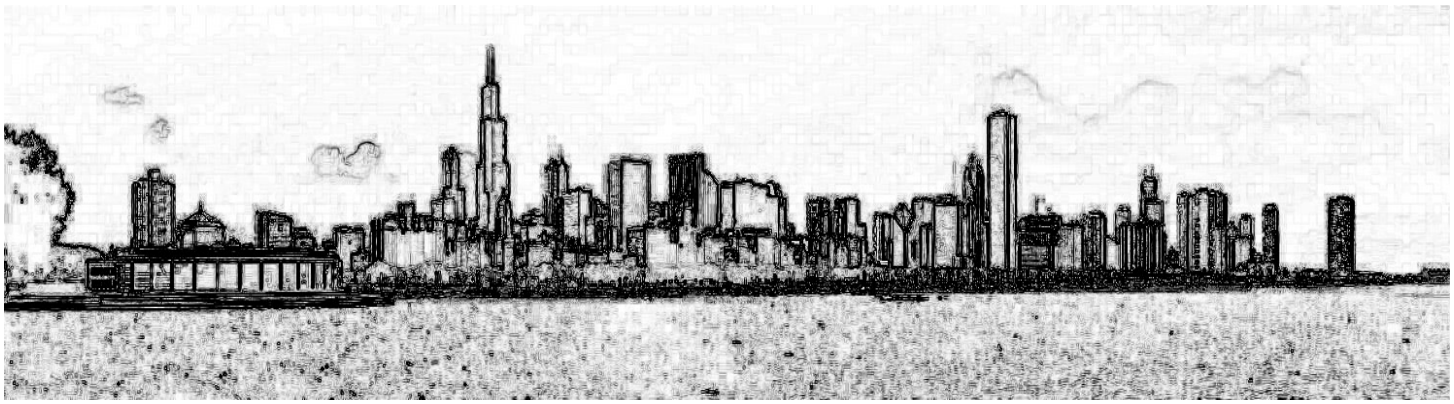
2005
Conference on
Gastrointestinal Function



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Program and Abstracts

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2005 CONFERENCE ON GASTROINTESTINAL FUNCTION

Welcome to the 2005 Conference on Gastrointestinal Function.

Prior to 2000 this meeting convened biennially as 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. Over time, 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. 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, in 2003 we revised the name, theme and scope of RFC to accommodate these emerging areas of research. In the 2005 Conference we hope to expand the scope further to include broader areas of gastrointestinal function and health, including those of humans. It is envisioned that the Conference on Gastrointestinal Function will serve as a mechanism for exchange of ideas, meeting the needs of scientists and foster the formation of many new collaborative efforts and research partnerships.

At the 2003 Conference we inaugurated 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. The first Bryant lecture was delivered by Milton Allison, a long time colleague of Marvin's. This year's lecture will be delivered by Joel Doré, his last student and esteemed researcher of the microbial ecology of the human gastrointestinal tract.

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, APRIL 11 **GOLD ROOM**

7:00 – 10:00 p.m. MIXER

TUESDAY, APRIL 12 **GOLD ROOM**

9:00 a.m. #1 OMICS APPROACHES TO STUDY *LACTOBACILLUS* – HOST INTERACTIONS.
Willem M. de Vos, Wageningen Centre for Food Sciences and Laboratory of Microbiology, Wageningen University, The Netherlands.

9:40 a.m. #2 DEGRADATION OF WHEAT STRAW BY *FIBROBACTER SUCCINOGENES* S85: A LIQUID AND SOLID STATE NMR STUDY.
M. Matulova[1,3], R. Nouaille[1,2], P. Capek[3], M. Péan[4], A.-M. Delort[1] and E. Forano[2]. [1]Laboratoire de Synthèse et Etude de Systèmes à Intérêt Biologique, UMR 6504 Université Blaise Pascal - CNRS, 63177 Aubière cedex, France; [2]Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France; [3]Institute of Chemistry, Slovak Academy of Sciences, Bratislava, 845 38 Slovak Republic; [4]DEVM/GRAP, CEA Cadarache, France.

