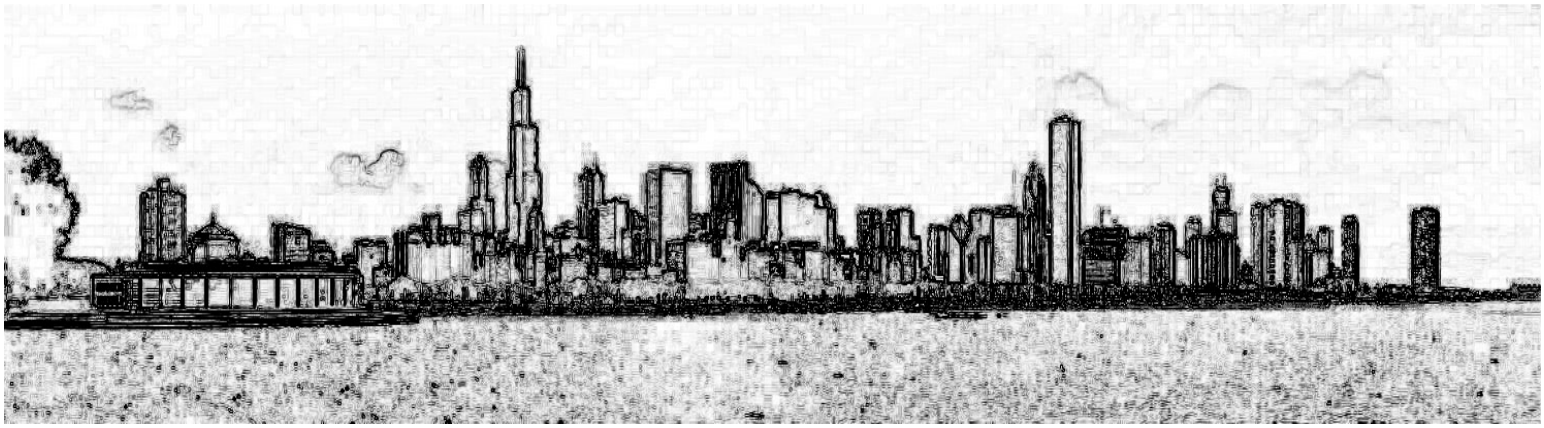
2003
Conference on
Gastrointestinal Function

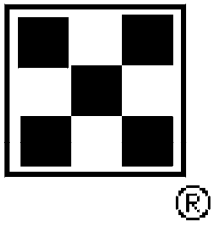


Congress Hotel
Chicago, Illinois
March 10-12, 2003
Program and Abstracts

This proceedings publication is a courtesy of

Purina Mills, LLC

St. Louis, Missouri



2003 CONFERENCE ON GASTROINTESTINAL FUNCTION

Welcome to the 2003 Conference on Gastrointestinal Function, formerly the Conference on Rumen Function. The Rumen Function Conference (RFC) was originally convened for research scientists to discuss problems associated with bloat and this remained a central theme until 1961. Since bloat was of uncertain, but multifactorial, etiology the program was divided into four research areas involved in research on bloat namely Rumen Microbiology, Ruminant Nutrition, Pathophysiology and Agronomy. The intention was for scientists from these four different areas to focus on the etiology and solution of the bloat problem. Until recently the RFC still maintained all four of these research areas on the program. Over the past ten years, the Conference broadened its program to address other factors that influence rumen fermentation and physiology. Much of the seminal work describing digestive processes in the rumen and the roles of gastrointestinal anaerobes in the nutrition and health of the animal were first presented at RFC and further served as models for later advances in our knowledge of these important processes in other gastrointestinal environments [e.g. humans, swine, and poultry, as well as termites]. For these reasons, RFC has been regarded as the "Gordon Conference" for those interested in ruminal microbiology, and has consistently attracted a large number of national and international scientists, as well as those with interests outside the specific area of ruminal function.

While fundamental and practical questions in ruminant digestion and microbiology remain, new problems and challenges face animal agriculture. Most notable are the demands to reduce or eliminate the use of antibiotics for prophylaxis and growth-promotion in livestock and poultry, the microbiological issues pertaining to pre- and post-harvest food safety, and the impacts from animal agriculture on the environment, specifically in terms of animal manure and odor control. In a number of instances, scientists who previously concentrated on ruminal microbiology research are now at the forefront of research in these areas. For these reasons, we have revised the name, theme and scope of RFC, to accommodate these emerging areas of research. It is envisioned that the new Conference on Gastrointestinal Function will serve as a mechanism for exchange of ideas, meeting the needs of agricultural scientists and foster the formation of many new collaborative efforts and research partnerships.

At this year's Conference will also inaugurate the Marvin P. Bryant Memorial Lecture. Marv was regarded as the foremost rumen bacteriologist in the world for his pioneering research on the ecology, physiology and metabolism of anaerobic rumen bacteria. He is also recognized for his many contributions to our understanding of anaerobic microbiology in general including the concept of interspecies hydrogen transfer, obligate proton reducing bacteria and their role in complete anaerobic degradation of organic matter, and the taxonomy and phylogeny of many anaerobic bacteria. He was a founding member and regularly attended the Rumen Function Conference and served as Microbiology Panel Chair for many years. This year's lecture will be delivered by Milt Allison, a long time colleague of Marvin's.

Additional information regarding the Conference on Gastrointestinal Function and future meetings can be accessed via the internet at http://www.ncaur.usda.gov/fbt/GIF/Default.htm . I hope that this year's Conference will provide a platform and foundation to launch another half century of innovative research and practical application to the field of gastrointestinal health and function.

Michael A. Cotta, Chairman
USDA-ARS
National Center for Agricultural Utilization Research
Peoria, Illinois

AGENDA

MONDAY, MARCH 10 **GOLD ROOM**

7:00 - 10:00 p.m. MIXER

TUESDAY, MARCH 11 **GREAT HALL**

9:00 a.m. #1 EFFECTS OF DIET COMPOSITION ON SPLANCHNIC METABOLISM AND NUTRIENT SUPPLY IN RUMINANTS.
C. K. Reynolds, Department of Animal Sciences, The Ohio State University, OARDC, Wooster, OH.

9:40 a.m. #2 RUMINAL DIGESTION IN LACTATING DAIRY COWS FED A HIGH-CONCENTRATE DIET SUPPLEMENTED WITH SUNFLOWER, LINSEED, OR FISH OIL.
K. Ueda[1], J. J. Loor[1,2], A. Ferlay[1], J. Chabrot[1], Y. Chilliard[1] and M. Doreau[1]. [1]INRA, 63122 St. Genès Champanelle, France, [2]Department of Animal Sciences, University of Illinois, Urbana, IL.

10:00 a.m. #3 *IN VITRO* RUMEN PLANT ENZYME MEDIATED LIPOLYSIS: GRASS V. RED CLOVER.
M. R. F. Lee, E. M. Martinez and M. K. Theodorou, Department of Plant, Animal and Microbial Sciences, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, UK.

10:20 a.m. BREAK

10:40 a.m. #4 RUMINAL FERMENTATIVE CHANGES ASSOCIATED WITH SUB-ACUTE ACIDOSIS; RUMEN STATUS ON PH, VFA AND LACTATE.
L. T. Mydland[1], A. Steen[2], E. Prestløkken[1] and H. Volden[1]. [1]Agricultural University of Norway, Aas, Norway and [2]The Norwegian School of Veterinary Science, Oslo, Norway.

11:00 a.m. #5 FRACTIONAL RATES OF VOLATILE FATTY ACID ABSORPTION AND LIQUID PASSAGE FROM THE RUMEN AND THEIR CONTRIBUTION TO RUMINAL VFA CONCENTRATION IN DAIRY COWS.
J. A. Voelker and M. S. Allen, Michigan State University.

11:20 a.m. #6 ACIDO-BASIC BALANCE IN RUMEN AND BLOOD IN INDUCED ACIDOTIC SHEEP.
L. Brossard[1,2], C. Martin[1], I. Fernandez[1], B. Michalet-Doreau[1]. [1]URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France and [2]Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France.

11:40 - 1:00 p.m.		POSTER SESSION LUNCH
2:00 p.m.	#7	PREBIOTICS AND PROBIOTICS THROUGH THE FOOD CHAIN: AGRICULTURAL USES FOR GUT FLORA MODULATION. **G. Gibson**. Food Microbial Sciences Unit, School of Food Biosciences, The University of Reading, Reading, UK.
2:40 p.m.	#8	AN EXOGENOUS PROTEASE INCREASES ENZYMIC ACTIVITIES, MICROBIAL NUMBERS AND FIBER DEGRADATION BY MIXED RUMINAL MICROORGANISMS IN CONTINUOUS CULTURE. **D. Colombatto**[1], G. Hervás[2] and K. A. Beauchemin[1]. [1]Agriculture and Agri-Food Canada, Lethbridge, AB, Canada and [2]CSIC, Leon, Spain.
3:00 p.m.	#9	THE CELLULASE AND HEMICELLULASE SYSTEM OF THE POLYCENTRIC RUMEN ANAEROBIC FUNGUS *ORPINOMYCES* SP. STRAIN PC-2. **X. Li**[1] and L. G. Ljungdahl[2]. [1]Fermentation Biotechnology Research Unit, NCAUR, USDA-ARS, Peoria, IL and [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA.
3:20 p.m.		BREAK
3:40 p.m.	#10	GENOME MINING TO ELUCIDATE THE MECHANISM OF DNA REPLICATION IN METHANOGENS: A POTENTIAL MEANS TO CONTROL METHANOGENESIS. **J. B. Robbins**, B. A. White, R. I. Mackie and I. K. O. Cann. Department of Animal Sciences, University of Illinois, Urbana, IL.
4:00 p.m.	#11	IDENTIFICATION AND CHARACTERIZATION OF ANAEROBIC PYRROLIZIDINE ALKALOID DEGRADING BACTERIA. **S. L. Lodge-Ivey**[1], M. Rappe[2], R. Bohlken[1] and A. M. Craig[2]. [1]College of Veterinary Medicine, [2]Department of Microbiology, Oregon State University, Corvallis, OR.
4:20 p.m.	#12	RUMINAL HISTAMINE PRODUCTION, LAMINITIS AND *ALLISONELLA HISTAMINIFORMANS* GEN. NOV., SP. NOV. **M. R. Garner** and J. B. Russell. Section of Microbiology, Cornell University and Agricultural Research Service, USDA, Ithaca, NY.
4:40 p.m.	#13	FROM POISONED SHEEP TO PREVENTION OF KIDNEY STONES: THE OXALOBACTER STORY. **M. J. Allison**. Department of Microbiology, Iowa State University, Ames, IA.
5:20 p.m.		DINNER

WEDNESDAY, MARCH 12 GREAT HALL

9:00 a.m. #14 IS THERE A RELATIONSHIP BETWEEN PERFORMANCE PRODUCTIVITY AND GASTROINTESTINAL FUNCTION IN POULTRY?
W. W. Robey and A. M. Jones. Cargill Feed Applications, Minnetonka, MN.

9:40 a.m. #15 THE EFFECTS OF TYLOSIN ON BACTERIAL MUCOLYSIS, *CLOSTRIDIUM PERFRINGENS* GROWTH, AND INTESTINAL BARRIER FUNCTION IN A CHICK MODEL OF NECROTIC ENTERITIS.
C. T. Collier[1], J. D. van der Klis[2], B. Deplancke[1], D. B. Anderson[3] and H. R. Gaskins[1]. [1]University of Illinois, Urbana, IL, [2]ID TNO Lelystad, The Netherlands and [3]Elanco Animal Health, Greenfield, IN.

10:00 a.m. #16 A NOVEL GENOMIC APPROACH FOR COMPARING COMPLEX MICROBIAL COMMUNITIES.
E. A. Galbraith, D. A. Antonopoulos and B. A. White. Department of Animal Sciences, University of Illinois, Urbana, IL.

10:20 a.m. BREAK

10:40 a.m. #17 DOMAIN SHUFFLING AND EVOLUTIONARY RATES IN THE POLYUBIQUITIN GENES OF RUMEN CILIATES.
N. R. McEwan[1], F. M. McIntosh[1], N. A. Thomas[1], N. Nelson[1], J. Jouany[2], E. Nsabimana[2], D. Macheboeuf[2], M. Makoto[3], T. Nagamine[4], K. Ushida[5], J. H. P. Hackstein[6] and **C. J. Newbold**[1]. [1]Rowett Research Institute, Greenburn Road, Aberdeen, Scotland, [2]INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Recherches de Clermont Ferrand-Theix, France, [3]National Institute of Livestock and Grassland Science, Tsukuba, Norindanchi, Ibaraki, Japan, [4]Rumen Microbiology Team, STAFF-Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki-ken, Japan, [5]Laboratory of Animal Science, Kyoto Prefectural University, Shimogamo, Japan and [6]Department of Microbiology and Evolutionary Biology, University of Nijmegen, Nijmegen, The Netherlands.

11:00 a.m. #18 ADVANCEMENTS TOWARDS THE VERIFICATION OF A MOLECULAR-BASED ASSAY TO PREDICT FLOW AND RUMEN POOL SIZE OF PROTOZOAL NITROGEN.
J. T. Sylvester, J. L. Firkins and M. Morrison. Department of Animal Sciences, The Ohio State University, Columbus, OH.

11:20 a.m. #19 MOLECULAR TAXONOMY: REVEALING UNSEEN DIVERSITY WITH THE GI TRACT.
P. A. Lawson[2], T. R. Whitehead[1], M. A. Cotta[1] and M. D. Collins[2]. [1]Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, Peoria, IL and [2]School of Food Biosciences, University of Reading, Reading, UK.

11:40 - 1:00 p.m. BUSINESS MEETING
LUNCH

1:00 p.m. #20 APPROACHES IN THE SAFETY EVALUATIONS OF VETERINARY ANTIMICROBIALS IN FOOD TO DETERMINE THE EFFECTS ON THE HUMAN INTESTINAL MICROFLORA.
C. E. Cerniglia. Division of Microbiology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR.

1:40 p.m. #21 EVALUATION OF DIVERSITY AND QUANTITATION OF TETRACYCLINE RESISTANCE GENES ON PIG FARMS WITH AND WITHOUT ANTIBIOTIC USE.
A. Mehboob, S. A. Kocherginskaya, R. I. Aminov and R. I. Mackie. University of Illinois, Urbana, IL.

2:00 p.m. #22 FLUORESCENT DETECTION OF FECAL CONTAMINATION ON ANIMAL CARCASSES.
M. A. Rasmussen[1], T. A. Casey[1] and J. W. Petrich[2]. [1]NADC/ARS/USDA and [2]Iowa State University, Ames, IA.

2:20 p.m. #23 *ESCHERICHIA COLI* 0147: AN EMERGING SEROGROUP OF EDEMA DISEASE OUTBREAKS.
A. F. Helgerson[1], R. Schroeder[1], V. Sharma[2], K. Post[3] and N. A. Cornick[1]. [1]College of Veterinary Medicine, Iowa State University, Ames, IA, [2]National Animal Disease Center, Ames, Iowa and [3]Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture and Consumer Services, Raleigh, NC.

2:40 p.m. #24 UNRAVELING THE PURITY AND COMPOSITION OF MSC™.
M. Gerds, K. Fenster, E. Emond and J. Leedle. Chr. Hansen Inc., Milwaukee, WI.

3:00 p.m. ADJOURN

POSTERS

#25 PROFILES OF CONJUGATED LINOLEIC ACIDS (CLA), *TRANS*-18:1 ISOMERS, AND *TRANS*11,*CIS*15-18:2 IN RUMEN FLUID FROM COWS FED A HIGH-CONCENTRATE DIET SUPPLEMENTED WITH SUNFLOWER, LINSEED, OR FISH OIL.
J. J. Loor[1,2], K. Ueda[1], A. Ferlay[1], Y. Chilliard[1] and M. Doreau[1]. [1]INRA, 63122 St. Genès Champanelle, France and [2]Department of Animal Sciences, University of Illinois, Urbana, IL.

#26 DEGRADATION OF ALFALFA CELL WALL POLYSACCHARIDES BY PURE CULTURES OF FIVE RUMEN BACTERIAL SPECIES.
H. G. Jung[1], P. J. Weimer[2] and F. M. Engels[3]. USDA-ARS, [1]St. Paul, MN and [2]Madison, WI and [3]Wageningen University, The Netherlands.

