meaningful and useful range. Milk samples were freeze-dried and FA methyl esters prepared using a one-step method. Two bulk milk samples were taken (1 month apart) from each of 12 farms during the period December 1998 to February 1999. There were useful ranges in the levels of most of the odd-chain FA (% of total FA): pentadecanoic acid (C15:0): 0.96 – 1.65; *iso* methyltetradecanoic acid (*iso* C15:0): 0.14 – 0.34; *anteiso* methyltetradecanoic acid (*anteiso* C15:0): 0.90 – 1.52; heptadecanoic acid (C17:0): 0.59 – 0.89; *iso* methylhexadecanoic acid (*iso* C17:0): 0.15 – 0.24; *anteiso* methylhexadecanoic acid (*anteiso* C17:0): 0.33 – 0.45; and heptadecenoic acid (C17:1): 0.22 – 0.45. Bulk samples were taken from a further 3 herds, at least monthly, over 8-month periods. Statistical analysis was undertaken according to whether cows were consuming winter rations (silage-based) or were grazing at pasture. Total odd-chain FA were higher when cows were grazing (5.1 vs 4.3% of total FA; SED=0.14; $P < 0.001$), with a particularly marked increase in *anteiso* C15:0 (1.7 vs 1.2% of total FA; SED=0.07; $P < 0.001$). This may relate to our earlier observation of higher levels of this FA in the bacteria harvested from the liquid phase of rumen contents.

#29 VARIATION IN THE CONCENTRATIONS OF ODD-CHAIN FATTY ACIDS IN RUMEN BACTERIA. ^{1,2}G. S. Bae, ²M. B. Chang, ³W. J. Maeng, ¹R. J. Dewhurst, ¹D. R. Davies and ¹R. J. Merry. ¹Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK. (+44 1970 823072), ²Department of Animal Science and Technology, Chung-Ang University, Ansong, Korea and ³College of Animal Husbandry, Kon-Kuk University, Seoul 143-701, Korea.

We are investigating less-invasive alternatives to current *in vivo* techniques for rumen studies, which are often difficult to conduct and interpret, and usually involve the use of fistulated animals. Several odd-chain fatty acids (FA) are present in rumen micro-organisms and measurement of these compounds in milk might provide a qualitative description of rumen function. Six common rumen bacterial species were grown in triplicate in pure culture on a mixed substrate, with and without supplementary fat (linseed oil or calcium salts of palm oil). Cells were harvested after 24h, freeze-dried and analyzed for FA. Additional dietary fat had no significant effect on microbial FA. Five FA made up between 20 and 50% of total bacterial FA and there were distinctive patterns within the FA. In particular the *Prevotella* spp. had significantly ($P<0.001$) higher concentrations of *anteiso* C15:0 and *R. flavefaciens* had a significantly ($P<0.05$) higher concentration of *iso* C15:0. A wider range of micro-organisms needs studying to further develop the approach but it shows potential for use in diagnostic tests, and may have wider applications in studies of microbial colonisation of feed.

FA (as % of total FA)	C15:0	aC15:0	iC15:0	C17:0	iC17:0
<i>Selenomonas ruminantium</i> HD4	9.2	3.6	7.3	1.8	2.0
<i>Streptococcus bovis</i> 26	8.9	5.0	3.8	4.0	1.0
<i>Ruminococcus flavefaciens</i> 17	8.2	9.0	19.0	3.1	8.4
<i>Ruminococcus albus</i> J6	6.2	5.0	10.8	3.3	2.0
<i>Prevotella ruminicola brevis</i> B14	13.4	14.8	10.8	1.9	4.0
<i>Prevotella albensis</i> M384	8.3	23.2	11.6	0.9	3.4
SED	3.10	3.23	4.18	0.98	1.61
Significance	NS	$P<0.001$	$P<0.05$	$P<0.05$	$P<0.001$

- #30 EFFECTS OF FIBROLYTIC ENZYMES ON DEGRADATION OF ALFALFA HAY FIBER BY MIXED RUMEN MICROORGANISMS. V. L. Nsereko¹, D. P. Morgavi, L. M. Rode, K. A. Beauchemin, T. A. McAllister, Research Center, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1 (403-327-4561). ¹Present address: Pioneer Hi-Bred, Forage Additive Research, Johnston, IA 50131-1004.**

A study was conducted to investigate the mechanisms by which fibrolytic enzymes improve alfalfa hay NDF degradation (NDFD). Alfalfa hay was treated with Depol 40, Sumizyme X, Liquicell 2500, Multifect xylanase, and an experimental blend of enzymes (DP, SX, LQ, MX, PB, respectively), diluted in buffer (pH 6.5), or with buffer alone (control) at 39°C, without (0 h) or with a 2 h incubation. The hay was autoclaved to denature the enzymes, washed to remove hydrolysis products, dried and then incubated with mixed rumen microorganisms for 12 and 48 h. Treatment with MX, PB and SX for 0 h increased NDFD at 48 h ($P < 0.05$). Treatment with MX, PB and SX for 2 h increased NDFD at 12 h, but at 48 h, this effect was only evident for MX and PB ($P < 0.05$). Compared to the 0 h treatment, the 2 h treatment with DP, LQ, PB and MX increased NDFD at 12 h ($P < 0.05$); however, at 48 h, this benefit of incubation was only evident for MX ($P < 0.05$). For the 0 h treatment, NDFD at 12 h was positively correlated to acetyl esterase ($r^2 = 0.84$) and xylanase ($r^2 = 0.92$) activities, and at 48 h, degradation of cellulose was positively correlated to xylanase activity ($r^2 = 0.88$). We conclude that fibrolytic enzymes enhance NDFD by altering the structure of alfalfa hay. Xylanases and esterases are implicated as initiators of this stimulatory effect.

- #31 A TRICHODERMA FEED ENZYME PREPARATION ENHANCES ADHESION OF FIBROBACTER SUCCINOGENES TO COMPLEX SUBSTRATES BUT NOT TO PURE CELLULOSE. D. P. Morgavi, V. L. Nsereko¹, L. M. Rode, K. A. Beauchemin, T. A. McAllister, Y. Wang, Agriculture and Agri-Food Canada, Lethbridge, AB, T1J 4B1 (403 327-4561). ¹Present address: Pioneer Hi-Bred, Forage Additive Research, Johnston, IA 50131-1004.**

Feed enzyme additives, used to improve digestion in ruminants, can potentially compete with rumen fibrolytic bacteria for available adhering sites on feed substrates. We studied the effects of an enzyme preparation from *T. longibrachiatum* (TE) on adhesion of *F. succinogenes* F85 to crystalline cellulose, corn silage, and alfalfa hay. Substrates were pre-incubated with incremental levels of TE for 2 h at 4°C, washed and then incubated with bacterial suspensions at 39°C for 30 min. Adhesion was determined by difference in OD of the bacterial suspension before and after sedimentation of the substrates. TE concentration was negatively related to adhesion of *F. succinogenes* to cellulose ($P < 0.05$). At the highest concentration used, TE reduced adhesion to cellulose from 65% to 39%. For corn silage and alfalfa hay TE stimulated adhesion at low levels ($P < 0.05$) but this effect was lost at higher levels. We conclude that the hydrolytic enzymes in TE competed with *F. succinogenes* for available binding sites on cellulose. However, for complex plant substrates, TE increased bacterial adhesion probably by modifying feed structure.