10:00 a.m. #3 CONTRIBUTION OF *SELENOMONAS RUMINANTIUM* TO FIBER DIGESTION IN THE RUMEN.
S. Sawanon, S. Koike, and Y. Kobayashi. Graduate School of Agric., Hokkaido Univ., Sapporo, 060-8589, Japan.

10:20 a.m. BREAK

10:40 a.m. #4 QUANTIFICATION OF *FIBROBACTER SUCCINOGENES* CELLULASE AND XYLANASE GENE EXPRESSION IN THE RUMEN OF A GNOTOBIOTIC LAMB BY REAL-TIME RT-PCR.
C. Béra-Maillet, A. Kwasiborski**,** P. Mosoni and E. Forano, Unité de Microbiologie, Institut National de la Recherche Agronomique, CR de Clermont-Fd/Theix, 63122 St-Genès-Champanelle, France.

11:00 a.m. #5 DETECTING CHANGES IN BACTERIAL AND PROTOZOAL POPULATIONS IN RUMINAL AND OMASAL SAMPLES FROM COWS FED SUPPLEMENTAL METHIONINE.
S.K.R. Karnati, J.T. Sylvester, Z. Yu, N.R. St-Pierre and J.L. Firkins. Department of Animal Sciences, The Ohio State University, Columbus, Ohio 43210.

11:20 a.m. #6 BOVICIN HC5, A NOVEL BACTERIOCIN FROM *STREPTOCOCCUS BOVIS.*
J.B. Russell, H.C. Mantovani, A.J. Houlihan, M.D. Flythe and B.M. Xavier, ARS/USDA and Dept. of Microbiology, Cornell University, Ithaca, NY 14853.

11:40 a.m. LUNCH

1:00 p.m. #7 LIVE RECOMBINANT LACTIC ACID BACTERIA FOR USE IN HUMAN HEALTH.
Annick Mercenier, Nestlé Research Center, Department of Nutrition and Health, Lausanne, Switzerland.

1:40 p.m. #8 OXALATE CONSUMPTION BY COMMERCIAL PROBIOTICS.
R. Cox and S. L. Daniel, Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920.

2:00 p.m. #9 PROBIOTICS FOR ARCTIC RUMINANTS.
K.E. Præsteng[1], S.D. Mathiesen[2], R.I. Mackie[3], I.K.O. Cann[3] and M.A. Sundset[1], [1]Dept. of Arctic Biology, University of Tromsø, 9037 Tromsø, Norway, [2]Section of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, 9292 Tromsø, Norway, [3]Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL, USA.

2:20 p.m. BREAK

2:40 p.m. #10 FIBROLYTIC BACTERIA FROM THE FORESTOMACH OF KANGAROOS.
D. Ouwerkerk[1], A. V. Klieve[1], A. J. Maguire[1] and R. J. Forster[2], [1]Department of Primary Industries and Fisheries, Animal Research Institute, Yeerongpilly Qld Australia and [2]Lethbridge Research Centre, Agriculture and Agri-Food, Lethbridge, Alberta, Canada.

3:00 p.m. #11 ANALYSIS OF NOVEL ARCHAEAL AND BACTERIAL DIVERSITY ASSOCIATED WITH GALAPAGOS LAND AND MARINE IGUANAS.
M. Mori[1], B. Merchen[1], M. C. Wikelski[2], I. K. O. Cann[1] and R. I. Mackie[1], [1]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [2]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544.

3:20 p.m. #12 REASSESSMENT OF THE HUMAN INTESTINAL MICROBIOTA – FROM PHYLOGENETICS TO METAGENOMICS.
Joël M. Doré and Marion Leclerc**,** INRA, Unité d'Ecologie et Physiologie du Système Digestif. 78352 Jouy-en-Josas Cedex, France.

4:00-6:30 p.m. POSTER SESSION

WEDNESDAY, APRIL 13 GOLD ROOM

9:00 a.m. #13 HOST AND INTESTINAL MICROBIOTA NEGOTIATIONS IN THE CONTEXT OF ANIMAL GROWTH EFFICIENCY.
H. Rex Gaskins, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.