#27 CHEMOTAXIS OF RUMEN HOLOTRICH PROTOZOA TOWARDS SOLUTIONS OF VARIOUS SUGARS AND ORGANIC ACIDS.
M. Rivera, W. Hu and M. R. Murphy. Department of Animal Sciences, University of Illinois, Urbana, IL.

#28 EFFECTS OF COMBINATIONS OF PROPYNOIC ACID AND CROTONIC ACID OR 3-BUTENOIC ACID ON RUMINAL DIGESTIBILITY AND MICROBIAL EFFICIENCY IN VITRO.

E. M. Ungerfeld, S. R. Rust and R. Burnett. Michigan State University, East Lansing, MI.

#29 RANKING OF WHOLE PLANT CORN SILAGES FOR SUBACUTE RUMINAL ACIDOSIS.

B. Michalet-Doreau, I. Fernandez and C. Martin. URH-DVA, INRA, Theix 63122 Saint-Genès-Champanelle, France.

#30 VALIDATION OF CONTINUOUS RUMINAL PH MEASUREMENTS BY INDEWLING PROBES.

L. Brossard[1,2], M. Fabre[1], C. Martin[1] and B. Michalet-Doreau[1]. [1]URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France and [2]Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France.

#31 FIBER DIGESTION AND MICROBIAL FIBROLYTIC ACTIVITY IN THE RUMEN.

C. Martin, B. Michalet-Doreau. URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France.

#32 ON THE CHARACTERISTICS AND POSSIBLE MODE OF ACTION OF AN EXOGENOUS PROTEASE FOR RUMINANT DIETS.

D. Colombatto and K. A. Beauchemin. Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.

#33 CHARACTERIZATION OF THE MICROBIAL POPULATION AND FERMENTATIVES ACTIVITIES IN THE FORESTOMACH OF YOUNG GUANACOS AND IN THE RUMEN OF YOUNG CALVES.

L. C. Arakaki[1], O. Balbuena[2], G. Osacar[1], V. Olivera[1], G. Marcoppido[1], A. Maguire[3] and G. Berra[1]. [1]INTA Instituto de Patobiologia, CC 78, Castelar, Argentina, [2]INTA Colonia Benitez, CP 3505, Argentina and [3]Fundación Habitad, Buenos Aires, Argentina.

#34 DNA SEQUENCE ANALYSIS OF THE TETW GENE FROM *RUMINOCOCCUS FLAVEFACIENS* JM1.

K. S. Scopel, D. A. Antonopoulos and B. A. White. Department of Animal Sciences, University of Illinois, Urbana, IL.

#35 CLONING OF THE *O*-ACETYLHOMOSERINE SULFHYDRYLASE GENE FROM THE RUMINAL BACTERIUM *SELENOMONAS RUMINANTIUM* HD4.

X. Qin and S. A. Martin. Department of Animal and Dairy Science, The University of Georgia, Athens, GA.

#36 EXPERIMENTAL CHLORATE PRODUCT TREATMENT TO REDUCE FOOD BORNE PATHOGENIC BACTERIA IN RUMINANT AND MONOGASTRIC FOOD ANIMALS.
T. R. Callaway, R. C. Anderson, T. S. Edrington, Y. S. Jung, K. J. Genovese, K. M. Bischoff, T. L. Poole, R. O. Elder, R. B. Harvey and D. J. Nisbet. Food and Feed Safety Research Unit, ARS/USDA, College Station, TX.

#37 ATLANTIC SALMON AND RAINBOW TROUT GASTROINTESTINAL MICROBIAL DIVERSITY.
J. M. Fisher[1], T. Bradley[2] and B. A. White[1]. [1]Department of Animal Sciences, University of Illinois, Urbana, IL and [2]Departments of FAVS/CMB, University of Rhode Island, Kingston, RI.

#38 COMPARATIVE GENOMICS OF A BIFUNCTIONAL CELLULASE SEQUENCE AT THE STRAIN, SPECIES, AND GENUS LEVEL IN RUMINAL MICROORGANISMS.
D. A. Antonopoulos and B. A. White. Department of Animal Sciences, University of Illinois, Urbana, IL.

#39 SUB-ACUTE ACIDOSIS IN DAIRY COWS; ANALYSIS OF CHANGES IN THE BACTERIAL COMMUNITIES IN RUMEN LIQUID BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE).
L. T. Mydland[1], Z. Yu[3], H. Volden[1], A. Steen[2] and M. Morrison[3]. [1]Agricultural University of Norway, Aas, Norway, [2]The Norwegian School of Veterinary Science, Oslo, Norway and [3]Ohio State University, Columbus, OH.

#40 INFLUENCE OF AROMATIC AMINO ACIDS AND PROLINE ON XYLAN FERMENTATION BY RUMINAL MICROORGANISMS *IN VITRO*.
A. Y. Guliye, C. Atasoglu, N. McKain and R. J. Wallace. Rowett Research Institute, Bucksburn, Aberdeen, UK.

#41 GENOME MINING TO ELUCIDATE THE MECHANISMS OF NITROGEN REGULATION IN THE RUMEN ANAEROBE *RUMINOCOCCUS ALBUS* 8.
K. R. Amaya, I. Cann and R. I. Mackie. Department of Animal Sciences, The University of Illinois, Urbana, IL.

#42 PHYLOGENETIC DIVERSITY OF NITROGEN FIXATION GENES IN RUMEN BACTERIA.
B. Cheng, I. K. O. Cann and R. I. Mackie. Department of Animal Sciences, University of Illinois, Urbana, IL.

#43 EFFICIENCY AND DOSE EFFECT OF LEVUCELL® SC TO IMPROVE RUMINAL PH IN INDUCED ACIDOTIC SHEEP.
L. Brossard[1,2], C. Martin[1], F. Chaucheyras-Durand[2] and B. Michalet-Doreau[1]. [1]URH-DVA, INRA, Theix, 63122 St. Genès-Champanelle, France and [2]Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France.

#44 MOLECULAR ANALYSIS OF THE BACTERIAL COLONISATION OF FRESHLY-INGESTED GRASS AND WHITE CLOVER.

E. J. Kim[1], R. J. Dewhurst[1], R. J. Merry[1], D. R. Davies[1], J. A. Brookman[1], M. J. Nicholson[1], S. A. Kocherginskaya[2], M. K. Theodorou[1] and R. I. Mackie[2]. [1]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, UK and [2]Department of Animal Sciences, University of Illinois, Urbana, IL.

#45 PRESENCE AND EXPRESSION OF TETRACYCLINE RESISTANCE GENES IN SALMONELLA.

C. J. Ziemer and E. Dang. Swine Odor and Manure Management Research Unit, ARS, USDA, Ames, IA.

#46 CHEMICAL COMPOSITION AND SMALL INTESTINAL DIGESTION OF PROTEIN FROM VARIOUS RUMINAL MICROBIAL FRACTIONS.

K. K. Nelsen, G. I. Crawford, R. L. K. Hulbert and M. D. Stern. Department of Animal Science, University of Minnesota, St. Paul, MN.

#47 EFFECTS OF A LIQUID BYPRODUCT N SOURCE ON FERMENTATION BY RUMINAL MICROBES IN CONTINUOUS CULTURE FERMENTERS.

G. I. Crawford[1], M. D. Stern[1], R. L. K. Hulbert[1] and P. A. Summer[2]. [1]Department of Animal Science, University of Minnesota, St. Paul, MN and [2]Ajinomoto USA, Eddyville, IA.

#48 EVALUATION OF INORGANIC AND CHELATED TRACE MINERAL SUPPLEMENTATION TO EARLY LACTATION AND DRY COW DIETS ON MICROBIAL FERMENTATION IN CONTINUOUS CULTURE.

R. L. K. Hulbert[1], G. I. Crawford[1], M. D. Stern[1] and M. T. Socha[2]. [1]Department of Animal Science, University of Minnesota, St. Paul, MN and [2]Zinpro Corporation, Eden Prairie, MN.

#49 *HESPELLIA STERCORISUIS* GEN. NOV. SP. NOV., AND *HESPELLIA PORCINUS* SP. NOV., ISOLATED FROM MANURE STORAGE PITS.

P. A. Lawson[1], M. A. Cotta[2], M. D. Collins[1] and T. R. Whitehead[2]. [1]School of Food Biosciences, University of Reading, Reading, UK and [2]Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, Peoria, IL.

#50 COMPARATIVE GENOME ANALYSIS OF A CELLULOLYTIC AND A NON-CELLULOLYTIC STRAIN OF *RUMINOCOCCUS ALBUS*.

C. Reveneau, V. Parisi, Z. Yu and M. Morrison. The MAPLE Research Initiative, The Ohio State University, Columbus, OH.

ABSTRACTS

#1 Effects of diet composition on splanchnic metabolism and nutrient supply in ruminants.

C. K. Reynolds, Department of Animal Sciences, The Ohio State University, OARDC, Wooster, OH 44691 (330-263-3793).

The portal-drained viscera (PDV; gut, pancreas, spleen and associated fat) and liver, or the splanchnic tissues, are extremely metabolically active and account for as much as half of body heat production in ruminants. This intensive oxidative metabolism arises in part from the costs of digestion, nutrient assimilation, and the high rate of protein turnover and ion flux associated with these functions. These metabolic costs are paid for by absorbed nutrients, thus net nutrient supply for production is determined not only by amounts of nutrients derived from fermentation and digestion, but also metabolic requirements of the splanchnic bed. These requirements are determined both by diet composition and relative intake level, which determine both the mass and activity of the PDV and liver. For example, differences in the efficiency of metabolizable energy (ME) use for net energy (NE) between grasses, legumes and cereal grains have long been attributed to changes in the pattern of ruminal VFA and a reduced energetic efficiency with increased acetate absorption relative to propionate. More recent studies have shown that differences in NE/ME between these ration types is largely a consequence of differences in PDV metabolism attributable to changes in tissue mass. In addition, increases in body heat production attributable to feeding N in excess of requirement are a consequence of increased oxidative metabolism of both the PDV and liver. In the case of increased N consumption, a pronounced increase in ammonia abosorption and urea cycling between the PDV and liver is typically observed. This N recycling represents an efficient scavenging mechanism and evolutionary advantage for browsing ruminants, but may incur a metabolic cost when higher quality feeds and excess protein are consumed. Increased fermentable energy supply reduces ammonia absorption from both the rumen and hindgut, but the impact on metabolizable protein supply and amino acid absorption is masked by splanchnic amino acid metabolism. Therefore, methods to quantify diet effects on peripheral amino acid supply must account for splanchnic sequestration. The splanchnic tissues play a crucial role in determining the supply of nutrients for production and the efficiency of their utilization.

#2 RUMINAL DIGESTION IN LACTATING DAIRY COWS FED A HIGH-CONCENTRATE DIET SUPPLEMENTED WITH SUNFLOWER, LINSEED, OR FISH OIL. **K. Ueda[1], J. J. Loor[1,2], A. Ferlay[1], J. Chabrot[1], Y. Chilliard[1], and M. Doreau[1]. [1]INRA, 63122 St. Genès Champanelle, France, [2]Department of Animal Sciences, University of Illinois, Urbana, USA.**

Digestibility, ruminal VFA and protozoa concentration were investigated in lactating dairy cows when offered diets supplemented with three different oil sources rich in n-6 or n-3 polyunsaturated fatty acids. The experiment was conducted as a 3 x 3 Latin square with three lactating Holstein cows. Cows received a high-concentrate [concentrate/forage (grass hay) = 65/35] diet supplemented with 5% (DM basis) sunflower oil (S), 5% linseed oil (L), or 2.5% fish oil (F). Total fecal excretion and duodenal digesta flow were measured to obtain total tract and ruminal digestibility for OM, NDF, ADF, and starch. Rumen fluid was collected before and after the morning meal, and analyzed for VFA and protozoa concentration. Total tract digestibility of OM (S, 75.7, L, 73.6, F, 75.2%), NDF (S, 64.1, L, 59.9, F, 64.4%), and ADF (S, 59.3, L, 56.1, F, 59.0%) was not affected by oil source. True ruminal OM digestibility also was not affected by oil source (S, 58.8, L, 53.3, F, 64.3%). However, there was a tendency for a lower ruminal NDF (P=0.15; S, 46.6, L, 34.7, F, 49.6%) and ADF (P=0.11; S, 40.0, L, 25.7, F, 42.3%) digestibility with diet L. Starch was mostly digested in the rumen (82% of total tract digestion). Ruminal starch digestibility was not affected by oil source (S, 80.0, L, 81.8, F, 81.3%). Total VFA concentration in rumen fluid was lower for diet L than diet S or F (P<0.01; S, 86.6, L, 70.0, F, 91.5 mmol/L). Molar ratio of acetate was higher for diet S or L than diet F (P<0.01; S, 66.0, L, 65.8, F, 62.7). Molar ratio of propionate was lower with diet S than diet L or F (P<0.05; S, 20.3, L, 21.3, F, 22.0). Molar ratio of n-butyrate was higher for diet F than diet S or L (P<0.01; S, 10.9, L, 10.1, F, 11.8). Ruminal concentration of total protozoa was higher for diet F than diet S or L (P<0.01; S, 90.9, L, 60.4, F, 145.6 10^3/mL). Differences in VFA and protozoa concentration due to supplemental oils were associated with lower ruminal digestibility when diet L was fed.

#3 *IN VITRO* RUMEN PLANT ENZYME MEDIATED LIPOLYSIS: GRASS *V.* RED CLOVER. M. R. F. Lee, E. M. Martinez and M. K. Theodorou, Dept. of Plant, Animal and Microbial Sciences, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, UK, SY23 3EB, +44 (0)1970 823084.

It is generally accepted that microbial enzymes are responsible for lipolysis and the destruction of plant membranes in the rumen. However, we question this assertion and tested the hypothesis that in ruminants grazing fresh pastures, the first stages of lipolysis are mediated by plant lipases. Additionally, we evaluated differences in lipolytic activity between grass and red clover, as the latter has been shown to reduce biohydrogenation in the rumen.

Grass (cv. AberElan) and red clover (cv. Milvus) from experimental plots, were harvested (3cm above ground level), bruised by minimal crushing, cut into 5mm segments and incubated in 25ml antibiotic-containing (5.0mg chloramphenicol/ml in 50% v/v ethanol) anaerobic phosphate buffer (50 mM Na_2HPO_4 and 50 mM KH_2PO_4) at 39^{o}C for up to 24 h. Rumen micro-organisms were not included in the incubation buffer. At different times (0, 1, 2, 3, 4, 5, 6 and 24 h) liquid samples were taken for VFA and lactate analysis and 25ml of isopropanol : chloroform (1:1 v/v) along with 1ml of internal standard (2.5mg C19:0 / ml chloroform) added. The mixture was left for 24 h, filtered under vacuum, rotary evaporated (at 60 ^{0}C) and re-extracted in chloroform : methanol : saline (8:4:3). The lipid extract was collected, lipid fractions separated by thin layer chromatography and the individual layers methylated and analysed by gas chromatography. Lipolysis was calculated by expressing the decrease in free fatty acid % of the polar fraction between the initial time point T_0 and incubation time point T_x, as a proportion of total fatty acid (polar lipid + triacylglycerol + free fatty acids + diglycerides) at T_0.

Table 1.

	Incubation time (T_x) in hours						
Lipolysis	1	2	3	4	5	6	24
Grass	0.09	0.27	0.33	0.37	0.39	0.40	0.63
(sed)	(0.024)	(0.033)	(0.040)	(0.017)	(0.056)	(0.026)	(0.016)
Red Clover	0.11	0.22	0.26	0.29	0.35	0.41	0.55
(sed)	(0.013)	(0.043)	(0.025)	(0.014)	(0.034)	(0.023)	(0.011)

The data (table 1) suggests a curvilinear response for both grass and red clover, however red clover appears to have a lower overall lipolytic activity. Trace amounts of acetate (2.0 mmol/l) were found in the buffer after 24 h, but the low level and presence of antibiotic made it unlikely that plant bacterial activity was responsible for the observed changes. These results support the view that plant lipases play a role in lipolysis in the early stages of digestion of forages in the rumen

#4 RUMINAL FERMENTATIVE CHANGES ASSOCIATED WITH SUB-ACUTE ACIDOSIS; RUMEN STATUS ON pH, VFA AND LACTATE.