#32 EFFECT OF LEVELS OF WHOLE COTTONSEED ON RUMINAL VOLATILE FATTY ACIDS, PH AND AMMONIA. O. Balbuena, C. L. Arakaki, C. D. Kucseva and G. A. Kosa. INTA Colonia Benitez, CP 3505, ARGENTINA. (054-3722-493044).

A 4x4 Latin Square design was used to study the effect of whole cottonseed (WCS) in the supplement on ruminal variables. Iso-energetic and iso-nitrogen supplements were formulated with ground sorghum, WCS (20% EE) and urea, and were fed to steers (458 kg BW) given a basal diet of low-quality stargrass hay (4.4% CP, 70 % NDF, 44% ADF and 8.1% lignin). Diets were restricted to maintenance level (about 1.3% BW of DM, 0.74% BW hay DM). Treatments were levels of WCS: 0 (C); 0.75 (L); 1.5 (M) and 2.25 (H) kg DM. Ruminal fluid was collected every 3 hours during a 24 hour period. Sampling were performed at the end of 21-day periods. There were no treatment by time interactions for the variables measured, except for caproic acid and ammonia-N. Ruminal pH was not affected by level of WCS (mean 5.76). Treatments C and L had higher acetate (71.2 and 71.8 vs 68.6 and 68.1 mol%, $P=0.011$), lower propionate (15.7 and 17.3 vs 20.4 and 20.7 mol%, $P=0.013$) and higher acetate to propionate ratios (4.54 and 4.28 vs 3.35 and 3.45, $P=0.005$) than M and H treatments. At 3 hours after feeding, ammonia-N concentrations differ among treatments (C= 48.8; L=35.7; M=26.5 and H=19.1 mg/dL, $P=0.001$). However, at 18 hour post-feeding, ruminal ammonia-N was lower ($P=0.01$) for C (5.5 mg/dL) than for the other treatments (9.0; 9.9 and 12.9 mg/dL for L, M and H, respectively). Feeding 1.5 to 2.25 kg of WCS (5.2 to 7.8% added fat in the diet) decreased acetate to propionate ratios.

#33 EFFECT OF LEVELS OF WHOLE COTTONSEED ON RUMINAL PROTOZOA. C. L. Arakaki, O. Balbuena, C. D. Kucseva and G. A. Kosa. INTA Instituto de Patobiologia Castelar, ARGENTINA. (054-11-46211289).

A 4x4 Latin Square design was used to study the effect of whole cottonseed (WCS) in the supplement on ruminal protozoa. Iso-energetic and iso-nitrogen supplements were formulated with ground sorghum, WCS (20% EE) and urea, and were fed to steers (458 kg BW) given a basal diet of low-quality stargrass hay (4.4% CP, 70 % NDF, 44% ADF and 8.1% lignin). Diets were restricted to maintenance level (about 1.3% BW of DM, 0.74% BW hay DM). Treatments were levels of WCS: 0 (CERO); 0.75 (LOW); 1.5 (MEDIUM) and 2.25 (HIGH) kg DM. Ruminal contents were sampled every 6 hours after feeding, during a 24 hour period for total protozoa counts. With samples taken at feeding, the generic composition of ruminal protozoa was determined. Sampling were performed at the end of 21-day periods. There was a lineal effect of WCS amount and protozoa counts: 13.51; 9.29; 6.47 and 4.38 protozoa $\times 10^{*4}$ for CERO, LOW, MEDIUM and HIGH, respectively (protozoa count= $12.94 - 4.028 * \text{kg WCS}$, adjusted rquare= 0.96). No marked differences were observed in the generic composition of protozoa among the four treatments. *Entodinium* was the predominant genus in all treatments: CERO (92.5%), LOW (93.7%), MEDIUM (94.6%) and HIGH (95.3%). *Isotricha* and *Epidinium* were: 2.6; 2.1; 3.4 and 2.9% and 2.8; 3.1; 1.7 and 1.4% for CERO, LOW, MEDIUM and HIGH, respectively. Increasing WCS levels decreased total protozoa counts with minor effects on protozoa generic composition.

- #34 MICROBIAL ACTIVITY IN GRASS-FED *IN VITRO* CONTINUOUS CULTURES IN RESPONSE TO INFUSION OF GRADED LEVELS OF SOLUBLE SUGARS. M. R. F. Lee¹, J. M. Moorby¹, J. C. Macrae², M. K. Theodorou¹, R. J. Merry¹, D. R. Davies¹ and N. D. Scollan¹, ¹ Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK. (++44 1970 823060) ² Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB2 9SB, UK.**

Novel lines of high sugar ryegrass have been shown to increase the efficiency of N use for milk production in dairy cattle. An experiment was carried out to determine whether this was in part due to an increase in the efficiency of rumen function in response to the amount and the availability of water soluble carbohydrate (WSC). An *in vitro* continuous culture system (RUSITEC) was fed with fresh, chopped and bruised perennial ryegrass (cv. Aberlan; 15g DM/day; containing 22 and 249 g/kg DM of total N and WSC respectively). Artificial saliva was infused into the culture vessels (2 per treatment) either alone (control) or with inulin and sucrose (80:20, w:w), to increase WSC inputs by 1.2 (A), 1.5 (B) and 1.8 (C) times the level in the basal grass feed. To simulate the pattern of release of WSC from grass the saliva/sugar solution was infused at a constant rate for 14h after feeding the forage, followed by artificial saliva until the next feed after 24h. (¹⁵N H₂)₂ SO₂ was infused as a microbial marker and samples of washed bacteria and effluent were taken after steady state had been reached. When no additional sugars were infused (control) the effluent pH was 6.4 but it was significantly ($p < 0.05$) lower at 6.3, 6.2 and 6.0 for levels A, B and C respectively. Ammonia-N concentrations were significantly lower ($p < 0.001$) when sugars were infused, with values of 53, 31, 15 and 5 mg/l for the control and A, B and C respectively. The efficiency of microbial synthesis increased significantly ($p < 0.001$) from 9.8 g N/kg OM apparently digested in the control to 10.8 and 12.7 for levels A and B, but fell to 7.1 at level C. Our results suggest that microbial N flow from the rumen in grazing cattle may be improved by feeding high sugar grasses.