9:40 a.m. #14 RACTOPAMINE SUPPLEMENTATION DECREASED FECAL SHEDDING OF *E. COLI* O157:H7 IN NATURALLY-INFECTED BEEF CATTLE.
T.S. Edrington[1], T.R. Callaway[1], K.J. Genovese[1], C.L. Schultz[1], T.H. Welsh[2], L.A. Soliz[2], S.B. Schroeder[2], R.C. Anderson[1] and D.J. Nisbet[1], [1]USDA-ARS-FFSRU and [2]Texas A&M University, College Station, TX 77845.

10:00 a.m. #15 THE EFFECTS OF LARCH EXTRACT ON PERFORMANCE AND FECAL FLORA IN NURSERY PIGS.

J.E. Wells and J.T. Yen, USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933.

10:20 a.m. BREAK

10:40 a.m. #16 COCCIDIA-INDUCED MUCOGENESIS PROMOTES *CLOSTRIDIUM PERFRINGENS* GROWTH AND SEVERITY OF DISEASE IN A CHICK MODEL OF NECROTIC ENTERITIS.

C. T. Collier[1], C. L. Hofacre[4], A. M. Payne[5], D. B. Anderson[5], P. Kaiser[6], R. I. Mackie[1,3] and H. R. Gaskins [1,2,3] Departments of Animal Sciences[1] and Veterinary Pathobiology[2], Institute for Genomic Biology[3], University of Illinois at Urbana-Champaign, Urbana-Champaign, IL; Poultry Diagnostic and Research Center, Department of Avian Medicine, University of Georgia, Athens, GA[4]; Elanco Animal Health, Greenfield, IN[5]; Institute for Animal Health, Compton, UK[6].

11:00 a.m. #17 EVALUATION OF DIETARY LACTOSE ON THE MICROBIAL ECOLOGY IN POULTRY WITH NECROTIC ENTERITIS.

J. L. McReynolds, J. A. Byrd, S. E. Duke, L. F. Kubena, and D. J. Nisbet. USDA/ARS/SPARC, Food & Feed Safety Research Unit, College Station, TX 77845.

11:20 a.m. #18 EVALUATION OF PERFORMANCE AND PCR-DGGE ANALYSIS OF CECAL MICROFLORA OF NATUSTAT-SUPPLEMENTED TURKEYS CHALLENGED WITH *HISTOMONAS MELEAGRIDIS*.

Sinéad M. Waters[1], Cepta F. Duffy[1], Michael D. Sims[2] and Ronan F.G. Power[3]. [1]Alltech Ireland Ltd., Sarney, Summerhill Rd., Dunboyne, Co. Meath, Ireland. [2]Virginia Diversified Research Corporation, Harrisonburg, VA 22801, USA. [3]Alltech, Inc., 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA.

11:40 a.m. LUNCH

1:00 p.m. #19 FERMENTATION PRODUCTS IN COLON HEALTH: MEDIATORS OF CELL KINETICS AND GENE EXPRESSION.

N.D. Turner, C.A. Warren, K.J. Paulhill, L.M. Sanders, M.Y. Hong, K.L. Covert, L.A. Davidson, R.S. Chapkin, and J.R. Lupton, Faculty of Nutrition, Texas A&M University, College Station, TX 77843.

1:40 p.m. #20 FATTY ACID METABOLISM, SENSITIVITY AND MECHANISM OF BUTYRATE FORMATION ARE LINKED IN DIFFERENT *BUTYRIVIBRIO* ISOLATES.

R. J. Wallace[a], D. Paillard[a], N. McKain[a], L. C. Chaudhary[a], N. D. Walker[a], N. R. McEwan[a], I. Koppova[b], J. Kopecny[b] and P. E. Vercoe[c], [a]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [b]Institute of Animal Physiology & Genetics, Prague, Czech Republic; [c]Animal Science Group, University of Western Australia, Crawley, WA 6009, Australia.