L.T. Mydland[1], A. Steen[2], E. Prestløkken[1] and H. Volden[1].
[1]Agricultural University of Norway, Aas, Norway and [2]The Norwegian School of Veterinary Science, Oslo, Norway.

The severity of nutritionally induced acidosis in lactating dairy cows is variable and ranges from acute to sub-acute or chronic At calving the diet changes from roughage based to a diet high in readily fermentable nonstructural carbohydrates, to which the rumen bacteria are not highly adapted. The rapid increase in VFA production may induce the risk of ruminal acidosis, which results in decreased DMI and a negative impact on milk production. Most studies of rumen acidosis are performed using experimentally induced acute cases where the lactate production and accumulation is very high. In this experiment, however, we gradually increased the amount of a barley-/oats-based concentrate mixture (from + 0,3 kg/day to + 1,2 kg/day) to 8 fistulated, multiparous dairy cows until sub-clinical acidosis was achieved. Cows were fed concentrate three times a day and grass silage was fed ad libitum. Ruminal pH was monitored every 5 minutes after morning feeding by direct, automatic measurements. This was done by placing a pH-electrode in a perforated tube into the rumen approximately 20 cm from the bottom of the ventral sack. The number of pH-hour under a certain pH was calculated using trapezoid integration. Rumen liquid samples were collected at pH minimum after morning feeding. The samples were analyzed for volatile fatty acids (VFAs; acetate, propionate, iso-butyrate, butyrate, iso-valerate and valerate), D- and L-Lactate and glucose. The number of protozoa was counted. The gradually increment of concentrate in the diet probably gave the ruminal bacteria more time to adjust, resulting in a relatively low ruminal concentration of lactate through the experiment. There was, however, an increase in ruminal VFA concentration, resulting in an average total VFA concentration of 144 mM the day before acidosis. Although protozoal populations decreased with increased ruminal acidity, complete defaunation did not occur.

#5 FRACTIONAL RATES OF VOLATILE FATTY ACID ABSORPTION AND LIQUID PASSAGE FROM THE RUMEN AND THEIR CONTRIBUTION TO RUMINAL VFA CONCENTRATION IN DAIRY COWS.

J.A. Voelker and M.S. Allen, Michigan State University (517-432-1386)

Fractional rates of absorption of valeric acid and passage of Co from the rumen were determined in an experiment in which dried beet pulp was substituted at 0, 6, 12, and 24% of diet DM for high-moisture corn in four diets fed to eight lactating dairy cows in a Latin-square design. Valeric acid and Co-EDTA were pulse-dosed into the rumen two hours after feeding. Rumen fluid was sampled immediately before dosing and every 30 min for 8 h after dosing. Co passage rate was determined using nonlinear regression of Co concentration over time. Valerate absorption rate was determined by nonlinear regression of the ratio of valerate concentration to Co concentration over time. Twenty-eight cow-period observations were used to correlate variables related to VFA production and disappearance. Within this data set, fractional valerate absorption rate ranged from 22 to 61%/h, and fractional liquid passage rate ranged from 11 to 23%/h. Valerate absorption rate was positively correlated with milk yield, possibly because greater mixing and/or energy use created a steeper concentration gradient across the rumen wall. Surprisingly, valerate absorption rate was positively correlated with mean daily ruminal pH. This might be because of a positive relationship between ruminal motility and ruminal pH, and a greater contribution to variation in valerate absorption rate from ruminal motility than from fraction of undissociated VFA, which decreases as pH increases. Valerate absorption rate decreased as moisture content of ruminal contents decreased, probably because liquid mixing was reduced. Most importantly, valerate absorption rate was strongly, negatively correlated ($r = -0.69$, $P < 0.001$) with ruminal VFA concentration, which ranged from 126 to 153 mM. In stepwise regression, 87% of variation in ruminal VFA concentration was accounted for by valerate absorption rate, Co passage rate, mean daily ruminal pH, ruminal content DM concentration, and yield of 3.5% fat-corrected milk. This provides further evidence that the valerate/Co method provides valid estimates for rates of VFA absorption and passage from the rumen. The ability to estimate VFA absorption from a rumen functioning under normal conditions can clarify effects of treatments on VFA concentration and turnover in the rumen.

#6 ACIDO-BASIC BALANCE IN RUMEN AND BLOOD IN INDUCED ACIDOTIC SHEEP. **L. Brossard (1,2), C. Martin (1), I. Fernandez (1), B. Michalet-Doreau (1), (1) URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France (33 4 73 62 40 55), (2) Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France**

In ruminant, ingestion of high cereals diets involves large ruminal modifications leading to a pH fall and to the appearance of latent acidosis states. In this study, 4 ruminal cannulated sheep received consecutively a control diet H (100% hay) during one week, an acidotic diet W (60% wheat + 40% hay) during two weeks, and again the control diet during two weeks. We studied variations of ruminal pH in relation with the major components of buffer system in rumen and in blood. Ruminal pH was measured continuously by indwelling probes. Titrimetry measurements used to identify components of acid-base status were realized on ruminal liquid, before, 3h and 6h after feeding. Blood pH, bicarbonates concentration and base excess were measured on venous samples 3h after feeding using a blood pH/gas analyzer. Mean, minimum and maximum ruminal pH were lower, and time and area under pH 6.0 were higher on the W diet ($P < 0.001$) than on the H diet. These pH parameters varied within a range usually defined as latent acidosis. Titrimetry allowed to identify VFA, the two acid functions of bicarbonates, and ammonia as major components of ruminal buffer system, but only VFA and bicarbonates showed significant variations between diets. Ruminal lactate was not detected, indicating a non lactic latent acidosis. Before feeding, the drop in rumen pH on the W diet was correlated with an increase in the VFA buffering capacity (BC, $R = -0.84$) and with a decrease in the BC of both carbonic acid species ($R = 0.72$ and 0.74, respectively). After feeding, acidotic diet effect on rumen pH could not be explained by variations in the BC of either of these chemical species. All these ruminal parameters recovered to initial levels often as early as the first week of the H diet redistribution following the acidotic diet period. In parallel with the ruminal observations, pH, bicarbonates concentration and base excess in blood tended to decrease during and after acidotic state, suggesting a decrease in body alkaline reserves and a longer recovery time of these reserves in blood than in rumen.

#7 **Prebiotics and Probiotics Through the Food Chain: Agricultural Uses for Gut Flora Modulation**.

Glenn Gibson.
Food Microbial Sciences Unit

School of Food Biosciences
The University of Reading
Reading. UK

Diet is currently recognised to have an important role in health and disease. There is increasing evidence that the gastrointestinal microbiota can contribute positively towards nutrition and improved health status. There is now much interest in the use of foods that are able to modulate the composition of the gastrointestinal microflora of humans and animals in a manner that improves health. For example, live additions of health promoting bacteria are made to many feed products (as probiotics), whilst the fermentation of certain carbohydrates (prebiotics) can selectively enhance beneficial components of the gut microbiota, such as lactobacilli or bifidobacteria.

The former approach has been widely advocated, and has successfully used for poultry, cows and pigs. Moreover, there has also been a shift towards probiotic applications in domestic animals. For humans, dairy products are the most popular delivery route. Both on the farm and in the human situation lyophilised probiotics exist in sprays, tablets, capsules, powders, etc. Despite the success in improved yield and the reduction in infection rate as a response to probiotics, doubts still exist over strain survival in the harshly competitive gut ecosystem. Also, many products do not contain their stated content in terms of microbial composition and/or numbers. The most reliable products will be those that have enhanced survival characteristics and proven data to support their application.

For humans, it is likely that in the next few years a great number of food products will exploit the prebiotic approach. This is because their survivability is not questionable and the product range susceptible to prebiotic addition is wide, with virtually any carbohydrate based material being susceptible. Principle types of prebiotics currently available include fructooligosaccharides, lactulose and galactooligosaccharides – all of which after a short feeding period markedly stimulate populations of bifidobacteria.

The agricultural use of prebiotics is less well developed but has several attractions from both the economic and logistical viewpoints. Functional food ingredients such as prebiotics could effect a beneficial modification in the composition and activities of gut microflora of farm animals, perhaps in combination with probiotics (as synbiotics). The aim of this presentation is to compare and contrast the probiotic and prebiotic approaches in different target animals. The health and nutritional bonuses that may accrue will also be described.

#8 AN EXOGENOUS PROTEASE INCREASES ENZYMIC ACTIVITIES, MICROBIAL NUMBERS AND FIBER DEGRADATION BY MIXED RUMINAL MICROORGANISMS IN CONTINUOUS CULTURE. D. Colombatto, G. Hervás† and K. A. Beauchemin. Agriculture and Agri-Food Canada, PO Box 3000, T1J 4B1, Lethbridge, AB, Canada (+1-403-3172235), and † CSIC, Apdo. 788, 24080 León, Spain.

The effects of pH and addition of a protease mixture on the fermentation characteristics of a total mixed ration (TMR) were investigated in a dual-flow continuous culture apparatus, using a 4 x 4 Latin Square design with a 2 x 2 factorial arrangement of treatments. The diet (DM basis) consisted of 30% alfalfa hay, 30% corn silage and 40% rolled corn. The silage and the grain were milled fresh, mixed with the alfalfa and treated with the enzyme (1.5 μL/g feed) daily. Ruminal fluid was collected 2 h post-feeding from 3 lactating dairy cows fed a TMR. Fermenters were fed 80 g DM/d in equal portions every 12 h. Treatments were control (C) and enzyme-treated (T) TMR at either high pH (HC and HT) or low pH (LC and LT). The pH was altered by diluting the artificial saliva to 60% of its original composition. Enzymic activities and total and cellulolytic bacterial numbers were determined on the liquid phase of the fermenter contents, 6 h post-feeding. Fiber degradation was determined from the outflow residues. Enzyme addition increased ($P < 0.05$) xylanase, xylosidase, endoglucanase, and protease activities (608 vs. 750; 0.48 vs. 0.80; 82 vs. 112; and 1.2 vs. 7.5 units for C and T, respectively), whereas it tended ($P < 0.12$) to increase exoglucanase and glucosidase activities (0.8 vs. 1.4; and 4.7 vs. 5.9 units). However, enzyme did not affect ($P = 0.18$) arabinofuranosidase activity (5.2 vs. 6.8). Total microbial numbers (expressed as Log_{10}) were increased ($P < 0.05$) at low pH (9.13 vs. 9.36) but enzyme had no effect ($P = 0.13$). Cellulolytic bacteria were reduced ($P < 0.02$) at low pH (3.91 vs. 2.79), with no effect ($P = 0.88$) of enzyme. Low pH reduced ($P < 0.001$) NDF, ADF, and cellulose degradation. NDF degradation was increased ($P < 0.01$) by enzyme addition (20% vs. 27%) but ADF was unaffected ($P < 0.20$), resulting in an increase ($P < 0.001$) in hemicellulose degradation. It is speculated that the enzyme removed structural barriers present in the feed, allowing a more rapid colonization of the fiber by ruminal microorganisms.

#9 The Cellulase and Hemicellulase System of the Polycentric Rumen Anaerobic Fungus *Orpinomyces* sp. Strain PC-2

Xin-Liang Li
Fermentation Biotechnology Research Unit, NCAUR, USDA-ARS, 1815 N. University St., Peoria, Illinois, 61604, USA. Phone: (309) 681-6327; Fax: (309) 681-6427

Lars G. Ljungdahl
Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602, USA

The polycentric anaerobic fungus *Orpinomyces* PC-2, isolated from cow rumen, is capable of growing on and utilizing plant cell wall structures. This ability is attributed to the production of a number of highly active hydrolytic enzymes. The enzymes are either secreted into culture medium or attached on the mycelial surface as high molecular mass complexes. The complexes are analogs of the cellulosomes produced by various species of cellulolytic anaerobic bacteria. We have recently isolated high molecular mass complexes from cellulose grown cultures of *Orpinomyces* PC-2. The complexes displayed cellulase, xylanase, mannanase, beta-glucosidase, beta-glucanase, and phenolic esterase activities. The isolated complexes were separated by size exclusion chromatography into two fractions containing structures of around 2,000 and 50,000 kDa. The size variation of the complexes was also visualized under scanning electron microscopy (SEM). Furthermore, SEM demonstrated that the complexes bind tightly to cellulose microfibrils. The binding was disrupted by repeated water washes. Through molecular biological techniques, more than fifteen cellulase and hemicellulase genes have been cloned from the fungus. Some of the genes have been over-expressed in heterologous hosts and their gene products explored for commercial applications.

#10 Genome mining to elucidate the mechanism of DNA replication in Methanogens: A potential means to control methanogenesis.

J. B. Robbins, B. A. White, R. I. Mackie, and I. K.O. Cann
Dept. of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, IL 61801 (217-333-2090)

Regulation of fermentation and redox balance is maintained within the gastro-intestinal tract (GIT) through conversion of hydrogen to methane by methanogenic archaea. In the ruminant this process also represents a loss of energy (5-12%) to the animal. In addition, methane is a greenhouse gas and is one of the culpable agents in global warming. Thus, controlling methanogenesis has both potential economic and environmental impact on ruminant animal production in the future. There are two prospective routes for controlling these microorganisms: targeting enzymes involved in methanogenesis or limiting cell proliferation by arresting DNA replication. Our approach involves understanding DNA replication in the methanogens and developing a means to regulate it. We have chosen *Methanosarcina acetivorans* (Mac) as the model organism based on availability of complete genome sequence and a gene transfer system. *Methanosarcina barkeri* and other methanogens have been isolated from the GIT. Using a bioinformatic approach, we have identified several genes coding for proteins involved in DNA replication. Each gene has been cloned, expressed and the protein purified. We are currently characterizing three unique single-stranded DNA-binding proteins (SSBs). The functional homologs of these SSBs have been shown to play essential roles in cellular DNA transactions in bacteria and eukaryotes. The three SSBs in *M. acetivorans* have been designated RPA-1, RPA-2, and RPA-3. These RPAs preferentially bind to single-stranded DNA with an affinity more than fifty times that for double-stranded DNA. The apparent molecular weights of RPA-1, RPA-2, and RPA-3 are 54, 47, and 49 kDa, respectively. Subunit analysis by gel filtration reveals that RPA-1 exists as dimers and tetramers in solution while RPA-2, and RPA-3 are dimers. Homologs of the *Mac* RPAs have been identified in other methanogenic archaea. The uniqueness of these SSBs make them potential targets for limiting proliferation of methanogens in the rumen environment and hence controlling methanogenesis.

#11 IDENTIFICATION AND CHARACTERIZATION OF ANAEROBIC PYRROLIZIDINE ALKALOID DEGRADING BACTERIA

S. L. Lodge-Ivey[1], M. Rappe[2], R. Bohlken[1], and A. M. Craig[2], [1]College of Veterinary Medicine, [2]Department of Microbiology, Oregon State University, Corvallis, OR 97331 (541) 737-6541

Pyrrolizidine alkaloids (PA) found in tansy ragwort (*Senecio jacobaea*) constitute an important source of livestock mortality in the northwestern United States and throughout the world. Animals susceptible to PA toxicosis, namely cattle and horses, develop chronic terminal hepatic disease after consuming 5-10% of their body weight in tansy ragwort plant material. Sheep are resistant to PA toxicosis and do not show signs of clinical, serological, or histopathological effects after ingesting 200% of their body weight in tansy ragwort plant material. The primary protective factor in sheep is believed to be microorganisms present in the rumen. This hypothesis is supported through animal studies using sheep in which PA extracts were infused into the portal vein of the liver to bypass the liver and PA toxicosis was detected. In order to investigate the diversity organisms capable of degrading PA, anaerobic bacteria were isolated from ruminal fluid collected from sheep consuming a forage diet with 5.0% (as fed) tansy ragwort. Enrichments were evaluated for PA degradation. The enrichment selected for further characterization was able to degrade all PA present in approximately 4 h when compared to an autoclaved control. Bacteria within the enrichment were isolated anaerobically and evaluated for PA degradation. The isolated bacteria were not able to degrade PA alone. Individual members of the enrichment were identified and classified using LH-PCR and RFLP. Seven different peaks were observed with LH-PCR and seven RFLP patterns resulted. Further analysis of the cloned 16S rRNA gene (rDNA) sequence of the isolated members revealed seven phylogenetic groups based on sequences available in public databases: *Desulfovibrio desulfuricans* subgroup, *Bacteriodes* group, *Anaerobaculum thermoterrunum* group, *Clostridium leptum* subgroup, *Clostridium coccoides* group, *Sporomusa* group, and *Veillonella parvula* subgroup. This consortium of PA degraders has been used experimentally to prevent PA toxicosis in cattle consuming tansy ragwort.