- #35 EFFECTS OF BUFFERS ON pH AND MICROBIAL METABOLISM IN CONTINUOUS CULTURE OF RUMEN CONTENTS. L. M. Aga, R. J. Koski and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108 (612-624-9296)**

Eight dual-flow continuous culture fermenters were used to study the effects of sodium bicarbonate and Acid Buf, a calcified seaweed product, on pH, microbial nitrogen metabolism and feed digestion. Dietary treatments were: 1) control diet, 2) control diet with .78% Acid Buf, 3) control diet with .49% Acid Buf, and 4) control diet with .78% sodium bicarbonate. The control diet had a 60:40 concentrate to forage ratio. The buffered diets did not alter ($P > .05$) pH of the fermenters compared with the control diet. The pH of the fermenters averaged below 6.0 for each of the diets throughout the experiment. No differences ($P > .05$) among treatments were observed for digestion of DM, OM, CP, NDF, or ADF. Molar percentages or total production of VFA were not different ($P < .05$) among treatments. Ammonia-N production, effluent N flow, and bacterial synthesis of CP did not differ ($P > .05$) among treatments. No differences ($P > .05$) were found among treatments for CP and OM composition of the microbes, however, purine concentration (mg RNA/g N) was greater ($P < .05$) for .49% Acid Buf compared with .78% sodium bicarbonate. The low concentration of buffers in the diets may explain the lack of response among treatments.

#36 INFLUENCE OF PATULIN LEVEL ON FERMENTATION BY RUMINAL MICROBES IN CONTINUOUS CULTURE. M. O. Tapia, M. J. Murphy, R. J. Koski and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108 (612-624-9296)

Penicillium species of mold can be contaminants of fermented feeds that can produce mycotoxins, including patulin that are toxic to a wide range of living organisms including microbes, plants and animals. Eight single-flow continuous culture fermenters were used to evaluate the influence of patulin on fermentation by ruminal microbes. There were two 7-day experimental periods, with 4-days for adaptation followed by 3 days of patulin inoculation and sampling. Substrate for microbial metabolism was a diet consisting of 38% alfalfa hay, 28% corn silage, 27% cracked corn, 5% soybean meal and .6% of a mineral mix on a DM basis. During each period, two fermenters each were inoculated with 0, 30, 60 or 90 ppm of patulin every 12 hours for the last 3 days. Addition of patulin to the fermenters at all 3 levels ($P < .05$) decreased true OM, crude protein and ADF digestion by an average of 25, 23 and 44%, respectively with no differences ($P > .05$) among patulin levels. Bacterial N flow (g/d) was 1.13, .65, .65 and .44 for the 0, 30, 60 and 90 ppm patulin levels, respectively and was greater ($P < .05$) for the 0 ppm treatment compared with 30, 60 and 90 ppm. Individual volatile fatty acid concentrations were also affected by patulin, most notably there was a decrease in acetate due to patulin addition. Results from this experiment indicate that patulin can have adverse effects on ruminal microbes and fermentation.

#37 EFFECTS OF RUMEN-DEGRADABLE AND -UNDEGRADABLE PROTEIN ON FINISHING STEER PERFORMANCE. C. J. Fu and M. S. Kerley, Department of Animal Sciences, University of Missouri, Columbia, MO 65211 (573-882-0834)

A 2 x 2 factorial experiment was used to evaluate the effects of supplemental ruminal degradable (RDP) and undegradable (UIP) protein on steer performance in a 170-d growth study involving 72 steers (325 kg). Four dietary treatments were: 1) Low RDP low UIP diet (LL); 2) Low RDP high UIP diet (LH); 3) High RDP low UIP diet (HL); and 4) High RDP high UIP diet (HH). The diets were corn-based and corn silage was used as roughage source. The RDP and UIP level were adjusted by soybean meal (SBM) and blood meal (BM), respectively. The crude protein content was 12%, 16%, 16%, and 20% (DM basis) for the four treatments, respectively. The average daily gain (ADG) trended to increase ($P < .08$) and feed efficiency (F/G) trended to improve ($P < .13$) for the steers receiving the LH diet compared to the steers receiving the HL diet during the first month. The whole period (17-0 days) ADG did not differ among treatments, but the LH and the HL treatment showed the best and worst feed efficiency, respectively (F/G: 5.63 vs 6.10, $P < .06$). The steers receiving the LL diet showed no differences compared to the other three treatments for ADG and F/G. The results indicated that the UIP was deficient for the steers during the early finishing stage. The low CP (LL) could meet the requirement of the steers at the current growth rate. The UIP showed a potential to improve the feed efficiency compared to RDP in finishing steers.

#38 CHOPPING FINENESS OF MAIZE SILAGE AND REDUCTION OF PARTICLE SIZE IN RUMEN. I. Fernandez, A. Garcia-Rodriguez, B. Michalet-Doreau, INRA, URH-DIM, 63122 St Genès Champanelle, FRANCE

Mean retention time of digesta in the rumen depends on intake level and composition of diet. On the other hand, the particle size is a key determinant of particle passage rate. To investigate this relationship between particle size and particle passage rate, an experiment was realized to study the consequences of variations in chopping fineness of maize silage on chewing behavior and particles size reduction, when intake level and composition of diet were the same. Four lactating cows fitted with a ruminal cannula received according to a 4x4 Latin square design four maize silages which differed by the chopping fineness and maize maturity stage, at 90% ad libitum intake level. Diets contained 80% maize silage, 10% soybean meal and 10% concentrate mix. Chewing behavior was recorded for each animal on 3 consecutive days. The proportion of large particles (>4 mm), obtained by wet sieving, was higher with the coarse chopping in maize silage (59 vs 45%), in bolus (35 vs 27%) and in rumen content (23 vs 16%). The proportion of large particle strongly decreased after ingestive mastication in comparison with effect of ruminative mastication and microbial breakdown, and this decrease was higher for coarse chopping silage than fine one. However eating time did not vary significantly between silages (295 vs 279 min). The efficiency of ingestive mastication would be higher for coarse chopping silage.

#39 IN SITU DISAPPEARANCE OF FIRST HARVEST ALFALFA HAY AS AFFECTED BY STAGE OF MATURITY AND CUTTING TIME. H. S. Hussein¹, H. F. Mayland², J. P. Tanner¹, H. Tokuyama¹, and G. C. J. Fernandez³. School of Veterinary Medicine¹ and Department of Applied Economics and Statistics³, University of Nevada-Reno, Reno, NV 89557 and USDA-ARS², Kimberly, ID 83341 (775-784-1708).