2:00 p.m. #21 LINOLEIC ACID BIOHYDROGENATION BY HUMAN INTESTINAL MICROORGANISMS AS A MIXED FLORA AND AS ISOLATED BACTERIAL STRAINS.

E. Devillard, F.M. McIntosh and R.J. Wallace, Rowett Research Institute, Bucksburn, Aberdeen, UK.

2:20 p.m. BREAK

2:40 p.m. #22 QUANTITATIVE MONITORING OF DISSEMINATION OF TETRACYCLINE RESISTANCE GENES FROM MANURE LAGOONS INTO GROUNDWATER UNDERLYING THE SWINE FARMS.

S. Koike[1], H. D. Oliver[1], I. G. Krapac[2], J. C. Chee-Sanford[3] and R. I. Mackie[1], [1]Dept. of Animal Science and [3]Dept. of Crop Science, University of Illinois, Urbana, IL 61801, [2]Illinois State Geological Survey, Champaign, IL 61820.

3:00 p.m. #23 QUANTIFICATION OF GENES ENCODING TETRACYCLINE RESISTANCE IN MICROBIAL COMMUNITIES BY REAL-TIME PCR ASSAYS.

Zhongtang Yu[1], Frederick Michel[2], Glenn Hansen[3], Tom Wittum[3], and Mark Morrison[1]. [1]The MAPLE Research Initiative, Dept. of Animal Sciences, [2]Dept. of Food, Agri. and Biol. Engineering, and [3]Dept. of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210.

3:20 p.m. CGIF BUSINESS MEETING

POSTERS

#24 FODDER LEAVES RICH IN TANNIN, SAPONIN AND NON-STARCH POLYSACCHARIDE MODULATES RUMEN FERMENTATION *IN VITRO*.

M. R. Amin***,** N. Selje, E. M. Hoffmann and K. Becker, Institute for Aquaculture Systems and Animal Nutrition in the Tropics and Subtropics, University of Hohenheim, Fruwirthstr. 12, D-70599 Stuttgart, Germany.

#25 EFFECT OF INTRARUMINAL NITROETHANE ADMINISTRATION ON METHANE-PRODUCING ACTIVITY AND VOLATILE FATTY ACID PRODUCTION *IN VIVO*.

R. C. Anderson[1*], G. E. Carstens[2], T. R. Callaway[1], C. L. Schultz[1], T. S. Edrington[1] and D. J. Nisbet[1], [1]USDA/ARS, SPARC, FFSRU, College Station, TX and [2]Dept. of Animal Science, Texas A&M University, College Station, TX.

#26 MICROBIAL DEGRADATION OF OXALATE IN THE GASTROINTESTINAL TRACTS OF CATS AND DOGS.

T. N. Talarico, T. Millis, and S. L. Daniel, Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920.

#27 CALCIUM REQUIREMENTS FOR GROWTH AND CELLULOSE DEGRADATION BY RUMEN BACTERIA.

Maria Sol Morales and Burk A. Dehority. Dept. Animal Sciences, OARDC, The Ohio State University, Wooster, OH.

#28 EFFECTS OF VARIOUS METHODS USED TO PROCESS SOYBEAN MEAL ON PROTEIN DIGESTION IN THE RUMEN AND SMALL INTESTINE.

M. D. Stern[1], M. Ruiz Moreno[1] and C. A. Macgregor[2], [1]Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108, [2]Grain States Soya, Inc., West Point, NE.

#29 THE EFFECT OF DIET ON THE POPULATIONS OF RUMEN METHANOGENS AND CILIATE PROTOZOA IN RED DEER.

M. J. Nicholson, N. D. Walker, P. Evans and K. N. Joblin, Rumen Microbiology, AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.

#30 EFFECT OF PARTICLE SIZE ON PASSAGE RATES FROM THE RUMEN TO THE DUODENUM OF LACTATING DAIRY COWS.