#12 Ruminal histamine production, laminitis and *Allisonella histaminiformans* gen. nov., sp. nov. M.R. Garner and J.B. Russell, Section of Microbiology, Cornell University and Agricultural Research Service, U.S. Department of Agriculture, Ithaca, New York 14853 (607-255-4508)

Histamine appears to be a key factor in bovine laminitis. In 1950's, Dougherty noted that histamine could accumulate in ruminal fluid, and shortly thereafter, Rodwell isolated histidine decarboxylating lactobacilli. It was assumed that lactobacilli were responsible for ruminal histamine accumulation, but our ruminal histidine enrichments produced histamine even if carbohydrates were not added. The isolates were obligate histidine decarboxylating, Gram-negative, monensin-resistant, ovoid-shaped cells. 16s rRNA gene sequencing indicated that they were most closely related to low G+C Gram-positive bacteria (firmicutes), but similarities were $\leq$ 94%. Because the most closely related bacteria (*Dialister pneumonsintes, Megasphaera elsdenii* and *Selenomonas ruminantium)* did not produce histamine, we propose a new genus and species, *Allisonella histaminiformans*. Dairy cows fed a commercial dairy ration had large populations (>10^6 cells per ml) of *A. histaminiformans,* but *A. histaminiformans* could not be isolated from cattle fed diets consisting only of hay. When stationary phase *A. histaminiformans* MR2 cultures were serially diluted into autoclaved ruminal fluid from cattle fed hay, histamine was not detected at dilutions greater than 10^{-2} even if histidine was added. In contrast, histamine was detected in the 10^{-9} dilution if the autoclaved ruminal fluid was obtained from cattle fed the commercial ration and supplemented with histidine. The commercial ration contained large amounts of alfalfa and corn silages, and water-soluble silage extracts stimulated the growth of *A. histaminiformans* MR2 in vitro. Alfalfa silage extract was at least 8-fold more stimulatory than the corn silage extract, and extract from only 5 mg of alfalfa dry matter promoted maximal histamine production in 1 ml of culture medium. Because non-ensiled alfalfa did not stimulate histamine production nearly as much as the alfalfa silage, the factor that stimulates the growth of *A. histaminiformans* appears to be a product of silage fermentation.

#13 FROM POISONED SHEEP TO PREVENTION OF KIDNEY STONES: THE OXALOBACTER STORY. Milton J. Allison, Dept. of Microbiology, Iowa State University, Ames, IA 50011 (515-294-3439)

Oxalate poisonings occur worldwide and thousands of sheep in the Rocky Mountain region of the US have been poisoned by halogeton (*Halogeton glomeratus*), a highly palatable plant that may contain oxalate at more than 25% of its dry wt. This lecture will trace the path of research that began in the 1970's at the National Animal Disease Center as part of an effort to gain an understanding of the physiology of oxalate poisoning in ruminants. The story illustrates how USDA, ARS policies allowed the investigations to follow where the Science led. I will tie together the work of graduate students: Karl Dawson, Steve Daniel, Carla Kuhner, and Nancy Cornick; and collaborators: Herb Cook, Lynn James, E.T. Littledike, Al Baetz, Peter Maloney, Ammon Peck, Harmeet Sidhu, Sylvia Duncan and Colin Stewart.

The gradual adaptation/tolerance to oxalate that develops in gut populations is explained by selection of *Oxalobacter formigenes,* a specialist that requires and uses only oxalate as substrate for growth. *O. formigenes* appears to be the only important oxalate-degrading organism in anaerobic gut habitats. The energetics of growth of an anaerobe on a substrate as oxidized as oxalate has been explained through description of membrane transport proton-motive force processes. *O. formigenes* has been found in cecal or colonic populations from horses, pigs, rabbits, guinea pigs, wild rats, birds, and man. About 60-75% of adult humans are colonized by *Oxalobacter*, but the incidence of colonization is much less in populations that have had multiple episodes of calcium oxalate kidney stones or in populations with hyperoxaluria related to enteric disease. Loss of colonization following antibiotic therapies seems certain, although evidence about the extent of this is anecdotal. Kidney stones affect millions of adults worldwide and calcium oxalate stones are the most important of these uroliths. Several studies support the concept of *Oxalobacter* use as a probiotic to reduce the risk of hyperoxaluria and its complications. Ixion Biotechnology, Inc is conducting pilot clinical trials to evaluate the safety and efficacy of *Oxalobacter* supplementation in hyperoxaluric disorders.

#14 IS THERE A RELATIONSHIP BETWEEN PERFORMANCE PRODUCTIVITY AND GASTROINTESTINAL FUNCTION IN POULTRY? W.W. Robey and Alison M. Jones, Cargill Feed Applications, Minnetonka, MN 55343 (952-984-0332)

An animal's gastrointestinal tract is a complex, metabolically active organ that plays a vital role in the normal nutritional, physiological, immunological, and protective functions of its host. It is subjected to extensive and continual inputs, including dietary substances and their breakdown products as well as microorganisms and exogenous toxins. Accordingly, it must permit the exchange of dietary substances between the gut lumen and the systemic circulation, but at the same time limit the penetration of pathogenic agents and toxins from the body. The gastrointestinal microbial community differs in composition and density along the length and across the diameter of the tract, and is comprised of both rapidly transiting and colonizing strains. This microbial community is impacted by the physiology of its environment, and, in turn, the intestinal microbes significantly influence the biochemistry, physiology, immunology, and disease resistance of the host. The gastrointestinal immune system is the host's 'first line of defense' to "non-self" molecules. In response to localized microbial pathogens, for example, the gastrointestinal tract may activate a systemic immune response in which lymphocytes, phagocytes, and cytokines play a role. The heightened vigilance and activity of the host's immune system requires that nutrients and energy be partitioned away from the host and directed towards fighting disease – clearly a performance drag on the host. The absorptive regions of the gastrointestinal tract contain villi / microvilli which increase the surface area for nutrient absorption. These "finger-like" structures are "vascularized" in their core, and are the structures through which nutrients pass from the gastrointestinal lumen for subsequent delivery to the liver. Gut morphometry may change in response to a myriad of factors including antibiotics (*i.e*., increase length and thickness) or stress (*i.e.,* becomes sloughed and /or the surface area is reduced during piglet weaning). In addition, as is well known, form drives function. What actually happens in the gastrointestinal ecosystem, and how / to what extent these processes can be manipulated has yet to be fully explored. However, it is reasonable that optimizing its function in food production animals may lead to enhanced productivity and improved health.

#15 The effects of tylosin on bacterial mucolysis, *Clostridium perfringens* growth, and intestinal barrier function in a chick model of necrotic enteritis

C. T. Collier[1], J. D. van der Klis[2], B. Deplancke[1], D. B. Anderson[3], and H.R. Gaskins[1]: [1]University of Illinois at Urbana-Champaign; [2]ID TNO Lelystad, The Netherlands; [3]Elanco Animal Health, Greenfield, Indiana

Necrotic enteritis (NE) is a poultry disease caused by *Clostridium perfringens* types A and C. Disease risk factors include concurrent coccidia infection and the dietary inclusion of cereal grains high in nonstarch polysaccharides (NSP). Here, a chick NE infection model was used in which Ross 508 broiler chicks (0-28 days of age) were inoculated with the coccidian *Eimeria acervulina* at d 10 and then *C. perfringens* on d 14, 15 and 16. To test the effects of tylosin (100 ppm) and a dietary NSP on microbial diversity as it relates to NE, treatments included a basal wheat/barley diet (W/B), W/B + tylosin, W/B + pectin (2%) and W/B + pectin + tylosin. Luminal and mucosal samples (n = 6 birds/treatment) were collected from the jejunum and ileum on d 17, 20 and 24. Microbial community structure was analyzed by comparing band numbers and banding patterns generated by PCR-DGGE using V3-16S rDNA primers. At d 17 and 20, tylosin reduced ($P < 0.05$) band numbers and selected for a more homogeneous ($P < 0.05$) microbiota that differed ($P < 0.05$) from the microbial profiles of control birds. At d 24, band numbers were increased ($P < 0.05$) for all birds, independent of treatment. The growth of *C. perfringens* was analyzed with a SYBR Green-based quantitative PCR (qPCR) assay using 16S rDNA-specific primers (Cp177F and Cp456R). Tylosin reduced ($P < 0.05$) *C. perfringens* growth on d 17 and 21, which was not detectable on d 24, regardless of treatment. The growth of mucosal associated bacteria (MAB) on mucin-limiting medium was determined as a percentage of MAB colonies (n = 20 colonies/bird) grown on habitat-simulating basal medium 10. Tylosin reduced ($P < 0.05$) the percentage of mucolytic bacteria on all days, independent of diet. The tylosin-mediated inhibition of mucolytic MAB in general and *C. perfringens* specifically, correlated in a temporal manner with a reduction ($P < 0.05$) in the occurrence of NE lesions and an improvement ($P < 0.05$) in barrier function. The inclusion of pectin did not significantly affect the variables measured. These data demonstrate that tylosin prevents NE by reducing *C. perfringens* growth and suggest a role for bacterial mucolysis in NE pathogenesis.

#16 A NOVEL GENOMIC APPROACH FOR COMPARING COMPLEX MICROBIAL COMMUNITIES. E. A. Galbraith, D. A. Antonopoulos, and B. A. White, Dept. Animal Sciences, University of Illinois, Urbana, IL 61801. 217-333-2091.

Molecular techniques previously used for genome comparisons of closely related bacterial species could prove extremely valuable for comparisons of complex microbial communities, or metagenomes. Suppressive Subtractive hybridization (SSH) is a PCR-based method for amplification of DNA fragments present in one genome but absent from another. In this technique, genomic DNA is isolated from a sample of interest (tester) and a comparison sample (driver). Each sample is digested with a particular restriction enzyme and unique adaptor sequences are ligated onto the ends of digested tester DNA fragments. Tester and driver DNA are mixed, and through a series of hybridizations followed by PCR amplification, tester-specific DNA fragments are isolated. Recently, SSH has been used to examine disparities in the genomic content of closely related species. Our study aimed to determine the breadth and value of SSH in a broad scale analysis of metagenomic DNA from complex communities of microorganisms in the rumen. SSH was performed on samples of total genomic DNA isolated from rumen fluid samples of two hay -fed steers, and arbitrarily designated as tester or driver. Ninety-six subtracted DNA fragments from the tester metagenome were amplified, cloned, and the DNA sequences were determined. Verification of the isolation of DNA fragments unique to the tester metagenome was accomplished through dot blot and Southern blot hybridizations. Tester-specific SSH fragments were found in 95 of 96 randomly selected clones. DNA sequences of subtracted fragments were analyzed by computer assisted DNA and amino acid comparisons. Using e-values $\leq 10^{-20}$, eight out of fourteen sequences had closest homology with methanogenic DNA. Additional comparative analyses of these sequence data propose a difference in methanogen populations from these two rumen samples. Our study exposes a novel approach to comparative analyses of environmental and ecological microbial communities through the use of SSH. Furthermore, this simple technique offers a glimpse into the wealth of information in the structure and function of complex microbial niches.

#17 DOMAIN SHUFFLING AND EVOLUTIONARY RATES IN THE POLYUBIQUITIN GENES OF RUMEN CILIATES

Neil R. McEwan [1], Freda M. McIntosh [1], Nadine A. Thomas [1], Nancy Nelson [1], Jean-Pierre Jouany [2], Elie Nsabimana [2], Didier Macheboeuf [2], Mitsumori Makoto [3], Takafumi Nagamine [4], Kazunari Ushida [5] Johannes H.P. Hackstein [6], and C. Jamie Newbold [1]

[1] Rowett Research Institute, Greenburn Road, Aberdeen, AB21 9SB, Scotland (+44 1224 712751), [2] I.N.R.A., Station de Recherches sur la Nutrition des Herbivores, Centre de Recherches de Clermont Ferrand-Theix, France, [3] National Institute of Livestock and Grassland Science, Tsukuba, Norindanchi P.O. Box 5, Ibaraki, 305-0901 Japan, [4] Rumen Microbiology Team, STAFF-Institute, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki-ken, 305-0854. Japan, [5] Laboratory of Animal Science, Kyoto Prefectural University, Shimogamo, Japan, [6] Department of Microbiology and Evolutionary Biology, University of Nijmegen, Nijmegen, The Netherlands

The polyubiquitin genes from five species of rumen ciliates (*Epidinium ecaudatum*, *Polyplastron multivesiculatum*, *Dasytricha ruminantium, Isotricha prostoma* and *Entodinium caudatum*) were investigated for their relationship to each other, both for the numbers of each codon used across the complete domains of the gene, and also for the position of the codons within the domains. As in other organisms ciliate polyubiquitins were found to be made up of a highly conserved tandem repeated domains of 76 amino acids. The frequency of use of each codon suggested that the species all use a relatively similar codon usage pattern. When the codons were assessed relative to their position within the domains it was clear that two distinct branches could be seen, with the holotrichs and entodiniomorphs branching separately. Furthermore, the domains clustered in a species-specific manner, rather than in a manner governed on the basis of their position within the gene, suggesting that this is the result of domain shuffling within the full-length gene. In addition the rate of sequence divergence appears more rapid than would be anticipated over the timescale proposed for the divergence of these organisms, suggesting that the time point for evolution of rumen ciliates requires re-consideration.

This project was supported by EU infrastructure grant QLRI-CT-2000-01455: www.ercule.com. The Rowett is supported by the Scottish Executive Rural Affairs Department.

#18 Advancements towards the verification of a molecular-based assay to predict flow and rumen pool size of protozoal nitrogen. J.T. Sylvester, J.L. Firkins and M. Morrison Department of Animal Sciences, The Ohio State University, Columbus, OH 43210 (614-688-3089)

Direct approaches to quantify intra-ruminal N recycling and protozoal flow post-ruminally have been limited by marker specificity. The current objectives are to report progress towards the verification of a molecular-based assay using 18S rDNA as a protozoal specific marker. A two-part DNA isolation method was derived, consisting of extraction and purification with a DNA binding column (QIAamp DNA Stool Mini Kit). Ciliate protozoal specific PCR primers were used to amplify a 1.5-kb fragment of the small subunit rRNA gene by conventional PCR and quantified for use as a standard. A second set of internal primers was used to amplify an approximate 300-bp fragment at each PCR cycle using real-time PCR (rt-PCR). Comparing the predicted 18S rDNA copies from genomic DNA before vs. after passing through the column gave a recovery of 98%. Duodenal digesta was evaluated with rt-PCR to determine sample size and storage conditions to yield the greatest rDNA copies. A 500-ml sample of duodenal digesta was divided into four fractions: two stored at –20^{o}C and two at –80^{o}C. Predicted rDNA copies were compared from replicates of fresh and frozen samples; there was no difference between –20^{o}C vs. –80^{o}C storage after one thaw cycle, but more than half of the copies were lost if samples were thawed twice. Sample size used for DNA extraction was evaluated by the same method comparing 1.0 ml, 0.5 ml and 0.25 ml of starting material used in DNA extraction. The 0.5 ml sample yielded (100%) more copies/ml than did 1.0 ml, but 0.25 ml decreased quantitatively (half the yield of 0.5ml). Total recoverable rDNA was determined using slope-recoveries of known quantities of protozoal cells spiked into duodenal samples. The recovery of protozoal rDNA averaged 82%. Duplication and replication C.V. averaged 18.6 and 21.2%, respectively. At least three PCR duplications of three replicated DNA extractions provided repeatedly similar mean values for rDNA copies. More work is being done in vivo to evaluate the method's performance predicting protozoal N flow and protozoal pool size in the rumen.