Two ruminally-cannulated steers were used to determine the effects of stage of maturity and cutting time on ruminal disappearance of DM, OM, NDF, ADF, cellulose, hemicellulose (Exp. 1), and N (Exp. 2). In both experiments, alfalfa was harvested at three stages (vegetative, early bud, and 1/10 bloom) of maturity and at three times (0600, 1500, and 2100) of cutting. The substrates were incubated for 24 and 48 h in Exp. 1 and for 0, 2, 4, 8, 12, 16, and 24 h in Exp. 2. In Exp. 2, a non-linear exponential model with no lag time (Orskov and McDonald, 1979; J. Agric. Sci. [Camb.] 92:499) was used to estimate kinetics of N disappearance. No interactions ($P > .05$) between stage of maturity and harvest time were detected. Cutting time also had no effect ($P > .05$). Disappearance of DM, OM, and fiber across incubation times were influenced ($P < .05$) by stage of maturity (vegetative > early bud > 1/10 bloom). The rapidly solubilized N fraction (A) was greater (46.5%; $P < .05$) for the vegetative than for the remaining stages (42.8%). The slowly solubilized but potentially digestible N fraction (B) was not affected ($P > .05$) by stage of maturity. First order rate constant (K) for disappearance from fraction B was lowest ($P < .05$) for 1/10 bloom (.153 h⁻¹) than for the less mature stages (average .232 h⁻¹).

#40 IN SITU DISAPPEARANCE OF THIRD HARVEST ALFALFA HAY AS AFFECTED BY STAGE OF MATURITY AND CUTTING TIME. H. S. Hussein¹, H. F. Mayland², S. L. Lake¹, H. Han¹, and G. C. J. Fernandez³. School of Veterinary Medicine¹ and Department of Applied Economics and Statistics³, University of Nevada-Reno, Reno, NV 89557 and USDA-ARS², Kimberly, ID 83341 (775-784-1708).

Two ruminally-cannulated steers were used to determine the effects of stage of maturity and cutting time on ruminal disappearance of DM, OM, NDF, ADF, cellulose, hemicellulose (Exp. 1), and N (Exp. 2). In both experiments, alfalfa was harvested at three stages (vegetative, early bud, and 1/10 bloom) of maturity and at three times (0600, 1500, and 2100) of cutting. The substrates were incubated for 24 and 48 h in Exp. 1 and for 0, 2, 4, 8, 12, 16, and 24 h in Exp. 2. In Exp. 2, a non-linear exponential model with no lag time (Orskov and McDonald, 1979; J. Agric. Sci. [Camb.] 92:499) was used to estimate kinetics of N disappearance. No interactions ($P > .05$) between stage of maturity and harvest time were detected. Disappearance of DM, OM, and fiber decreased ($P < .05$) by advancing maturity (vegetative > early bud > 1/10 bloom). Cutting hay at 1500 increased ($P < .05$) disappearance of DM and OM but not fiber. Disappearance kinetics of N were not altered ($P > .05$) by stage of maturity or cutting time. The average values for rapidly solubilized N (fraction A), slowly solubilized but potentially digestible N (fraction B), and first order rate constant for disappearance from B (K) were 40.7%, 59.1%, and $.151 \text{ h}^{-1}$, respectively.

#41 REDUCING NITROGEN LOSSES FROM STORED COW SLURRY USING ORGANIC TREATMENTS. D. F. McCrory¹, P. J. Hobbs¹, E. Bakewell² and R. J. Merry² Institute of Grassland and Environmental Research, ¹North Wyke Research Station, Okehampton, Devon, EX20 2SB, UK, ²Aberystwyth Research Centre, Plas Gogerddan, Aberystwyth, SY23 3EB, UK.

Loss of nitrogen (N) through ammonia (NH₃) volatilisation from cow slurry reduces its fertiliser value and is increasingly a source of environmental pollution. Reducing slurry pH through the direct addition of acids is an effective control mechanism but they are hazardous to use and not acceptable in organic farming systems. This study evaluated the potential of 3 different additive treatments, glucose, amylose as soluble potato starch and a cellulose rich waste product to stimulate fermentation and thus organic acid production in cow slurry and consequently reduce both pH and NH₃ loss. Treatments were applied at a level of 2% (w/v) to 5 litres of cow slurry in triplicate and stored at 20°C for 45 days. Results indicated that all treatments significantly ($P < 0.05$) reduced total-N loss compared to an untreated slurry by 46, 40 and 26% respectively. A significant ($P < 0.05$) positive correlation between the average pH of each treatment over the incubation period and loss of total-N suggested that the effectiveness of each treatment depended upon the associated reduction in slurry pH. Slurry pH was positively correlated ($P < 0.05$) with the production of organic acids stimulated by each treatment. The dominant bacterial groups in the waste varied with treatment and time and numbers of glucose- and starch-degrading bacteria appeared to be closely related to the production of these acids when the corresponding substrates were used. Glucose and amylose appear to offer an environmentally friendly and effective means to reduce N loss from cow slurry, particularly if cheap sugar-rich by-products are used.

#42 AMMONIA FLUXES FROM LAGOONS AND FEEDING AREAS OF A 190-COW DAIRY. R. L. Kincaid¹, B. Rumburg², G. H. Mount², J. Havig², K. A. Johnson¹, H. Westberg², and B. Lamb², ¹Dept. of Animal Sciences, and the ²Laboratory for Atmospheric Research, Dept. of Civil and Environmental Engineering, Washington State University, Pullman, WA 99164-6351 (509-335-2457).

Emissions of ammonia into the air at the WSU dairy facility were measured during winter, spring and summer. The facility houses about 190 mature cows and 185 heifers. Diets for lactating cows had 19 to 20% CP; dry cows, 13%; and heifers, 16%. Storage capacity for manure slurry is 284 m³ in the settling pond; 5,678 m³ for lagoon 2; and 37,857 m³ for lagoons 3 and 4; the surface areas are 7710, 5110 and 5110 m² for lagoons 2, 3, and 4, respectively. The concentration of total-N in lagoon slurries averaged 0.07% N and ranged from 0.03% to 0.106%. Atmospheric ammonia concentrations were measured using short-path spectroscopic absorption in the near ultraviolet region of the spectrum with up to a few second time resolution and 1 ppbv sensitivity. Concentrations ranged from 50 ppbv directly over lagoon 2 in winter to more than 2 ppmv during summer. Ammonia concentrations in air increased with temperature and air stagnation. Concentrations of ammonia downwind from the cow holding areas were similar to those measured from lagoon 2. Ammonia emission fluxes were determined by a trace gas technique for lagoon 2 and the cow holding area. Because of the extensive amount of ammonia that is released into the atmosphere from dairy operations, ammonia release must be quantified from various parts of the dairy to accurately estimate N balance.