A. B. Peterson[1], R. L. Baldwin, VI[2] and R. A. Kohn[1]. [1]Dept. of Animal and Avian Sciences, College Park, MD 20742. [2]Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville, MD 20705

#31 AN ANALYSIS OF THE CONTROL OF THE VOLATILE FATTY ACID PROFILE IN THE RUMEN.

E. M. Ungerfeld and R. A. Kohn, Dept. of Animal & Avian Sciences, University of Maryland, College Park, MD 20742.

#32 CHARACTERIZATION OF MICROBIAL POPULATIONS OF IN VITRO RUMINAL PROTOZOAL CULTURES BY 16S rDNA SEQUENCE ANALYSES.

T. R. Whitehead[1] and B. A. Dehority[2], [1]USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604, and [2]Dept. of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691.

#33 EVALUATION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* SURVIVAL IN THE ENVIRONMENT.

K. Cook[1], J. Britt[2], and A. Pike[2], [1]USDA-ARS and [2]Western Kentucky University, Bowling Green, KY.

#34 PHYLOGENETIC ANALYSIS OF RUMEN BACTERIAL POPULATIONS IN REINDEER.

M. A. Sundset[1], I.K.O. Cann[2], S.D. Mathiesen[3] and R.I. Mackie[2], [1]Dept. of Arctic Biology, University of Tromsø, 9037 Tromsø, Norway, [2]Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL, USA, [3]Section of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, 9292 Tromsø, Norway.

#35 PHAGE IN THE GUT ECOSYSTEMS OF GRAZING LIVESTOCK.

N.D. Walker and K.N. Joblin. Rumen Microbiology Group, AgResearch Grasslands, Palmerston North, New Zealand.

#36 PLANT OILS AND UREASE INHIBITORS AS SOLUTIONS FOR ENVIRONMENTAL ISSUES ASSOCIATED WITH CONFINED ANIMAL FEEDING OPERATIONS.

V.H. Varel and J.E. Wells, USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933.

#37 USE OF ACIDIFIERS IN FEEDING OF BROILERS AND COMPARISON WITH USE OF VIRGINIAMYCIN.

Seyed Mohammad Hashemi, Morteza Beiki. Scientific Member of Qom Agriculture and Natural Resources Research Center Qom, P.O. Box 37185 779, Qom, Iran.

#38 PREVALENCE AND GENETIC TYPING OF *ESCHERICHIA COLI* O157 IN THE RECTOANAL MUCOSAL REGION OF BEEF CATTLE.

J. T. Fox, X. Shi and T. G. Nagaraja. Dept. of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS 66506.

#39 IN VITRO GROWTH AND GAS PRODUCTION OF RUMEN BACTERIA AND POTENTIAL BLOAT MITIGATION WITH CONDENSED TANNINS IN WINTER WHEAT.

B.M. Min[1], R.A. Anderson[2], and W.E. Pinchak[1]. [1]Texas Agri. Exp. Station, Vernon, TX 76385. [2]USDA-ARS, College Station, TX 77845.

#40 MOLECULAR MONITORING OF TYLOSIN- AND ERYTHROMYCIN-RESISTANCE-GENES IN LAGOONS AND GROUNDWATER UNDERLYING TWO SWINE PRODUCTION FACILITIES.

H. D. Oliver[1], S. Koike[1], I. G. Krapac[2], J. C. Chee-Sanford[3] and R. I. Mackie[1], [1]Dept. of Animal Science and [3]Dept. of Crop Science, University of Illinois, Urbana, IL 61801, [2]Illinois State Geological Survey, Champaign, IL 61820.

#41 EFFECT OF DIETARY SOURCES ON RUMEN ECOLOGY OF SWAMP BUFFALOES (BUBALUS BUBALIS).

M. Wanapat, S. Wora-anu, C. Yuangklang, P. Chanjula and O. Puongchompu, Dept. of Anim. Sci., Fac. of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.

#42 AMYLASE AND CALCIUM CHELATOR INTERACT DURING NEUTRAL DETERGENT FIBER ANALYSIS.