#19 Molecular Taxonomy: Revealing unseen diversity with the GI tract. Paul A. Lawson[2], Terry R. Whitehead[1], Michael A. Cotta[1], and Matthew D. Collins[2]. [1]Fermentation Biochemistry Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service, 1815 N. University Street, Peoria, U.S.A;[2] School of Food Biosciences, University of Reading, Reading, UK

The intestinal tract of both man and animals harbours an enormous diversity of microorganisms that play a decisive role in the health and physiology of the host. However, the microbial ecology of these ecosystems is not well understood due to the inadequacy of classical, culture-dependent microbiological methods. The vast majority of organisms within the gut flora consist of obligate bacterial anaerobes most of which can be assigned to 3 major phylogenetic lineages viz: *Bacteroides* group, *Clostridium coccoides* group and *Clostridium leptum* subgroup. Molecular taxonomic inventories obtained directly from human and animal faeces have shown that the vast majority (60–80%) of generated rDNA sequences do not correspond to known organisms and clearly derive from hitherto unknown species within the gastrointestinal tract. However, it is not known if this 'hidden' flora are non-culturable or if they represent organisms which have so far eluded identification using traditional phenotypic taxonomic methods.The application of improved diagnostic tools, in particular the combined use of phenotypic approaches such as miniaturised biochemical testing and protein profiling, and molecular based methodologies such as 16S rRNA gene sequencing has facilitated a plethora of organisms belonging to completely unknown genera being scientifically described. Until the majority of organisms present in the GI tract have been described, the complex interrelationships between both the bacterial flora themselves and in addition, with the host, cannot be fully understood. Non-culture based methodologies have given us an invaluable insight into the enormous diversity present within this environment yet to be elucidated. However, it is only by recovering organisms in pure culture and subjecting them to the appropriate analyses that questions of how the intestinal bacterial community responds to perturbations such as different diets and the use of antibiotics, probiotics, etc. can be addressed.

#20 APPROACHES IN THE SAFETY EVALUATIONS OF VETERINARY ANTIMICROBIALS IN FOOD TO DETERMINE THE EFFECTS ON THE HUMAN INTESTINAL MICROFLORA. **C.E. Cerniglia, Division of Microbiology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079. (Telephone: 870-543-7341)**

The use of veterinary antimicrobial agents in food animals allows for the potential of antibiotic residues to enter the food supply and be consumed by humans. To assess the safety of ingested antimicrobial residues to the consumer, national and international committees evaluate data on the chemical, pharmacological, toxicological, and antimicrobial properties of the veterinary drugs derived from studies of experimental animals and observations in humans. Currently, European and United States guidelines for veterinary drug registration recommend that microbiological hazards from antimicrobial residues must take into account the potentially harmful effects of the drug residues on the human gastrointestinal microflora. The main adverse effects of antimicrobial drugs on the human intestinal microflora are selection of resistant bacteria and disruption of the colonization barrier of the resident intestinal microflora. The Veterinary International Cooperation on Harmonization (VICH) Committee with representatives from the European Union, the United States, Japan, and Australia/New Zealand are currently reviewing all information available on testing methods and protocols for the safety evaluation of antimicrobial drug residues in food. The ultimate goal of the VICH is to harmonize the approaches in the determination of a microbiological allowable daily intake (ADI) when assessing the effects and safety of veterinary antimicrobial residues on the human intestinal microflora. In my presentation, I will describe the *in vitro* and *in vivo* methods that are used for determining no-observable-effect levels (NOEL's) and setting ADIs for antimicrobial residues. Furthermore, I will discuss current research needs and a proposed comprehensive approach in the evaluation of veterinary antimicrobial residues in food for their potential to affect human intestinal microflora.

#21 EVALUATION OF DIVERSITY AND QUANTITATION OF TETRACYCLINE RESISTANCE GENES ON PIG FARMS WITH AND WITHOUT ANTIBIOTIC USE. A. Mehboob, S. A. Kocherginskaya, R. I. Aminov, R. I. Mackie: University of Illinois, Urbana, IL

Our objective was to determine if the suggested link between the use of antibiotics in farm animal diets and the spread of antibiotic resistance in the environment is real. A phylogeny-aided molecular ecology approach was used for the detection and quantitation of tetracycline resistance genes within the ribosomal protection protein and efflux pump protein gene families in a set of 30 samples obtained from two farms. Samples (n=23) were collected from Farm-HF, where chlortetracycline was routinely used for growth promotion and prophylaxis, and 7 samples were obtained from an Organic Farm where no antibiotics have been used since 1993. Samples were obtained from feed, fecal, waste and soil samples on each farm, and bulk genomic DNA extracted. Feed tetracycline resistance genes on both the farms examined, was genetically contaminated with diverse tetracycline resistance genes (tet MOQWCHZ). The containment buildings and waste handling facilities consistently produced a strong PCR signal, confirming the presence of a wide variety of both ribosomal protein and efflux pump tetracycline antibiotic resistance genes. The Real-Time PCR technique was used to calculate the copies of genes for each specific class. As an internal control, a primer set targeting a conserved region of the 16S rRNA gene molecules was used to calculate the bacterial concentration in each sample. The concentration range of 16S rRNA genes detected on Farm-HF was $139x10^3$ to $448x10^5$ molecules/ul and in Organic Farm it was $538x10^3$ to $793x10^4$ molecules/ul. Concentrations of tetracycline resistance genes in Farm-HF and Organic Farm samples were as follows: Tet H was found to be $133x10^2$ to $129x10^5$ and 406 to $165x10^3$ molecules/ul, TetZ was 51 to $106x10^5$ and 918 to $154x10^3$ molecules/ul, Tet C was 120 to $371x10^5$ and 123 to 1684 molecules/ul and Tet Q was 36 to $477x10^5$ and 450 to $397x10^2$ molecules/ul respectively. Regardless of antibiotic use, tetracycline resistance genes were widely distributed but the level was 10-1,000 fold lower on the Organic Farm compared to Farm-HF depending on sampling location.

#22 FLUORESCENT DETECTION OF FECAL CONTAMINATION ON ANIMAL CARCASSES.

M.A. Rasmussen[1], T.A. Casey[1] and J.W. Petrich[2] , [1] NADC/ARS/USDA, and [2]Iowa State University, Ames, IA. 515-663-7350.

Contamination of animal carcasses with feces is an important source of foodborne pathogens such as *E. coli.*O157:H7. Meat processors require devices that can be used to determine the general level of carcass cleanliness. Current methods include unaided visual inspection of carcasses. However, it is difficult to thoroughly inspect carcasses visually given the rapid chain speeds used in modern meat processing. Improved methods or devices need to be automated for rapid data collection and real time analysis. Such devices should be capable of monitoring slaughter process interventions. Fluorescence spectroscopy has been previously applied to the detection of some contaminants on foods. Several fluorescent markers, which may be useful for the detection of feces, are known including the methanogenic cofactor F420. However, the degradation of green plants in the G.I. tract produces chlorophyll metabolites that are highly fluorescent and more useful markers for a fecal detection system. These metabolites have peak excitation and emission bands near 420 nm and 675 nm, respectively, and meat contributes little background fluorescence at these wavelengths. Chlorophyll metabolites are commonly present in the G.I. tract of herbivorous animals. We have exploited the fluorescent properties of these metabolites for the development of instruments, which can detect fecal contamination on meat carcasses in real time. Although diet influences the fluorescent signal, instruments have been designed with sufficient sensitivity for detection of feces from animals consuming commercial finishing diets. These instruments can augment other more time-consuming microbiological testing methods and can assist meat inspectors in their efforts to minimize contamination on meat.

#23 *ESCHERICHIA COLI* O147: AN EMERGING SEROGROUP OF EDEMA DISEASE OUTBREAKS. **A. F. Helgerson[1], R. Schroeder[1], V. Sharma[2], K. Post[3], N. A. Cornick[1], [1]College of Veterinary Medicine, Iowa State University, Ames, IA 50010 515-294-4096, [2]National Animal Disease Center, Ames, IA 50010, [3]Rollins Animal Disease Diagnostic Laboratory, North Carolina Department of Agriculture and Consumer Services, Raleigh, NC 27607**

Edema disease is a systemic disease of weanling pigs caused by *E. coli* strains that produce Stx2e, a variant of Shiga toxin 2. These strains usually produce an F18ab fimbriae and heat stable enterotoxin b (STb). Historically, edema disease strains from U.S. outbreaks have not produced heat stable enterotoxin a (STa) and have belonged to the serogroups O138, O139 and O141. Many isolates recovered from recent outbreaks of edema disease have the *sta* gene and belong to the serogroup O147. We hypothesized that these strains may be an emerging clone of *E. coli* producing edema disease in the U.S. To test this hypothesis 53 *E. coli* O147 strains, isolated between 1996 and 2002, were compared to 6 *E. coli* O147 strains, isolated between 1970 and 1987, using pulsed-field gel electrophoresis (PFGE). The presence of virulence genes was confirmed using a multiplex PCR. The chromosomal DNA was cut with *XbaI* and subjected to the PFGE under standard conditions. GelComparII software was used to compare the banding patterns and determine relatedness. The majority of isolates (44/53) were positive on PCR for *f18, sta, stb, stx2*; and 7 isolates were positive for *sta, stb, stx2*. Only one of the strains isolated prior to 1996 was positive for *f18, sta, stb, stx2*. PFGE divided the recent isolates into seven distinct groups. More than three quarters of the isolates (42/53) fell into a single group (A) with >85% similarity (based on DICE UPGMA coefficient), 7 additional strains exhibited between 80--95% similarity with group A and to one another. The four remaining strains were distinct from one another and from the other groups (<80% similarity). Our data suggests that many *E. coli* O147 strains with a virulence genotype of *f18, sta, stb*, and *stx2* are closely related to one another and may represent an emerging clone of *E. coli* producing edema disease in the U.S.

#24 UNRAVELING THE PURITY AND COMPOSITION OF MSC™. M. Gerds, K. Fenster, E. Emond and J. Leedle. Chr. Hansen Inc. 9015 W. Maple Street, Milwaukee, WI 53214, (414) 607-5700

MSC is a competitive exclusion product derived from cecal scrapings of specific pathogen-free chickens. The product is used to inoculate newly hatched chicks to help establish a healthy, adult gut microflora while the chicks are most susceptible to *Salmonella* colonization. Chr. Hansen, in partnership with Wayne Farms LLC, developed the product as an animal drug. To obtain FDA approval, extensive characterization of the MSC microbial community has been performed. Results from microbiological assessment, 16S rDNA analysis, repetitive DNA genomic fingerprinting (rep-PCR), and Denaturing Gradient Gel Electrophoresis (DGGE) have substantiated the purity and composition of MSC. A total of 300 bacterial colonies were picked, purified and characterized for cell and colony morphology, size, motility, gram reaction, oxygen tolerance, and gas production. The resulting isolates were categorized into groups based upon these classic microbiological characteristics. Analysis of the 16S rDNA sequence of the isolates identified 23 distinct phylogroups that belonged to the genera *Bacteroides*, *Clostridium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Megamonas* and *Veillonella*. To determine the diversity of the phylogenetic groups, rep-PCR genomic fingerprinting was performed and phylogroups were further broken down into 42 distinct Strain-Specific Clusters (SSC). A DGGE method was developed and optimized to monitor and assess the community composition (community DNA fingerprint). Individual DNA bands excised from DGGE gels were identified by sequence analysis and cross-referenced with the distinct phylogroups. DGGE analyses revealed that 5 manufacturing-scale productions from 1998 and 2002 were identical to each other with no observable additions or deletions of bands. These data support that the MSC final product can be reproducibly manufactured.

#25 PROFILES OF CONJUGATED LINOLEIC ACIDS (CLA), *TRANS*-18:1 ISOMERS, AND *TRANS*11,*CIS*15-18:2 IN RUMEN FLUID FROM COWS FED A HIGH-CONCENTRATE DIET SUPPLEMENTED WITH SUNFLOWER, LINSEED, OR FISH OIL. **J. J. Loor[1,2], K. Ueda[1], A. Ferlay[1], Y. Chilliard[1], and M. Doreau[1]. [1]INRA, 63122 St. Genès Champanelle, France, [2]Department of Animal Sciences, University of Illinois, Urbana, USA.**

Profiles of hydrogenation intermediates in response to 18:2*n*-6, 18:3*n*-3, or 20:5*n*-3 availability were evaluated using three lactating Holstein cows fed a high-concentrate diet (65:35 concentrate to forage) with 5% (DM basis) sunflower oil (SO), 5% linseed oil (LO), or 2.5% fish oil (FO). A 3 × 3 Latin square with three 4-wk periods was used. Grass hay and concentrate mixtures were offered separately at 0900, 1300, and 1700 h daily. Rumen fluid was collected via the rumen cannula at 0900, 1100, 1300, 1500, 1700, 2000, and 2400 h. Overall concentrations (% of total fatty acids) of *cis*9,*trans*11-18:2 (9/11CLA), *trans*10,*cis*12-18:2 (10/12CLA), *cis*9,*cis*11-18:2 (*c*9*c*11CLA), *trans*11,*trans*13-18:2 (11/13CLA), *trans*,*trans*-18:2 (*tt*CLA), or *trans*11,*cis*15-18:2 (11/15LA) did not differ due to time, but were affected by oil type. Feeding SO resulted in greater concentration of 9/11CLA (1.46%) and 10/12CLA (0.23%) compared with LO or FO (0.71%, 0.07%). However, feeding LO doubled concentration of *c*9*c*11CLA (0.15% vs. 0.08%) compared with SO or FO. Concentration of 11/13CLA was greater in response to FO or LO (0.21%) compared with SO (0.10%), whereas FO or SO increased *tt*CLA (0.31% vs. 0.18%) compared with LO. Concentration of 11/15LA, an intermediate of 18:3*n*-3 hydrogenation, ranked by treatment was LO (8.56%) > FO (2.93%) > SO (0.95%). Overall, *trans*10-18:1 and *trans*11-18:1 (TVA) did not differ due to time. Average concentration of *trans*10-18:1, however, was greater when FO or SO were fed (8.20%) compared with LO (3.06%). Oils did not affect TVA concentration (10.53%). Results indicate marked differences in production of hydrogenation intermediates due to the nature of dietary polyunsaturated fatty acids. A weak overall correlation (r = 0.51) between 10/12CLA and *trans*10-18:1 suggests other factors are involved in the production of this 18:1 isomer.

#26 DEGRADATION OF ALFALFA CELL WALL POLYSACCHARIDES BY PURE CULTURES OF FIVE RUMEN BACTERIAL SPECIES. H. G. Jung[1]*, P. J. Weimer[2], and F. M. Engels[3]. USDA-Agricultural Research Service, [1]St. Paul, MN 55108 and [2]Madison, WI 53706, and [3]Wageningen University, The Netherlands (612/625-8291).

The rumen bacterial strains *Fibrobacter succinogenes* S85 (Fs), *Ruminococcus albus* 7 (Ra), *R. flavefaciens* FD-1 (Rf), *Butyrivibrio fibrisolvens* H17c (Bf), and *Lachnospira multipara* 40 (Lm) were compared with rumen fluid for their ability to degrade alfalfa stem cell wall polysaccharides in pure culture and as a five-species mixture. Two maturity stages of alfalfa (bud and full flower) were utilized as substrates and incubation periods of 24- and 96-h were employed. The alfalfa stem substrates and fermentation residues were analyzed for cell wall concentration and composition by the Uppsala Dietary Fiber procedure. Cell wall glucose was considered to represent the cellulose fraction; the sum of xylose, mannose, and fucose residues composed the hemicellulose fraction; and pectin was the sum of uronic acids, galactose, arabinose, and rhamnose residues. Degradation of the immature alfalfa stems was significantly greater than for the mature stems for total cell wall polysaccharides and all polysaccharide fractions. The 96-h incubation period resulted in significantly more degradation of all cell wall polysaccharides than observed after 24-h. Mean degradability of cell wall polysaccharides across alfalfa maturities and incubation times are shown below. The Ra culture was able to degrade all cell wall polysaccharide fractions as well or better than any other culture, including rumen fluid. Co-culture of Ra with the other four rumen species did not improve degradation of any of the cell wall polysaccharides compared to Ra alone. Microscopic examination of stem thin sections indicated that Ra and the five-species mixture appeared to be able to degrade alfalfa tissues to almost the same extent as observed for rumen fluid.