#43 NUTRIENT MANAGEMENT OF BROILER LITTER FOR BEEF CATTLE ON PASTURE. J. P. Fontenot, N. B. Frank, R. K. Shanklin and V. G. Allen, Dept. of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (540/231-5136).

An experiment was conducted during the past 6 yr to compare recycling of nutrients from broiler litter by feeding or soil application. Each year growing beef steers grazed tall fescue and were fed hay as needed. Treatments were: 1) no feeding of broiler litter or soil application of litter or fertilizer; 2) feeding broiler litter; 3) soil application of broiler litter; and 4) soil application of inorganic fertilizer. The amount of litter applied to the soil (treatment 3) was the same amount as fed to cattle in treatment 2 the previous year. The inorganic fertilizer applied supplied the same amounts of N, P and K as in the litter applied. The average amount of broiler litter applied to the soil was 2220 kg/ha. There has been a gradual increase in soil P in the pastures in which litter was fed and litter or inorganic fertilizer was applied to the soil. Daily gains were lower ($P < .05$) for cattle on treatment 1, compared to those fed litter (treatment 2). Copper was usually higher in forage if broiler litter was fed or applied to the soil. Blood serum mineral levels were in normal ranges. Blood serum P and Cu were usually higher for cattle fed broiler litter. It appears that feeding broiler litter could be used to supply plant nutrients, and perhaps prevent overloading the soil with N and P.

- #44 EFFECTS OF pH ON VIABILITY AND GROWTH OF ENTODINIUM CAUDATUM, ENTODINIUM EXIGUUM, EPIDINIUM CAUDATUM, AND OPHRYOSCOLEX PURKYNJEI IN VITRO. Burk A. Dehority, Dept. Of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691 (330-263-3909)**

Cultures of *Entodinium caudatum*, *Entodinium exiguum*, *Epidinium caudatum*, and *Ophryoscolex purkynjei* were grown and transferred in poorly buffered media prepared using different concentrations of sodium bicarbonate and a nitrogen gas phase. By transferring every 12 or 24 h, culture pH was gradually decreased until the protozoa disappeared. The cultures were transferred by placing half of the culture into an equal volume of fresh medium, resulting in pH fluctuations similar to the changes associated with fermentation, eating and saliva production in the rumen. All species appeared to maintain their concentrations at pH values around 5.8, but numbers began to decrease as pH values fell to 5.6 and below. The four species were similar in that they all had a minimum pH value between 5.3 and 5.4. These results are somewhat surprising, since *Entodinium* species have been thought to be more tolerant to low pH than the higher ophryoscolecids. No adaptation to low pH was observed in *Epidinium* cultures after recovery from pH 5.4 medium containing only one or two viable cells. Unexpectedly, *Ophryoscolex* concentrations were slightly higher in culture media with pH values between 5.6 and 5.7, as compared to concentrations when media pH values were above 6.2.

- #45 EFFECT OF SUPPLEMENTAL PROTEIN TYPE ON RUMINAL BACTERIA POPULATIONS IN WHITEFACE WETHERS CONSUMING MEDIUM QUALITY GRASS HAY. S. L. LODGE, M. W. SALISBURY, T. T. ROSS, AND T. MAY, Department of Animal and Range Science, New Mexico State University, Las Cruces, NM 88001 (505-646-2016)**

Dormant forage produced on rangelands in the southwestern United States is generally inadequate in crude protein (CP) content to meet the NRC (1985) requirements of a growing lamb. This study was conducted to measure the influence of protein supplements containing two levels of degradable intake protein (DIP) on several microbial populations in sheep consuming a basal diet of 31% love grass and 69% blue gramma grass hay diet (7.1% CP). Five whiteface wethers fitted with ruminal and duodenal cannulas were randomly allotted to one of three treatments in a replicated 3 X 3 Latin square. Treatments consisted of no supplement (control), low DIP (161.2 g/d of DIP, LDIP) or high DIP (192.9 g/d of DIP, HDIP) in a supplement containing 219 g/d of protein. Approximately 25 ml of ruminal fluid was collected every 4 hours for 48 hours. A domain Bacteria probe (Eubacteria) was used to determine total bacterial population abundance. Oligonucleotide specific probes targeted to sites on the 16S rRNA of *Ruminococcus albus* 8, *Ruminococcus flavefaciens* FD-1, and *Fibrobacter succinogenes* S85 were used to study bacterial population changes. In general, sheep that received no supplement (control) had the highest, HDIP was intermediate and LDIP had the lowest abundance of total bacterial rRNA as well as *R. albus* 8, *R. flavefaciens* FD-1, and *F. succinogenes* S85.

#46 PROBIOTIC EFFECTS ON RUMEN FUNGI. R. Calza, J. Chang, J. Schmidt, S. Albright, and G. Calza. Department of Animal Sciences, Washington State University, Pullman, WA. 99164. (509-335-7051)

Effects of fermentation extracts derived from *Aspergillus oryzae*, Amaferm™, on the physiology and morphology of rumen fungi will be reported. Experimentation demonstrated an accelerated growth and secretion of plant degrading enzymes from rumen fungi at supplementation levels recommended for cattle. Dramatic inhibition of fungal physiology was recorded, however, when using higher concentrations of extract. Chemical analysis of extract proved at least one inhibitor compound was present. Inhibitor could be separated from extract using partitioning on thin layers. Inhibitor possessed a low (e.g. less than 8Kd) molecular weight and was soluble in organic (e.g. ethanol) solvents. Efforts to remove or inactivate inhibitor using heat or solvent extraction, respectively, were unsuccessful whereas membrane dialysis significantly decreased extract toxicity. Anion exchange chromatography allowed a partial separation of this 365nm absorbing component which was apparently synthesized at alkaline pH. Ongoing efforts to reformulate direct-fed microbial (DFM) products for increasingly consistent animal responses, and, possible relationships between fiber degradation and fungal growth stimulation in cattle caused by extract will also be presented.