R. A. Kohn, D. S. Brougher, and T. B. Oleas, Dept. of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742.

#43 BIPLOT ANALYSIS TO DESCRIBE THE RELATIONSHIPS BETWEEN PLANT AND MICROBIAL FATTY ACIDS IN INGESTED HERBAGE.

E. J. Kim, R. Sanderson, M. S. Dhanoa and R. J. Dewhurst, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK.

#44 PHYLOGENETIC ANALYSIS OF A CONSORTIUM OF OVINE RUMINAL MICROBES THAT BIODEGRADES PYRROLIZIDINE ALKALOIDS.

R. Rattray, S. Ivey-Lodge, and A.M. Craig, Dept. of Biomedical Science, Oregon State University, Corvallis, OR 97331.

#45 ENZYMES OF AMMONIA ASSIMILATION IN *RUMINOCOCCUS FLAVEFACIENS* FD-1 AND *RUMINOCOCCUS ALBUS* 8.

S.A. Kocherginskaya[1], K.R. Amaya[1], K.E. Nelson[2], M. Morrison[3], I.K.O. Cann[1], and R.I. Mackie[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL 61801, [2]The Institute for Genomic Research, Rockville, MD 20850, [3]The Ohio State University, Columbus, OH 43210.

#46 EFFECT OF FEED ADDITIVES ON RUMEN FERMENTATION CHARACTERISTICS AND FROTHY BLOAT IN STEERS GRAZING WHEAT PASTURE.

B.R. Min[1], W.E. Pinchak[1], J.D. Fulford[1], and R. Puchala[2]. [1]Texas Agri. Exp. Station, Vernon, TX 76385, [2]Langston University, Langston, OK.

#47 THE *RUMINOCOCCUS ALBUS pilA1-pilA2* LOCUS: EXPRESSION AND PUTATIVE ROLE OF TWO ADJACENT *pil* GENES IN PILUS FORMATION AND BACTERIAL ADHESION TO CELLULOSE.

Harivony Rakotoarivonina[a], Marilynn A. Larson[b], Mark Morrison[c], Jean-Pierre Girardeau[a], Brigitte Gaillard-Martinie[a], Evelyne Forano[a] and Pascale Mosoni[a]. [a]Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France; [b]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, 68198, USA; [c]The MAPLE Research Program, Department of Animal Sciences, The Ohio State University, 2027 Coffey Road, Columbus, OH 43210, USA.

#48 TETRACYCLINE RESISTANCE RESERVOIRS IN DIFFERENT SWINE FARMS AND AT DIFFERENT LOCATIONS WITHIN EACH FARM AS DETERMINED BY REAL-TIME PCR.

Jing Chen[1], Z. Yu[1], Qing Xia[1], Svetlana Kocherginskaya[2], Asma Mehboob[2], R.I. Mackie[2], and M. Morrison[1]. [1]The MAPLE Research Initiative, Dept. of Animal Science, The Ohio State University, Columbus, OH 43210; [2]Dept. of Animal Science, University of Illinois at Urbana-Champaign, IL 61801.

#49 RUMEN DEFAUNATION USING ESSENTIAL OILS.

S. Franklin, S. Carlson, M. Rasmussen, National Animal Disease Center, ARS, USDA, Ames, IA 50010.

#50 NOVEL CARBOHYDRATE-BINDING MODULES FROM THE *MANA* GENE OF *THERMOANAEROBACTERIUM POLYSACCHAROLYTICUM.*

S. Ohene-Adjei, S. Kocherginskaya, R.I. Mackie, and I.K.O. Cann. Department of Animal Sciences, University of Illinois at Urbana-Champaign.

#51 ANTIPROTOZOAL ACTIVITIES OF 'RUMEN-UP' PLANTS.

R. Ningrat, I. Hussy, C. P. Walsh and R. J. Wallace. Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK.

#52 RE-EVALUATION OF THE FROTHY BLOAT COMPLEX IN CATTLE GRAZING WINTER WHEAT IN THE SOUTHERN PLAINS: EVOLUTION OF A NEW INTEGRATED RESEARCH PARADIGM.