Culture	Total	Cellulose	Hemicellulose	Pectin
	---------- % ----------			
Fs	28.2^{e}	19.2^{d}	16.7^{c}	64.8^{bc}
Ra	61.1^{a}	62.5^{a}	40.3^{a}	78.1^{a}
Rf	31.2^{d}	23.8^{c}	19.0^{c}	64.3^{bc}
Bf	22.5^{f}	11.6^{e}	15.6^{c}	60.7^{c}
Lm	26.3^{e}	18.7^{d}	7.4^{d}	67.3^{b}
Mixture	37.4^{c}	26.4^{c}	24.5^{b}	81.5^{a}
Rumen fluid	54.1^{b}	55.0^{b}	26.7^{b}	78.5^{a}

#27 CHEMOTAXIS OF RUMEN HOLOTRICH PROTOZOA TOWARDS SOLUTIONS OF VARIOUS SUGARS AND ORGANIC ACIDS. **M. Rivera, W. Hu, and M.R. Murphy, Dept. of Animal Sciences, University of Illinois, Urbana, IL 61801 (217-333-0093)**

Rapid chemotaxis of ruminal protozoa towards soluble starch, fructosan, glucose, sucrose, fructose, galactose, maltose and cellobiose has been demonstrated. Holotrich protozoa, in particular, are attracted to solutions containing sucrose, glucose, or fructose. In addition to sugars, ruminant feeds sometimes contain relatively large concentrations of organic acids (malate, citrate, succinate, and fumarate). Our objective was to determine whether holotrich protozoa were attracted to 1m*M* solutions (in centrifuged and autoclaved rumen fluid with 0.1 % L-cysteine) of these organic acids or other soluble sugars (arabinose, galactose, and xylose), in addition to glucose and fructose. Chemotaxis into eight capillary tubes from fresh rumen fluid (collected before feeding each day) after 30 min was measured. Duplicate tubes of control, glucose, and two other solutions were tested twice in a single day. The experiment was repeated on subsequent days until all solutions had been tested. The results indicated that control tubes averaged 2.5 holotrich protozoa; whereas, fructose and glucose tubes contained 45.3 and 41.3, respectively (different from control based on square root transformed data, $P < 0.0001$). Citrate averaged 10.7 and galactose 9.6 holotrichs (compared to control, $P < 0.1$); whereas, counts in tubes containing xylose (6.0), fumarate (3.4), succinate (3.3), malate (3.0), or arabinose (1.9) did not differ from control ($P > 0.1$). Holotrich protozoa were highly attracted to fructose and glucose, but much less so to solutions of other sugars or organic acids. By competing with other microbes for fructose and glucose, holotrich protozoa may help reduce the rate of production of lactate and stabilize ruminal digestion.

#28 EFFECTS OF COMBINATIONS OF PROPYNOIC ACID AND CROTONIC ACID OR 3-BUTENOIC ACID ON RUMINAL DIGESTIBILITY AND MICROBIAL EFFICIENCY IN VITRO. E. M. Ungerfeld, S. R. Rust and R. Burnett, Michigan State University, East Lansing, MI 48824 (517 355 4070)

Propynoic acid has been shown to inhibit ruminal methanogenesis in vitro, but also had adverse consequences on fermentation. As crotonic acid and 3-butenoic acid seemed to stimulate fermentation, it was hypothesized that they could relieve the fermentation constraints caused by propynoic acid. In 1000 mL-Erlenmeyer flasks, 750 mL of a 4:1 mixture of buffer and ruminal fluid was delivered under O_2-free CO_2, and 6 g of grass hay used as substrate. Propynoic acid at 0, 2, and 4 mM initial concentration was combined with 3-butenoic acid (Exp. 1) or crotonic acid (Exp. 2) at 0 or 4 mM. Flasks were incubated at 39 C for 72 h. ^{15}N was used as a microbial marker. In Exp. 1, propynoic acid decreased ($P = 0.02$) OM digestibility by 5 percentage units, and tended ($P = 0.08$) to decrease N digestibility by 10 percentage units. Propynoic acid increased ($P < 0.01$) the efficiency of microbial OM and protein synthesis by 48 and 55%, respectively. 3-Butenoic acid had no effects on OM or N digestibility, or on microbial efficiency of OM or CP synthesis. In Exp. 2, propynoic acid decreased OM ($P = 0.04$) and N ($P < 0.01$) digestibility by 12 and 35 percentage units, respectively. Propynoic acid increased ($P < 0.01$) the efficiency of OM and N synthesis by 72 and 85%, respectively. Crotonic acid had no effects on OM or N digestibility, or on microbial efficiency of OM or CP synthesis. Both 3-butenoic and crotonic acid were ineffective in improving fermentation. It remains to be elucidated if the improvement in microbial efficiency of OM and CP synthesis caused by propynoic acid was a consequence of the change in H dynamics caused by the inhibition of methanogenesis, or a particular effect of propynoic acid on some microorganisms.

#29 RANKING OF WHOLE PLANT CORN SILAGES FOR SUBACUTE RUMINAL ACIDOSIS. B. Michalet-Doreau, I. Fernandez, C. Martin, URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France (33 4 73 62 40 76)

In Northern Europe, corn silage based diet is largely used to meet the nutrient requirements associated with the increased milk yields. Starch from corn silage is rapidly fermented in the rumen, and information is needed regarding how much it can be fed without compromising performance due to subacute ruminal acidosis. The objective of this trial was to evaluate whole plant corn silages (WPCS) throught their capacity to maintain ruminal pH within a tolerable range.

WPCS (N=8) were fed to four cows fitted with permanent ruminal cannulas. Diets were based on the experimental WPCS (77.1 % in mean) supplemented with 21.7 % concentrate and 1.2 % minerals. Ruminal pH was measured every two hours over a 24-period. Ruminal fluid was collected before and after feeding (+3h, +5h) on two consecutive days to determine by titrimetry major components of the ruminal buffer system. Chewing activity of cows was recorded for three consecutive days by a pressure gauge system.

Variations of mean pH were low (from 6.19 to 6.51), but time during pH stayed under 6.2 ($time_{pH<6.2}$) varied in large proportions (from 1.9 to 10.6h/d) with WPCS. These variations were not related to the chemical composition of diet ($P>0.10$). Titrimetry measurements allowed to identify VFA ($pK=4.8$) and the two functions of carbonates ($pK_1=6.25$ and $pK_2=10.25$) as the main components of the ruminal buffer system. A significant relationship was found between the $time_{pH<6.2}$ and the buffering capacity of the first carbonates function, but not with the ruminal buffering capacity of VFA. When the chewing time, expressed per unit of feed intake, increased, the $time_{pH<6.2}$ decreased significantly, and this parameter explained 70% variations of the $time_{pH<6.2}$.

The WPCS based diets involved large variations in $time_{pH<6.2,}$ were explained by changes in buffering capacity of carbonates in ruminal fluid, and may be related to chewing time and saliva production.

#30 VALIDATION OF CONTINUOUS RUMINAL pH MEASUREMENTS BY INDEWLING PROBES. **L. Brossard (1,2), M. Fabre (1), C. Martin (1), B. Michalet-Doreau (1), (1) URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France (33 4 73 62 40 55), (2) Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France**

Full implication of nutritional acidosis on overall health of the animal requires precise information on circadian variation in ruminal pH, including drop and duration of drop under a pH threshold. A study was made to evaluate a continuous pH measurement system. Two diets inducing different ruminal mean pH and post-prandial variations were distributed consecutively to four ruminal cannulated sheep. A 100% hay diet was given one week before and one week after distribution of an acidotic diet (60% wheat + 40% hay) during two weeks. Ruminal pH was measured continuously and recorded at 5-min intervals throughout the experiment on sheep fitted with individual indwelling pH probes. The self-cleaning flat kynar pH electrode had a 3-m wire extension. The wire and the probe were fixed to a 25-cm plastic stalk, ballasted by a 100-g weight to maintain the device in place in the ventral sac. The wire was thread through the cannula cover, blocked herein, and secured to the cannula. The other end of the wire was connected to a pH data logger which was placed in a dress handkerchief fixed to the back of the animals. The probes were calibrated at the beginning of the experiment, and checked at the end. Data were collected weekly by connecting each logger to a PC using a designed software. In parallel, ruminal samples were collected in ventral sac before, 3h and 6h after feeding on 3 consecutive days of each week. After filtration, the filtrate was maintained under magnetic stirring for immediate pH determination with a digital pH meter (Ag/AgCl electrode). All values obtained with pH meter were compared to those obtained at the same time with probes. Values of pH were highly correlated between the two systems (pH probe = 0.89 (SE 0.03) pH-meter + 0.71 (SE 0.17), R = 0.94 (SE 0.01), n = 144). Calibrations of probes were valid throughout the four weeks of experiment. Indwelling probes system is robust to measure complete and representative kinetics of ruminal pH. By suppressing ruminal content sampling, this system is also more convenient for user and less disturbing for animals than pH-meter system.

#31 FIBER DIGESTION AND MICROBIAL FIBROLYTIC ACTIVITY IN THE RUMEN. C. Martin, B. Michalet-Doreau, URH-DVA, INRA, Theix, 63122 Saint-Genès-Champanelle, France (33 4 73 62 40 55)

Cereal supplementation involves a quadratic decrease of plant cell walls digestion in the rumen. Reduced microbial cellulolysis in response to readily fermentable carbohydrates supplementation will not be ascribed to a weaker microbial colonization of plant particles, but rather to a fall in fibrolytic activity of solid-associated microorganisms (SAM), considered as the most active microbial population in the ruminal fiber digestion.

In five experiments (three with cattle and two with sheep) in which the proportion of rapidly degradable starch (RDS) in the diets varied between 0 and 50 %, we measured fibrolytic enzyme activity (xylanase) in the SAM. After enzymes extraction from SAM by physical treatments (freezing, sonication), xylanase activity was determined by measuring spectrophotometrically (410 nm) the amount of reducing sugars released after incubation (39°C, 60 min) of xylan with the enzyme preparation. Total xylanase activity (TXA) was expressed as the quantity of reducing sugars released per gram DM solid phase per hour.

We found a significant and quadratic decrease in microbial xylanase activity as the amount of RDS in the diet increased (TXA = -0.137 % RDS^2 (SE 0.091) + 0.033 % RDS (SE 3.762) + 280.4 (SE 31.4); R = 0.87; n = 13). The responses of fibrolytic enyme activity and fiber digestion in the rumen to readily fermentable carbohydrates supplementation appear similar. With high RDS supplement (> 30 %), TXA was very low suggesting the existence of a fibrolytic activity threshold beyond which fibrolytic activity will limit fiber digestion in the rumen. Devising ways to improve the ruminal fibrolytic activity seems a first step to improve the fiber digestion in ruminants when it is compromised by high readily fermentable carbohydrates supplement.

#32 ON THE CHARACTERISTICS AND POSSIBLE MODE OF ACTION OF AN EXOGENOUS PROTEASE FOR RUMINANT DIETS. D. Colombatto and K. A. Beauchemin. Agriculture And Agri-Food Canada, PO Box 3000, T1J 4B1, Lethbridge, AB, Canada (+1-403-3172235).

The biochemical properties and possible mode of action of a protease mixture (PM) established as effective to increase fiber degradation in the rumen were investigated. Protease activity assays (pH 6.0, 39°C) revealed that the PM was equally effective against gelatin, sodium casein or azocasein, but had no action against bovine serum albumin. Response of protease activity to pH (range 5-8) was determined using azocasein as substrate. Activity increased quadratically with pH, with the activity at pH 8.0 being three-fold of that determined at pH 5.0. Addition of 1 m*M* disodium EDTA did not inhibit the proteolytic action, whereas 3 m*M* phenylmethylsulfonyl fluoride inhibited protease by 36%, thus indicating the presence of serine proteases but absence of metalloproteases in the PM. Judged by SDS-PAGE, the PM contained a major band of 32 kDa, with other smaller bands of around 22 and 10 kDa. *In vitro* fermentation studies with ruminal fluid demonstrated that, added at 1.5 μL/g DM 2 h prior to ruminal fluid addition, the PM was effective at increasing the DM degradation (22 h incubation) of alfalfa hay by 11.8%. Furthermore, degradation was increased up to 21% with increasing application rates (up to 10 μL/g), however the relationship was quadratic ($P < 0.001$, $R^2 = 0.85$). Autoclaving destroyed this ability, indicating that the active component is heat-labile. Microscopy studies revealed that the protease increased the degraded areas of alfalfa hay after 18 h of incubation with ruminal fluid, with some effects also observed at 0 h (i.e., pre-treatment effects). It is speculated that the protease mixture removes structural barriers present in the forages, thus allowing a more rapid colonization and degradation of the fiber by ruminal microorganisms.

#33 Characterization of the microbial population and fermentatives activities in the forestomach of young guanacos and in the rumen of young calves

L. C. Arakaki[1], O. Balbuena[2], G. Osacar[1], V. Olivera[1], G. Marcoppido[1], A. Maguire[3] and G.Berra[1]

[1] INTA Instituto de Patobiología, CC 78, (1708) Castelar, Argentina. (054-11-4621-1289)
[2] INTA Colonia Benitez, CP 3505, Argentina. (054-3722-493044). [3]Fundación Habitad, Buenos Aires, Argentina.

The objetive of this study was to compare the microbial population and fermentative activities in the forestomach of young guanacos and in the rumen of young calves. The study was made using 6 guanacos (5 to 14 weeks of age) and 10 calves (4 to 9 weeks of age). During the week 4 the animals were fed milk and alfalfa in different amounts according to the specie. During the week 10 the guanacos were fed milk, alfalfa and grain, and the calves were fed milk, alfalfa and concentrates. Samples were collected by stomach tube before the morning feeding and transported inmediately to the laboratory for microbiological studies. During the experimental period, 4 calves from the original 10 group developed bloat. Bloating animals were no sampled. On average, the guanacos developed lower number ($p<0.01$) of total anaerobic bacteria and amilolytic bacteria than calves. No diferences were observed in the number of cellulolytic bacteria in the two species. Total VFA and molar proportion of propionic and butyric acids were higher ($p<001$) in calves than in guanacos. Acetate was higher in guanacos than calves, ($p<0.01$). Under similar feeding conditions, the pH levels were higher in guanacos than in the calves, 6.81 vs 6.18 , ($p<0.01$) respectively. Protozoa was observed in four from the 6 guanacos in the order of 10^3 -10^4 /ml at only 6 weeks of age. Among calves, no protozoa was detected during the first 9 weeks. The present study showed a more stable pH conditions and the presence of protozoa in the forestomach of guanacos at earlier stages than in the rumen of calves.

#34 DNA SEQUENCE ANALYSIS OF THE TetW GENE FROM *Ruminococcus flavefaciens* JM1. K. S. Scopel, D. A. Antonopoulos, and B. A. White, Dept. Animal Sciences, University of Illinois, Urbana, IL 61801. 217-333-2091.

Ruminococcus flavefaciens FD-1 utilizes cellulose degradation as a means of providing energy, but the regulation involved in this process is not well understood. In order to see how this organism is affected by the absence of a functional copy of specific genes, transposon mutagenesis can be used to interrupt the action of such genes. We are currently constructing a mariner transposon-based delivery system for mini-transposon mutagenesis in *R. flavefaciens* FD-1. In order to insure that the selection marker used will be expressed in *R. flavefaciens* FD-1, we are cloning and sequencing a tetracycline resistance marker from a different strain of *R. flavefaciens* (JM1). We have been able to amplify and sequence a section of the open reading frame of a tetracycline gene (TetW). The TetW gene fragment has 98% amino acid sequence identify with the *Butyrivibrio fibrisolvens* TetW sequence and 99% nucleotide sequence identity with the *Mitsuokella multiacidus* TetW sequence; sequence alignment data was obtained from the GenBank database. Using primers designed off of this fragment, we were able to amplify and sequence the entire open reading frame of the TetW gene. We are using inverse PCR to obtain the regions upstream and downstream of the TetW gene. The purpose of this is to acquire the sequence for the promoter region of the TetW gene and to determine if the TetW gene is part of a transposable element.

#35 CLONING OF THE *O*-ACETYLHOMOSERINE SULFHYDRYLASE GENE FROM THE RUMINAL BACTERIUM *SELENOMONAS RUMINANTIUM* HD4.