#47 ISOLATION, CHARACTERIZATION AND STUDY OF HYDROLYTIC ACTIVITIES OF ANAEROBIC RUMEN FUNGI FROM RIVERINE BUFFALO (*Bubalus bubalis*) Amit Singhal and S. Neelakantan, Dairy Microbiology Division, National Dairy Research Institute, Karnal - 132001, India (a.singhal@mailcity.com ; (+91-184-259102)

Ruminants are able to use plant residues with a high fibre content as feedstuffs, because of the breakdown of these materials by a complex microbial population in the reticulo-rumen, which is practically analogous to a large fermentation vat. Rumen anaerobic fungi have attracted considerable attention globally since its discovery in mid-seventies because of their ability to attack, penetrate and utilize all categories of plant biomass including highly lignified fibre. Several reports on the improved efficiency of utilization of nutrients within the ruminant system by appropriate rumen fungal inoculum form the basis of this study. A total of 10 cultures were isolated from Murrah breed of riverine buffalo, out of which 8 were found to be monocentric and 2 polycentric. Based on the morphological characteristics different fungal cultures were identified. Six cultures were of *Piromyces* spp., two of *Neocallimstix* spp., one each of *Anaeromyces* spp. and *Orpinomyces* spp. CMCase activity of cultures ranged from 2.7685 to 11.1234 mIU/ml, FPase activity of cultures ranged from 0.4369 to 1.9786 mIU/ml, cellobiase activity ranged from 1.5285 to 2.7399 mIU/ml where as xylanase activity ranged from 0.9594 to 3.1932 mIU/ml. Thus, the present investigation confirmed the presence of both monocentric and polycentric fungi in the rumen of riverine buffalo. Out of 10 isolates, B13 isolate (*Orpinomyces* spp.) showed maximum CMCase activity (11.1234 mIU/ml) and thus can be a good candidate probiotic fungus which can be used for increasing the fibre degrading ability of ruminants in the near future.

- #48 ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY IN PEA PROTEIN FERMENTS AND HYDROLYSATES** V. Vermeirssen, J. Van Camp and W. Verstraete, Lab. of Microbial Ecology and Technology, Lab. of Food Technology and Nutrition, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Ghent, Belgium.

ACE inhibitory peptides are bioactive peptides with possible blood pressure lowering effects in vivo. In this research microorganisms were screened on the formation of ACE inhibitory peptides during fermentation of pea protein. A simulation of protein digestion indicated the contribution of the proteolytic enzymes in the gastrointestinal tract to the formation of bioactive peptides. The biological effect was tested in vitro by an ACE inhibitory assay. A commercially available diagnostic kit for measuring Angiotensin Converting Enzyme activity from Sigma was transformed in an enzyme inhibition assay and optimized, which led to a more sensitive and less expensive assay. Hence, by this spectrophotometric method, ACE inhibition is measured using as substrate furanacryloyl tripeptide and as ACE source rabbit lung acetone extract. The ACE inhibitory assay was validated by captopril and enalapril, two antihypertensive drugs. Pea protein was fermented for 48 h in monoculture by different lactobacilli and *Sacharomyces cerevisiae*. During this screening phase, fermentation by the yeast resulted in a remarkable higher ACE inhibitory activity than the one observed in the lactobacilli ferments. However, effective production of ACE inhibitory peptides has not yet been documented. Moreover, the microbial fermentation appeared to be less effective in the formation of ACE inhibitory peptides compared to in vitro gastrointestinal digestion. Nevertheless, it seems of interest to further examine microbial hydrolysis products of pea protein for specific ACE effects.

- #49 HEMICELLULASE ENCODING GENES OF *F. SUCCINOGENES*** J. K. Ha, L. M. Malburg, Jr. H. Jeon, X. Wang, S. MacLellan and C. W. Forsberg, University of Guelph, Guelph, ON. cforsber@uoguelph.ca

To better understand the role of *Fibrobacter succinogenes* in plant cell wall digestion in the rumen we have cloned three xylanase and two acetylxylan esterase genes. The three family 10 xylanase genes *xynD*, *xynE* and *xynB* were located in series on the *F. succinogenes* chromosome. They code for enzymes with masses of 69.2, 68.9 and 65.1 kDa, respectively, that exhibited between 53 to 60% similarity. XynD was purified to homogeneity and was shown to have unique catalytic properties. One acetylxylan esterase *axeA* and a segment of a second esterase *axeB* with substantive similarity were also cloned. AxeA possessed a type 4 cellulose-binding domain, but bound with higher efficiency to insoluble xylan than to cellulose. The presence of similar xylanase and esterase enzymes in each family provides evidence that each family arose as a result of gene duplication. An important question is whether all of the related genes are expressed in cells growing on plant cell walls. The novel features of these enzymes help explain the basis for the key role of *F. succinogenes* in digestion of recalcitrant plant cell walls.

- #50 ISOLATION AND CHARACTERIZATION OF LIPOPOLYSACCHARIDE-LIKE AND CAPSULAR MATERIALS FROM A PREDOMINANT CELLULOLYTIC RUMINAL BACTERIUM *FIBROBACTER SUCCINOGENES* S85.** E. E. Egbosimba¹, E. Vinogradov², M. B. Perry², J. S. Lam¹, and C. W. Forsberg¹. ¹University of Guelph, Guelph ON, N1G 2W1 & the ²Natl Research Council of Canada, Ottawa, ON, Canada. (519-824-4120).

Fibrobacter succinogenes S85 must adhere to cellulose in order to carry out digestion of polymeric matrix. Capsular material and lipopolysaccharide (LPS) are normally at the surface of the cell, and could have an influence on cellulose digestion. We have therefore undertaken the characterization of LPS-like and capsular materials of S85 as an initial step in elucidation of their respective roles in cellulose digestion. The LPS-like and capsular materials of *Fibrobacter succinogenes* was isolated from outer membranes of cellulose grown cells by extraction with hot aqueous phenol. LPS was separated from the capsular polysaccharide to yield a rough type LPS with a mass of about 4.5 kDa and capsular materials with masses between 30 and 200 kDa. Monosaccharides rhamnose, galactose, glucose, mannose, xylose, and galacturonic acid were present in both LPS and capsular polysaccharides but in different ratios. Major fatty acids present in LPS included pentadecanoic acid, anteisopentadecanoic acid and heptadecanoic acid. The capsular polysaccharide contained only anteiso pentadecanoic acid. Structural analysis revealed the presence of two types of carbohydrate structures in both polymers, with capsular materials containing equal amounts of both carbohydrate structures, and LPS containing predominantly one type. Whether the capsular material characterized corresponds to that noted on the cell surface by electron microscopy in other studies remains to be determined.

- #51 THE EXTREME ACID RESISTANCE OF ESCHERICHIA COLI IS REGULATED BY AMINO ACID AVAILABILITY AS WELL AS VOLATILE FATTY ACIDS** G. N. Jarvis and J. B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, New York 14853 (607-255-4508)

Many bacteria are killed by the low pH of the gastric stomach, but *Escherichia coli* has an inducible mechanism of extreme acid resistance that can overcome this barrier. Previous work showed that the extreme acid resistance of *E. coli* could be induced by undissociated acids, but genetic studies indicated that amino acid decarboxylases are also involved. When a freshly isolated *E. coli* strain and O157:H7 were grown anaerobically on maltose with less than 0.25 mg/ml yeast extract or Trypticase, the acid shock (pH 2.0, 1 h) survival was less than 0.01%, but acetate (50 mM, pH 7.0) increased the survival 1000-fold. If cultures were supplemented with 1.5 mg/ml Trypticase, the acid shock survival was already so high (approximately 50%) that they no longer responded to acetate. When the strains were grown aerobically in LB (a common laboratory medium based on amino acids), acid shock survivals were nearly 100%. Stationary phase cells were 100-fold more resistant to acid shock than those growing exponentially, but refrigeration (7days, 5°C) did not trigger an increase in acid resistance. These results support the idea that the acid resistance of *E. coli* is regulated by amino acid availability as well as volatile fatty acids, but is unlikely that high concentrations of amino acids would ever be available in the GI tract.