X. Qin and S. A. Martin. Dept. of Animal and Dairy Science, The University of Georgia, Athens, GA 30602-2771 (706-542-1065)

The *O*-acetylhomoserine sulfhydrylase (OAHS) gene was cloned from a *Selenomonas ruminantium* HD4 Lambda ZAP® II genomic library by degenerative probe hybridization and complementation. Sequence analysis revealed an 869 bp ORF with a G + C content of 53%. The ORF had significant homology with enzymes involved in homocysteine biosynthesis. A CuraBLASTN™ homology search showed that the ORF has 63% nucleotide identity with the OAHS of *Bacillus stearothermophilus, Corynebacterium glutamicum,* and *Acremonium chrysogenum*, and has 58% identity with Met25 of *Saccharomyces cerevisiae* and MetZ of *Pseudomonas aeruginosa*. The deduced amino acid sequence exhibited 45% similarity with Met25 and MetZ. Further analysis predicted that the gene product was a member of the pyridoxal phosphate enzyme family. Complementation experiments using *Escherichia coli met*A, *met*B, and *met*C mutant strains showed that the *S. ruminantium* OAHS gene can complement the *met*C mutation and allow for growth on minimal media that contained either sulfate or thiosulfate as the sole source of sulfur. When the OAHS was disturbed by inserting an EZ:TN™ pMOD™-2<Apramycin> transposon, the complementation was lost. Therefore, these results suggest that the gene functions as OAHS in *S. ruminantium* HD4.

#36 EXPERIMENTAL CHLORATE PRODUCT TREATMENT TO REDUCE FOOD BORNE PATHOGENIC BACTERIA IN RUMINANT AND MONOGASTRIC FOOD ANIMALS.

T. R. Callaway*, R. C. Anderson, T. S. Edrington, Y. S. Jung, K. J. Genovese, K. M. Bischoff, T. L. Poole, R. O. Elder, R. B. Harvey, and D. J. Nisbet.

Food and Feed Safety Research Unit, ARS/USDA. College Station, TX 77845 (979-260-9374)

Pathogenic bacteria that cause human food borne illness can live within the gastrointestinal tract of food animals. Many of these bacteria do not affect animal production, but do pose a threat to consumers via meat and dairy products. Over 76 million cases of food borne illness occur each year in the U.S.; many of these cases are linked to consumption of contaminated meat products. Some of the most prevalent food borne pathogenic bacteria share a common trait, the ability to respire anaerobically using nitrate. This reduction is accomplished via the intracellular enzyme nitrate reductase; however, NR does not differentiate between nitrate and its valence state analog chlorate, which can be reduced to cytotoxic chlorite. When added to pure and mixed cultures, chlorate killed *E. coli* and *Salmonella* within 24 h. Preliminary in vivo studies indicated that chlorate supplementation reduced *E. coli* O157:H7, wild-type *E. coli* and *Salmonella* in cattle, sheep and swine, respectively. Therefore, an experimental chlorate-containing product (XCP) has been developed for use in monogastric and ruminant animals. XCP treatment significantly reduced *E. coli* concentrations including the enterohaemorrhagic *E. coli* O157:H7 concentrations in sheep, and significantly reduced wild-type *E. coli* concentrations in finishing cattle. XCP treatment also significantly reduced *Salmonella* concentrations in the gastrointestinal tract of growing swine but did not affect *Salmonella* contamination of the tonsils of swine experimentally inoculated with *Salmonella* during lairage. These results indicate that XCP could be a viable pre-harvest intervention strategy to reduce some food borne pathogenic bacteria in food animals.

#37 ATLANTIC SALMON AND RAINBOW TROUT GASTROINTESTINAL MICROBIAL DIVERSITY. **J. M. Fisher[1], T. Bradley[2], and B. A. White[1], [1]Dept. of Animal Scienes, University of Illinois, Urbana, IL 61801 (217-244-4305) and [2] Depts. of FAVS/CMB, University of Rhode Island, Kingston, RI 02881 (401-874-5404)**

Characterization of the microbial diversity of Atlantic salmon and rainbow trout gastrointestinal communities has previously relied on classical culture based microbiology techniques. The use of molecular techniques to compare the microbial diversity has several distinct advantages over culture-based approaches including identification of uncultured microorganisms. Therefore, the microbial community structures in the gastrointestinal tracts of these food fish were analyzed by denaturing gradient gel electrophoresis (DGGE). The microbial diversity in the gastrointestinal tracts of Atlantic salmon reared with rainbow trout, reared separately, and rainbow trout reared independently were compared. DGGE banding patterns were analyzed and relatedness trees were constructed using the UPGAMA algorithm. Prominent DGGE bands from a standard fed, freshwater raised Atlantic salmon sample were excised from the gel, reamplified, cloned, and sequenced for identification. The community structure of the Atlantic salmon reared with rainbow trout was similar to the Atlantic salmon raised independently. Although all the Atlantic salmon samples were similarly related, there was a distinction between Atlantic salmon reared with rainbow trout and independently raised salmon. In addition, one cohabited salmon sample revealed a close microbial diversity with independently raised rainbow trout. Over 50% of the gastrointestinal microbial diversity from rainbow trout reared with Atlantic salmon were closely related with individually raised Atlantic salmon. Sequence analysis from two prominent Atlantic salmon DGGE bands resulted in a 96% similarity to an uncultured marine bacterium and *Shewanella putrefaciens* and 100% similarity to *Aeromonas veronii/ Aeromonas sobria*. *Shewanella putrefaciens* is a known marine bacterium found in foodfish species and *Aeromonas veronii/ sobria* were first identified in a trout fish farm and stream.

#38 COMPARATIVE GENOMICS OF A BIFUNCTIONAL CELLULASE SEQUENCE AT THE STRAIN, SPECIES, AND GENUS LEVEL IN RUMINAL MICROORGANISMS. D. A. Antonopoulos and B. A. White. Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801

An 833 bp fragment exhibiting motifs associated with fiber-degradation was recovered from comparative studies of strains of *Ruminococcus flavefaciens* using suppressive subtractive hybridization. The technique allows amplification of regions of dissimilarity between simple prokaryotic genomes and has been applied to *R. flavefaciens* strains FD-1 and JM1. One of the cloned fragments was putatively identified as a bifunctional endo-1,4-beta-xylanase in initial BlastX comparisons with GenBank, but upon closer inspection two domains of different identities were identified in the same reading frame. One of the domains within the fragment displayed an extremely high-degree of identity (85-89% at the nucleotide level) with a xylanase domain from *R. flavefaciens* 17. However, the in-frame second domain displayed higher similarity with an endoglucanase from a different Ruminococcus organism, rather than the cognate region of the *R. flavefaciens* 17 sequence. Digested genomic DNA from strains of *R. flavefaciens*, *R. albus*, and *Fibrobacter succinogenes* were subjected to Southern blot hybridizations with the putative bifunctional xylanase fragment. As expected, no signal was present from the strain used as the source of driver DNA in the original subtractive hybridization study (driver DNA refers to the genome used to "subtract" the regions in common between the organisms being compared). An identical pattern of fragments appeared in the lanes representing *R. flavefaciens* FD-1, and R13e2. The patterns remained consistent between these three organisms regardless of the domain used as the probe. Our analysis indicates at this point a conserved 10 kb region containing this gene within these strains. Extra copies of these domains may appear elsewhere in the genomes as well.

#39 SUB-ACUTE ACIDOSIS IN DAIRY COWS; ANALYSIS OF CHANGES IN THE BACTERIAL COMMUNITIES IN RUMEN LIQUID BY DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE).

L.T. Mydland[1], Z. Yu[3], H. Volden[1], A. Steen[2], and M. Morrison[3].
[1]Agricultural University of Norway, Aas, Norway, [2]The Norwegian School of Veterinary Science, Oslo, Norway and [3]Ohio State University, Columbus, OH, USA.

Sub-acute acidosis generally occurs during the transition period. The condition is due to a rapid change in diet from a roughage-based dry cow diet to a lactation diet high in readily fermentable nonstructural carbohydrates, to which the rumen bacteria are not adapted. In essence, there is a lowering of ruminal pH (increased acidity) due to an accumulation of bacterial fermentation products (volatile fatty acids, VFAs), which can result in unfavorable conditions for several important groups of rumen bacteria's (e.g. methanogens, cellulolytic and lactate utilizing bacteria). In this experiment, we gradually increased the amount of a barley-/oats-based concentrate mixture (+ 0,3 kg/day to + 1,2 kg/day) to 8 fistulated, multiparous cows until sub-clinical acidosis were achieved. Cows were fed concentrate three times a day and grass-silage was fed ad libitum. Rumen liquid samples were collected at pH minimum after morning feeding. Four rumen liquid samples from each cow were chosen for total DNA extraction and further analysis (Start (S); at calving, Middle (M); the middle of the experimental period, Acidosis (A); at acidosis and Recovery (R); 2-3 days after acidosis). The V3 16S rDNA region was amplified using universal bacterial PCR primers (GC-Eub-357f and Eub-519r) and separated by denaturing gradient gel electrophoresis (DGGE). Major bands were excised, reamplified and sequenced. Computation of the similarity of the banding patterns between samples using BioNumerics indicated that although there were differences in the banding pattern of all samples, there was a closer similarity within cows and not within treatment groups (S, M, A and R). Of close to 150 bands sequenced, >75 % had a closest identity-match to the genus *Prevotella*. However, only approx. 40 % of these had a similarity of 95 % or higher, thus a certain identity of many of the bacteria in these samples cannot be established.

#40 Influence of aromatic amino acids and proline on xylan fermentation by ruminal microorganisms *in vitro*. **A. Y. Guliye, C. Atasoglu, N. McKain and R. J. Wallace, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK. Tel.: (+44)-1224-712751**

Although ruminal microbes use NH_3 as their main source of N, there is ample evidence that preformed amino acids stimulate microbial growth yield and increase fibre digestion. Recent *in vitro* incubations in our laboratory showed that deletion of phenylalanine, tryptophan and tyrosine from a complete mixture of all 20 common amino acids resulted in a decline in the rate of xylan fermentation. Here, we examined the cumulative effects on xylan fermentation of adding or deleting aromatic amino acids and proline, as a group. Ruminal fluid was taken, after overnight starvation, from four ruminally cannulated sheep receiving a mixed grass hay/concentrate diet. Samples (30 ml) of ruminal fluid diluted 1:2 in buffer were incubated with 200 mg of oat spelts xylan (Sigma) with, as N sources, 10 mM NH_4Cl and various mixtures of amino acids, each at 0.25 g/L. The addition of a complete mixture of 20 amino acids resulted in significantly (17%; $P<0.05$) higher initial (up to 8 h) gas production rate than with NH_3 alone. Deleting the aromatic amino acids and proline reduced the stimulation by 34%. Deleting the four amino acids had no significant influence on VFA production or microbial growth yield ($P>0.05$). Conversely, adding the four amino acids to the NH_3 incubation increased the rate of xylan fermentation by 36%. Thus, aromatic amino acids and proline are important amino acids for xylan fermentation, but they do not alone cause the stimulation observed with a complete amino acids mixture.

#41 Genome Mining to Elucidate the Mechanisms of Nitrogen Regulation in the Rumen Anaerobe *Ruminococcus albus* 8

Kensey R. Amaya, Isaac Cann, and Roderick I. Mackie

Department of Animal Sciences, The University of Illinois at Urbana-Champaign

Bacterial cells contain 65% protein (10% N) and acquisition of ammonia utilized for subsequent amino acid biosynthesis are critical for cell growth. Ammonia plays a central role as an intermediate in the degradation and assimilation of dietary nitrogen by gastrointestinal microorganisms. The key enzymes involved in the assimilation of ammonia are glutamate dehydrogenase (GDH), glutamate synthase (GOGAT), and glutamine synthetase (GS). The synthesis and activity of these enzymes in ammonia assimilation and the biosynthesis of amino acids has led to a need to understand and study how these enzymes are regulated in the ruminal cellulytic bacterium *Ruminococcus albus* 8. The sequenced genome of *R. albus* 8 and gene annotation list, allowed for the search and identification of genes involved in ammonia assimilation and regulation in *R. albus* 8. With this bioinformatic information a hypothetical model of nitrogen regulation for *R. albus* was created based on the regulatory scheme of the well-studied low G+C, gram-positive bacterium *Bacillus subtilis*. In *R. albus* 8 the key ammonia assimilation enzymes GDH, GOGAT, and GS typeI enzymes have been identified. In addition to these key enzymes, a novel glutamine synthetase typeIII (GSIII), not found in *B. subtilis*, was identified in the annotation list of *R. albus* 8. The novel *R. albus* 8 GSIII was further confirmed by the identification of conserved domains from other hypothetical and biochemically characterized GSIII's. The glnA gene was subsequently amplified by PCR from genomic DNA isolated from *R. albus* 8, cloned and expressed in the pET28 vector under a T7 promoter. A pure GSIII protein was obtained by sequential purification steps using affinity (cobalt) column, anion exchange, and gel filtration chromatography. γ–Transferase activity of the cloned and purified enzyme suggest that this protein plays a key role in ammonia assimilation and regulation of nitrogen regulation of *R. albus* 8.

#42 Phylogenetic Diversity of Nitrogen Fixation Genes in Rumen Bacteria

Biao Cheng, Isaac K. O. Cann, Roderick I. Mackie
Department of Animal Sciences, University of Illinois at Urbana-Champaign
Urbana, IL 61801

Nitrogen fixation by the symbiotic microbes inhabiting the gut of termites is important since they thrive on nitrogen poor diets (C:N ratio of 1000:1). However, nitrogen fixation has never been demonstrated in the rumen and high levels of ammonia (>5 mM) would repress nitrogenase activity. However, genome sequencing and annotation of *Fibrobacter succinogenes* S85 and *Ruminococcus albus* 8 revealed that both of them have nitrogen fixation (*nifH*) genes. This raises several questions of interest, *i.e.*, where do *nifH* genes in rumen bacteria originate, are they functional, and are they present in other rumen bacteria.

The *nifH* gene encodes the dinitrogenase reductase and is conserved among diverse nitrogen fixing bacteria and archaea. The *nifH* gene can be used to detect nitrogen fixation genes in microbial communities without the need for cultivation. In this study, the phylogenetic diversity of nitrogen-fixing (*nifH*) genes in rumen bacteria was investigated using a molecular approach. PCR primers targeting a conserved region of the *nifH* gene were designed and a ~400 bp fragment of dinitrogenase reductase gene (*nifH*) was directly amplified from DNA extracted from the mixed bacterial population in the rumen. Analysis of the sequences of cloned *nifH* fragment revealed the presence of *nifH* sequences in rumen bacteria. Phylogenetic analysis based on *nifH* sequences showed that cloned *nifH* fragments in rumen samples could be assigned to different *nifH* phylogenetic groups than *F. succinogenes S85* and *R. albus* 8 *nifH* sequences. *F. succinogenes* S85 and *R. albus* 8 could be assigned to different clusters within the anaerobic *nif* group consisting of *Clostridium* spp. and sulfur reducing bacteria. In contrast, the cloned *nifH* fragments from rumen samples form a distinct lineage within the alternative *nifH* (*anf*) group. This suggests that, if they are expressed, *nifH* genes in the rumen do not function in nitrogen fixation but are likely involved in another, as yet unknown, function in nitrogen metabolism.

#43 EFFICIENCY AND DOSE EFFECT OF LEVUCELL® SC TO IMPROVE RUMINAL pH IN INDUCED ACIDOTIC SHEEP. **L. Brossard (1,2), C. Martin (1), F. Chaucheyras-Durand (2), B. Michalet-Doreau (1), (1) URH-DVA, INRA, Theix, 63122 St Genès-Champanelle, France (33 4 73 62 40 55), (2) Lallemand Animal Nutrition, 19 rue des Briquetiers, BP 59, 31702 Blagnac Cedex, France**

Direct-fed microbial products with *Saccharomyces cerevisiae* have been used for their beneficial effect on ruminal fermentation. However, this effect depended on strain (characteristics and dose) and on diet, among others. The aim of this study was to investigate the effect of two doses of the additive Levucell ® SC (*Saccharomyces cerevisiae*, CNCM I-1077) on ruminal pH during induced latent acidosis in sheep. After an adaptation period to their diet, twelve ruminal cannulated sheep received twice daily an acidotic diet (60% wheat + 40% hay) during two weeks (W1 and W2). Animals were allocated in three groups: control group (L0; n = 5); 0.2 g/day of Levucell ® SC (L1, 2.10^{10} CFU/g; n = 3), and 2 g/day of Levucell ® SC (L10, 2.10^{11} CFU/g; n = 4). Yeasts were dosed intraruminally once daily before morning feeding. Ruminal pH was measured continuously and recorded at 5-min intervals by indwelling probes throughout the experiment. Ruminal pH kinetics were used to calculate daily mean pH, time and area under pH 6. For the control group, ruminal pH parameters varied within a range usually defined as latent acidosis, with a mean pH of 5.6, 18.5 h spent under pH 6, and an area under pH 6 of 10.5 unit pH.h. However, control animals were less affected by acidosis on W2 than on W1. Supplementation with yeast reduced ($P < 0.001$) severity of acidosis by increasing mean pH (5.8), and decreasing time (15.6 h) and area under pH 6 (7 unit pH.h), with a greater effect of yeast on W1 because of adaptation of control group on W2. An effect of the quantity of yeast supplementation was observed on the two weeks, mean pH being higher ($P < 0.05$) and time and area being lower ($P < 0.1$ and $P < 0.001$, respectively) with L1 dose than with L10 dose. Thus, supplementation with Levucell ® SC reduced severity of latent acidosis, L1 being the optimal dosage for the stabilization of the rumen microbial ecosystem, as shown by an improved ruminal pH.

#44 MOLECULAR ANALYSIS OF THE BACTERIAL COLONISATION OF FRESHLY-INGESTED GRASS AND WHITE CLOVER. **E. J. KIM[1], R. J. DEWHURST[1], R. J. MERRY[1], D. R. DAVIES[1], J. A. BROOKMAN[1], M. J. NICHOLSON[1], S. A. KOCHERGINSKAYA[2], M. K. THEODOROU[1] AND R. I. MACKIE[2], [1]Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB, UK (+44 1970 823000). [2]Dept. of Animal Science, University of Illinois, Urbana, Illinois 61801 (217 244 2526).**

The objective of this study was to identify and follow the succession of rumen bacterial populations colonising feed boli (the discrete mass of herbage formed during ingestion) from fresh grass and white clover, using Denaturing Gradient Gel Electrophoresis (DGGE) analysis, a DNA fingerprinting technique. We have developed a procedure for retrieving feed boli from the rumen of grazing cows, and propose that incubation of boli in the rumen *in sacco* could provide insights into microbial colonisation and succession. After rumen emptying and hand-feeding of forage to cows, the boli were collected and 150g portions immediately weighed into dacron bags, followed by incubation in the rumen for 0, 1, 2, 4, 8 and 24 hours. After removal from the rumen, bags were gently rinsed in cold water, freeze-dried and stored at -20°C prior to DNA extraction. Following PCR amplification of the variable region 3 (V3) of the 16S rDNA molecule, separation of the resulting V3 amplicons was performed using gels containing a linear gradient of 35 to 60% denaturant. Representative bands were excised from DGGE gels and sequenced for identification. The results from PCR-DGGE analysis demonstrated rapid (< 2 hours) and continued bacterial colonisation of both grass and white clover. Bacteria appeared to colonise plant substrates in succession and diversity differed according to both plant type and individual animal. However, further analysis of sequences retrieved from the gels revealed that many bands were of chloroplastic origin, especially at the early times (0-4 hours). Several standard primer sets for the study of bacterial diversity based on the 16S rDNA molecule were compared with the sequences of the maize chloroplast using BLASTn and most were very similar. The primer set targeting the V3 region of 16S rDNA is not suitable for the study of plant-microbe interactions in the rumen. Further analysis with a range of different primer sets is under way to resolve this problem.

#45 Presence and expression of tetracycline resistance genes in Salmonella.

C. J. Ziemer and E. Dang
Swine Odor and Manure Management Research Unit, ARS, USDA, Ames, IA 50011 (515-294-0197)

Mobility of antibiotic resistance genes is accepted; however, whether acquisition of these genes modifies expression of other genes is not clear. Modification of gene expression may contribute to differences among serovars of food borne pathogens. An initial investigation to determined tetracycline resistance and presence of common resistance genes in *Salmonella* was conducted. *Salmonella* strains screened were obtained from the Salmonella Genetic Stock Center (Alberta, Canada) and included 46 strains (27 serovars) of *Salmonella enterica* subspecies I and 2 strains each from *S. enterica* subspecies II, subspecies IIIa and IIIb, subspecies IV, subspecies VI, subspecies VII and *S. bongori*. Expression of tetracycline resistance was determined aerobically by plating on Muller-Hinton agar and anaerobically on Wilkens-Chalgren agar containing 0, 4, 6, 8, 10, 12, 16 or 64 Fg/ml chlortetracycline. Aerobically, 3 *Salmonella* strains were sensitive (#4 Fg/ml), 51 had intermediate resistance (6 to 12 Fg/ml) and 6 were resistant to tetracycline (16 and 64 Fg/ml). Expression of resistance was different in some strains when grown anaerobically; 14 sensitive, 34 intermediate and 12 resistant strains. Presence of tetracycline resistance genes (TRG) was determined by PCR for TRG efflux classes *tet*(A)-(E), (G), (H), (K) and (L) and ribosomal protection protein classes *tet*(M), (O), (Q), (S) and (W). TRG present were *tet*(C), *tet*(D), *tet*(A), *tet*(K), *tet*(H), *tet*(G), *tet*(L) and *tet*(B), in order of predominance. No *tet*(E) or ribosomal protection protein genes were detected in any strain. The association of TRG with plasmid preparations will be determined as well as confirmation of genes for *tet*(H), *tet*(K) and *tet*(L), not commonly reported in *Salmonella,* by gene sequencing. Multiple tetracycline resistance genes were detected in thirty one strains: 19 strains had 2 genes, 11 strains had 3 genes and one strain had 5 genes. In 3 of the resistant *Salmonella*, none of the TRG screened were present. The 3 tetracycline-sensitive strains were positive for resistance genes. The *Salmonella* studied appear to have a broader range of tetracycline resistance genes than other reports.

#46 CHEMICAL COMPOSITION AND SMALL INTESTINAL DIGESTION OF PROTEIN FROM VARIOUS RUMINAL MICROBIAL FRACTIONS. **K. K. Nelsen, G. I. Crawford, R. L. K. Hulbert and M. D. Stern, Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108 (612-624-9296)**

Whole ruminal contents were separated into various microbial fractions including solid-associated bacteria (SAB), liquid-associated bacteria (LAB) and protozoa. These microbial fractions were analyzed for ash, crude protein (CP), small intestinal digestion of CP and amino acids. Statistical analyses were performed on all values except on the amino acids because there was no replication of amino acid analysis. Ash concentration (% of DM) was lower ($P < 0.05$) for protozoa (4.2) compared with SAB (12.1) but was not different ($P > 0.05$) between LAB (9.9) and SAB. Crude protein (% of OM) differed ($P < 0.05$) between the bacterial fractions in this study with LAB containing higher CP (70.2) compared with SAB (51.6), however CP of LAB was not different ($P > 0.05$) from protozoa at 60.2. Small intestinal CP digestion estimated using the pepsin-pancreatin step in the procedure of Calsamglia and Stern (1995), was numerically greatest for protozoa (96.6%) followed by SAB (92.2%) and LAB (91.6%), however these results were not different ($P > 0.05$). Total amino acid concentration (μg/mg) was numerically greatest for protozoa (906.4) compared with SAB (782.8) and LAB (892.2). The most obvious difference in individual amino acid concentration (g/100 g of total amino acids) among microbial fractions was the relatively high amount of lysine for protozoa (11.0) compared with the SAB (7.7) and LAB (8.3) fractions. Results from this study demonstrated several differences in chemical composition of the microbial fractions and a greater than 90% estimated small intestinal digestion for bacterial and protozoal crude protein.

#47 EFFECTS OF A LIQUID BYPRODUCT N SOURCE ON FERMENTATION BY RUMINAL MICROBES IN CONTINUOUS CULTURE FERMENTERS.

G. I. Crawford*[1], M. D. Stern[1], R. L. K. Hulbert[1] and P. A. Summer[2], [1]Dept. of Animal Science, University of Minnesota, Saint Paul 55108 (612-624-6216), [2]Ajinomoto USA, Eddyville, IA 52553.

A liquid byproduct of monosodium glutamate (MSG) production, containing 13.7% N and 8.3% NH_3N (DM basis) was evaluated as a nitrogen source for rumen microbes using eight dual-flow continuous culture fermenters. Two dietary treatments were utilized, with the primary N source being provided by soybean meal (SBM) in the first treatment, and the MSG byproduct (MSGN) in the second treatment. Each treatment was formulated to contain approximately 17% CP, 30% NDF, 17.5% ADF, and 5% fat (DM basis). The experiment consisted of one 10 d experimental period, including a 7 d stabilization phase followed by 3 d of sampling. Fermenter pH was maintained between 5.8 and 6.5 throughout the experiment. Measured pH was higher ($P < 0.05$) for SBM (5.83) than for MSGN (5.82). Digestion of ADF was 63.4 and 69.3% for SBM and MSGN, respectively, and tended to be higher ($P = 0.06$) for MSGN. Organic matter, CP, and NDF digestion were not affected ($P > 0.05$) by treatment. Efficiency of bacterial synthesis averaged 20.5 g of N/kg of OM truly digested, and was not different ($P > 0.05$) between treatments. Effluent concentration of NH_3N was greater ($P < 0.05$) for MSGN (13.5 mg/100 mL) compared with SBM (2.4 mg/100 mL). Molar proportions of acetate ($P = 0.07$) and valerate ($P = 0.08$) tended to be higher for MSGN, while molar proportions of propionate tended ($P = 0.07$) to be higher for SBM. Total amino acid input and flow were higher for SBM, and total amino acid flow relative to input was greater for MSGN ($P < 0.05$). Results from this experiment indicate that the liquid byproduct of MSG production supported in vitro microbial growth similar to SBM and also increased ADF digestion.

#48 EVALUATION OF INORGANIC AND CHELATED TRACE MINERAL SUPPLEMENTATION TO EARLY LACTATION AND DRY COW DIETS ON MICROBIAL FERMENTATION IN CONTINUOUS CULTURE. **R. L. K. Hulbert*[1], G. I. Crawford[1], M. D. Stern[1] and M. T. Socha[2], [1]Dept. of Animal Science, University of Minnesota, St. Paul 55108 (612-624-6216), [2]Zinpro Corporation, Eden Prairie, MN 55344.**

Three early lactation and three dry cow diets were formulated with increasing levels of zinc (40, 80, 160 ppm), manganese (40, 80, 160 ppm), cobalt (0.1, 0.5, 1.0 ppm), and copper (10, 20, 40 ppm) sulfate. A fourth lactation diet and dry cow diet contained zinc (160 ppm) and manganese (160 ppm) methionine, copper (40 ppm) lysine, and cobalt (1.0 ppm) glucoheptonate. These eight treatments were evaluated in fermenters inoculated with ruminal fluid from a cannulated cow. There were four 10-day experimental periods to achieve four replications for each dietary treatment. Treatments were arranged as a 4 x 2 factorial completely randomized block design with four types of mineral supplementation and two diet types (early lactation *vs* dry cow). Various differences were observed between the early lactation *vs* the dry cow diet, respectively, including pH (6.21 *vs* 6.49), true OM digestion (53.8 *vs* 35.1%), NDF digestion (40.7 *vs* 34.6%), ADF digestion (51.6 *vs* 42.7%) and total VFA concentration (131.9 *vs* 74.3 mM). Greater *($P < 0.05$)* N flow (2.2 *vs* 1.6 g/d) and CP degradation (63.7 *vs* 47.6%) of the lactation *vs* dry cow diet resulted in greater *($P < 0.05$)* bacterial N flow (0.96 *vs* 0.66 g/d). However, efficiency of bacterial synthesis was not different *($P > 0.05$)* between diet types (26.6 *vs* 29.4 g of N/kg of OM truly digested). No differences *($P > 0.05$)* were observed for the effect of mineral supplementation for either diet type in regard to nutrient digestion, nitrogen metabolism and VFA concentrations. However there were several interactions *($P > 0.05$)* observed between types of diet and mineral supplementation for VFA molar proportions.

#49 *Hespellia stercorisuis* gen. nov. sp. nov., and *Hespellia porcinus* sp. nov., isolated from manure storage pits.

Paul A Lawson [1] , Michael A. Cotta [2], Matthew D. Collins [1], and Terence R. Whitehead [2]
[1] School of Food Biosciences, University of Reading, Reading, RG6 6AP, UK
[2]Fermentation Biochemistry Research Unit, National Center for Agricultural Utilization Research, USDA, Agricultural Research Service,Peoria, USA.

Intensive modern livestock farming practices has resulted in the concentration of generated waste products into increasingly smaller locations. Lagoon treatment or deep pit storage are among the more favoured methods used to handle liquid swine manure. The storage of swine manure is associated with the production of a variety of odorous chemicals, much of which is due to the incomplete digestion process associated with anaerobic systems. In addition, production of odorous compounds within confined facilities as used with swine can pose potential health problems to both the animals and human workers. Although production of these chemicals is the result of microbiological activity, little is known about the types of micro-organisms responsible for their production. During the course of an ongoing study into the microbial diversity present within manure storage pits (Whitehead & Cotta, 2000), we characterised four strictly anaerobic, Gram-positive, asporogenous, rod-shaped organisms of uncertain taxonomic position. Based on the results of a polyphasic taxonomic study, it is proposed that the isolates be assigned to a new genus *Hespellia*, as *Hespellia stercorisuis* sp. nov. and *Hespellia porcinus* sp. nov.

#50 Comparative genome analysis of a cellulolytic and a non-cellulolytic strain of *Ruminococcus albus.* Carine Reveneau, Victoria Parisi, Zhongtang Yu, and Mark Morrison, The MAPLE Research Initiative, The Ohio State University, Columbus OH, 43210.

A combination of suppressive subtractive hybridization (SSH) and representational difference analysis (RDA) has been used to reveal genomic differences between a cellulolytic strain (strain 8) and a non-cellulolytic strain (strain B199) of *Ruminococcus albus,* gram-positive anaerobes belonging to cluster IV of the *Clostridium* lineage of eubacteria. Both genomes were digested with *Rsa*I to construct the SSH libraries, and the average insert size of both libraries was similar (~250 basepairs). The percent G:C content of the cloned DNA fragments were also within the range expected for *R. albus* genomes. Forty six of the SSH clones (from a total of 75) sequenced so far from the strain 8 library produced significant hits with Genbank entries. Using the nomenclature developed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) more than half of these clones were related to environmental or genetic information processing, and involved with DNA replication, translation, and DNA transposition. Another 25% of the clones produced significant hits with genes encoding glycosyl hydrolases, including beta-1,4-endoglucanases (family 5 and 9) and two beta-1,4-xylanases (family 11). Forty four of the 75 clones sequenced from the strain B199 library matched the *R. albus* 8 genome sequence, but with low identity (<85%), and were considered divergent sequences. The remainder of the clones showed no match with genome sequence data from *R. albus* 8. Only 23 of the clones produced significant hits with Genbank entries, and the majority of the predicted functions were also related to genetic information processes, as described above. However, none of the clones were found to encode for glycosyl hydrolases, suggesting the endoglucanases and xylanases identified from the strain 8 library are, at least in part, responsible for the phenotypic differences between the two strains. For RDA analyses, the genomes from the two strains were digested with either *Hha*I or *Nla*III resulting in the production of four libraries. A combination of Southern and RFLP analyses show that all of the RDA libraries contain very few false positive clones, and unlike the SSH libraries, regions of genomic difference between 100 basepairs and 1.5 kilobases were obtained, and approximately 800 clones have now been sent for sequencing. The restriction enzymes used in RDA resulted in libraries with larger clones that may uncover more information regarding the genomic and phenotypic differences between strains 8 and B199. The preliminary results reveal that the genomic differences between the two strains extends beyond their degradative phenotypes, to include functions central to DNA replication and translation processes.

Author Index by Abstract Number

The Organizing Committee:

Michael A. Cotta, Chairman
Jeffrey L. Firkins
Mark Morrison
Terence R. Whitehead
Roderick I. Mackie, Treasurer
Jane Leedle, Secretary

2003 CGIF is partially funded by grant number 2003-35206-12860 from the NRI Competitive Grants Program/CSREES/USDA

Thanks to the following donors:

Purina Mills, LLC

Chr. Hansen Biosystems

Abbott Laboratories Ross Products Division

for sponsoring the
2003 Conference on Gastrointestinal Function