- #52 CHARACTERIZATION OF ISOLATED BACTERIAL STRAINS WITH ANTAGONISTIC PROPERTIES AGAINST FOOD-BORNE PATHOGEN LISTERIA MONOCYTOGENES. H. Roman, E. T. Ryser, S. Rust and M. T. Yokoyama. Department of Animal Science, Michigan State University, 2265F Anthony Hall, East Lansing, MI 48824-1225. (517-353-2299)**

Listeriosis in ruminants is most frequently implicated with improperly fermented silage. The causative agent is *Listeria monocytogenes*. We hypothesize that bacterial strains, antagonistic against this foodborne pathogen, can be isolated directly from the farm environment. Three wild bacteria strains (designated Ba-E, Bs-3 and Bs-13) antagonistic towards strains of this pathogen (CWD246 Brick silage and DGallup CWD95), were isolated from fresh corn silage and rumen fluid. Isolates were identified according to the API system, FAME and 16S rRNA, and also survived and grew in culture medium containing both rumen fluid and bile. Ammonium sulfate precipitation of these isolates revealed the presence of bioactive protein factors. SDS-PAGE analysis of two of these preparations (Bs-3 and Bs-13) showed that only one peptide band displayed antimicrobial activity. These peptides were sensitive to protease and gastric peptidases but tolerant of changes in heat, organic solvent and pH. We speculate that at least one of these bioactive peptides may be a new bacteriocin that can potentially be used both as an anti-microbial agent for silage and animal feed preparation to control *Listeria monocytogenes*. This research was supported by the National Food Safety & Toxicology Center, MSU.

- #53 FACTORS AFFECTING ON THE BINDING OF THE CLONED CELLULOSE-BINDING DOMAIN, DERIVED FROM *FIBROBACTER SUCCINOGENES* EGF, TO CARBOXYMETHYLCELLULOSE. Makoto Mitsumori, Hiroshi Kajikawa, and Sadahiro Ohomomo National Institute of Animal Industry, Tsukuba Norindanchi P.O. Box 5, Ibaraki, 305-0901, Japan (+81-298-38-8660)**

In previous studies, we showed that *Fibrobacter succinogenes* EGF bound to cellulose via its cellulose-binding domain (CBD). Since EGF bound to cellulose was detached by addition of carboxymethylcellulose (CMC), it was assumed that EGF strongly bound to CMC. In the present work, the interaction of the cloned CBD of EGF, termed AD4, with carboxymethylcellulose (CMC), immobilized on surface of a cuvette, was examined by IAsys optical biosensor. We examined the effect of glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, arabinose, maltose, starch, lichenan, CMC, methylcellulose (MC), and Ca^{2+} or Mg^{2+} , on the binding. Glucose, cellobiose, cellotriose, arabinose, maltose, and starch slightly inhibited binding, whereas cellopentaose, lichenan, CMC, and MC, greatly inhibited binding. Cellotetraose affected the binding, but did not completely inhibit the binding. Therefore, it has been clear that AD4 recognize a molecule comprised of 5 sugar units. Although Mg^{2+} did not affect the binding, the binding was greatly increased by Ca^{2+} . Therefore, it has been appeared that the binding of AD4 is Ca^{2+} -dependent.

#54 CLONING OF THE O-ACETYL SERINE LYASE GENE FROM THE RUMINAL BACTERIUM SELENOMONAS RUMINANTIIUM HD4. J. D. Evans, S. F. Al-Khaldi, and S. A. Martin, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706-542-1065)

The gene coding for O-acetylserine lyase (OASL) from *Selenomonas ruminantium* HD4 was cloned from a Lambda ZAPII genomic library. Sequence analysis revealed a 930 bp fragment with a G + C content of 53% that contained two open reading frames (ORF), ORF1 and ORF2, of 257 and 239 amino acids, respectively. While ORF2 revealed no significant homology to known protein sequences, ORF1 had significant protein homology with enzymes involved in cysteine biosynthesis. A CuraBLASTN_ homology search showed that ORF1 shared 66% nucleotide identity with the *cysK* of *Mycobacterium tuberculosis* and 63% nucleotide identity with the *cysM* of *Streptococcus suis*. Further analysis predicted that the gene product was a member of the pyridoxal phosphate enzyme family and of cytoplasmic origin. Phylogenetic analysis clustered the *S. ruminantium* gene product with the OASLa isoform of *Bacillus subtilis* and the OASLb isoforms of *Streptococcus suis*, *Escherichia coli*, and *Campylobacter jejuni*. The OASL of *S. ruminantium* HD4 was also able to complement the *cysM cysK* double mutations in *Escherichia coli* NK3 and allow for growth on minimal media that contained either sulfate or thiosulfate as the sole source of sulfur. These results suggest that the gene might function as a *cysM* in *S. ruminantium* HD4. In conclusion, this research describes the cloning and expression of an O-acetylserine lyase gene from the predominant ruminal anaerobe *S. ruminantium* HD4.

#55 CLONING OF THE L-LACTATE DEHYDROGENASE GENE FROM THE RUMINAL BACTERIUM SELENOMONAS RUMINANTIIUM HD4. J. D. Evans and S. A. Martin, Dept. of Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771 (706-542-1065)

A clone from a *Selenomonas ruminantium* HD4 lambda ZAP II genomic library was isolated by its ability to complement the anaerobic growth deficiency of an *Escherichia coli* (*pfl, ldh*) double mutant. The 1.0-kb insert from the clone was sequenced and revealed a single open reading frame (ORF, 975-bp) which was preceded by a putative Shine-Dalgarno (SD) sequence (AGGGGG). The potential SD sequence corresponded to 3' 16S rRNA sequences of various *Selenomonas* strains. The ORF was predicted to encode a protein of 318 amino acids with a calculated molecular mass of 34,975 Da and an isoelectric point of 5.54. In addition, the ORF contained 51 mol % G + C and this is consistent with the average G + C content (54%) of the *S. ruminantium* chromosome. The cloned *S. ruminantium* gene exhibited 60% nucleotide identity and 61% deduced amino acid similarity with L-lactate dehydrogenases (L-LDH) of *Pediococcus acidilactici* and *Bacillus megaterium*, respectively. Incorporation of the cloned *S. ruminantium* gene into *E. coli* DC1368 (*pfl, ldh*) restored anaerobic growth on glucose and L-LDH activity was detected in cell extracts. Because lactate accumulation within the rumen can be detrimental to animal performance, characterizing the gene(s) involved in lactate production by predominant ruminal bacteria will lead to a better understanding of lactate metabolism within the rumen.

#56 EFFECT OF ACACIA ANGUSTISSIMA ON BACTERIAL DIVERSITY IN THE RAT CECUM. A. H. Smith and R. L. Mackie, Department of Animal Sciences, University of Illinois, Urbana, IL, 61801 (217-244-2526)

Comparative studies were performed on cecal bacterial populations of rats fed a control diet, 7.5% *A. angustissima* diet and a diet containing a 70% acetone extract of *A. angustissima* using PCR amplification of the V3-16S rDNA and separation by denaturing gradient gel electrophoresis (DGGE). Selected bands were excised, reamplified and sequenced. Computation of the similarity between samples indicated that although there were differences in the banding patterns of all the samples there were similarities within treatment groups. Of the 28 clones sequenced, 22 (79%) belong to the CFB (*Cytophaga-Flexibacter-Bacteroides*) phylum. Eleven (69%) of the 16 sequences from the control group belong to the CFB phylum and eleven (92%) of the 12 sequences from the 70% acetone extract fed group belong to CFB phylum. Some of these cluster with *B. vulgatus*. Of the five non-CFB phylum sequences from the control group one is a novel sequence, one is related to *Acidovorax* species, one clusters with the Clostridia and two are related to the Lactobacilli. There is less diversity in the 70% acetone extract fed group based on the number of bands in the gel and supported by the sequence data, possibly due to a negative effect of condensed tannins of *A. angustissima*. Bacterial population differences between treatment groups was detected by PCR-DGGE analysis and a preliminary identity of the bacteria present was obtained from sequencing and alignment of the 200 base pair amplicons.

#57 COEXISTENCE OF RUMEN CELLULOLYTIC RUMINOCOCCUS , PREVOTELLA RUMINICOLA GA33 AND BUTYRIVIBRIO FIBRISOLVEN D1 GROWN IN BATCH CULTURES. J. K. Browne-Silva and Tammy May. Dept. of Animal and Range Sciences, New Mexico State University, Las Cruces NM 88003.

Six ruminal organisms, *Ruminococcus albus* 8, *Ruminococcus albus* sy3, *Ruminococcus flavefaciens* FD1, *Ruminococcus flavefaciens* RF1, *Prevotella ruminicola* GA33 and *Butyrivibrio fibrisolvens* D1, were grown as monocultures, dicultures and tricultures. This was done to determine the effect of strains of *Ruminococcus* on *Prevotella ruminicola* GA33 and *Butyrivibrio fibrisolvens* D1. The absorbance was taken at 600nm from two hours up to 30 hours. *B. fibrisolvens* D1, a rapid grower, reached the highest OD600 in 8 hours. In the di and tri-culture combinations, the OD readings were comparable to the highest reading of the individual bacteria as seen in the monocultures, mainly to those of *B. fibrisolvens* D1 and *P. ruminicola*. This suggests that GA33 and D1 were not inhibited. By the presence of the cellulolytics but dominated the growth of the others. Northern hybridization of RNA isolated from these cultures at the highest OD readings confirmed that neither GA33 nor D1 was inhibited. The growth of *R. albus* 8 was also not hindered. The protein concentration for each culture was determined and found to increase with time as the OD. Once more, the cultures with D1 contained the highest protein concentrations.

#58 STABILITY OF PORCINE FECAL BACTERIAL POPULATIONS AFTER INTRODUCTION OF *LACTOBACILLUS* SPP. J. M. Simpson, V. J. McCracken, H. R. Gaskins, and R. I. Mackie, Dept. of Animal Sciences, University of Illinois, Urbana, IL 61801 (217-244-4305)

Use of probiotics has been advocated as an alternative to inclusion of growth promoting levels of antibiotics. This requires an understanding of the impact on existing gastrointestinal populations. In the weaning piglet gastrointestinal tract, a relatively few species (or genera) of bacteria generally exert a major controlling influence on further colonization, by virtue of their numbers, metabolism or other activities. We investigated the impact of an introduced species of *Lactobacillus reuteri* upon the indigenous bacterial populations present during the weaning period and developed methods which allow monitoring of the introduced species. Four-week-old piglets weaned to an antibiotic-free diet were inoculated with *L. reuteri* MM53 (2.5×10^{10} cells/dose). Fecal samples were collected and analyzed for presence of inoculated strain by selective plate count enumeration. Samples were further analyzed using DGGE and 16S rRNA hybridization. Piglets averaged 1.5×10^6 CFU/g feces of *L. reuteri*. DGGE analysis revealed that initial shifts in *L. reuteri* patterns stabilized and remained constant throughout the 21 day experiment. There were other bacterial population shifts observed as the piglets matured and subsequent banding pattern shifts were primarily resultant from developmental events rather than from *L. reuteri*-induced changes.

#59 APPLICATION OF GROUP SPECIFIC AMPLIFIED R-DNA RESTRICTION ANALYSIS TO DIFFERENTIATE AMONG SWINE FECAL AND WASTE STORAGE PIT SAMPLES. C. J. Ziemer, M. A. Cotta, and T. R. Whitehead. NCAUR, ARS, USDA, 1815 University St., Peoria, IL 61604 (309-681-6273).

Incomplete anaerobic digestion of swine waste by microorganisms results in the production of various odorous compounds, however, little is known about microorganisms involved in these processes. Group specific amplified ribosomal-DNA restriction analysis (GS-ARDRA) was evaluated as a method to determine changes in microbial community structure among swine fecal and storage pit samples. PCR primer and probe sequences were evaluated for their potential for targeted DNA amplification using a set of target and non-target organisms. A number of primer sets were identified targeting Bacteroides-Prevotella, clostridia clusters IX & XI, clostridia clusters XIVa & XIVb, clostridia clusters I & II, Lactobacillus, Desulfovibrionaceae and Streptococcus-Lactococcus groups as well as a universal primer pair. Seven tetrameric restriction enzymes were screened for their ability to differential among organisms within target groups, in order to optimize a set of at least three restriction enzymes for each target group. Only the restriction enzyme *Msp* I was suitable for all target groups and four restriction enzymes were used except for clostridial cluster I & II group where only three restriction enzymes were found to be suitable. Differentiation among swine fecal and storage pit samples using GS-ARDRA was possible with all target groups with some restriction enzymes.

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