W. E. Pinchak[1], B. R. Min[1], D. P. Malinowski[1], J. W. Sij[1], R. J. Gill[1], R. Puchala[2] and R. A. Anderson[3]. [1]Texas Agricultural Experiment Station, Vernon, TX. [2]Langston University, Langston, OK. [3]USDA-ARS, College Station, TX.

#53 PHAGE INCIDENCE IN FEEDLOT CATTLE FECES, AND THE USE OF PHAGE TO REDUCE *E. COLI* O157:H7 *IN VIVO.*

T. R. Callaway[1], T. S. Edrington[1], K. J. Genovese[1], J. E. Keen[2], R. C. Anderson[1], A. D. Brabban[3], E. Kutter[3], T. L. Poole[1], and D. J. Nisbet[1], [1]USDA-ARS, Food and Feed Safety Research Unit, 2881 F&B Road, College Station, TX 77845; [2]USDA-ARS, Meat Animal Research Center, Clay Center, NE; [3]Evergreen State College, Olympia, WA.

#54 NOVEL GLYCOSYL HYDROLASE ARCHITECTURE FROM THE *RUMINOCOCCUS FLAVEFACIENS* FD-1 GENOME.

M. E. Berg[1], D. A. Antonopoulos[1, 4], M. Morrison[2, 4], K. E Nelson[3, 4], and B. A. White[1, 4]. [1]Department of Animal Sciences, University of Illinois, Urbana, IL 61801; [2]Department of Animal Science, The Ohio State University, Columbus, OH 43210; [3]The Institute for Genomic Research Rockville, MD 20850; and [4]The North American Consortium for Genomics of Fibrolytic Ruminal Bacteria.

#55 PCR-DGGE ANALYSIS OF PROKARYOTE DIVERSITY AND COMMUNITY STRUCTURE IN DIFFERENT FRACTIONS OF RUMEN DIGESTA.

R. Garcia-Gonzalez[1], Z. Yu[2], R.C. Larue[3] and M. Morrison[2]. Current address: [1]Dept. of Animal Production, University of Leon, 24071 Leon, Spain; [2]Dept. of Animal Sciences, The Ohio State University, Columbus, OH 43210; [3]Dept. of Microbiology, The Ohio State University, Columbus, OH 43210.

ABSTRACTS

#1 OMICS APPROACHES TO STUDY *LACTOBACILLUS* – HOST INTERACTIONS.
Willem M. de Vos, Wageningen Centre for Food Sciences and Laboratory of Microbiology, Wageningen University, The Netherlands.

The gastro-intestinal tract of human and other monogastric animals contains a vast number of microbes that are numerically dominated by gram-positive bacteria and at several sites by Lactobacilli. Recent 16S rDNA-based approaches have shown that the upper intestinal tract contains a variety of *Lactobaclllus* spp., some of which have not yet been cultured, notably at the mucosal interface. This highlights the capacity of *Lactobacillus* species to interact with the host, which is the basis for their application as probiotics in food and feed. To reveal the significance and specificity of these interactions, we have initiated an array of approaches to identify their molecular mechanism in model animals, *in vitro* cell lines and human systems. Results will be presented of several complementary research lines that all exploit functional or comparative genomics approaches. These include the identification, isolation and analysis of a new animal *Lactobacillus* species using specific genomic signatures, comparative studies on the spatial specificity of *L. plantarum* gene expression in model animals and human, and the analysis of the global transcriptional response of germ-free animals, cell-lines and healthy human volunteers to exposure of *L. plantarum* and other *Lactobacillus* spp. The presentation will be concluded by summarizing how these ~omics approaches are shaping our ideas on how *Lactobacillus*-host interactions operate at the mucosal interface.

#2 DEGRADATION OF WHEAT STRAW BY *FIBROBACTER SUCCINOGENES* S85: A LIQUID AND SOLID STATE NMR STUDY.
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Wheat straw degradation by the rumen bacterium *Fibrobacter succinogenes* was studied using NMR spectroscopy and chemolytic methods. This allowed us to investigate the activity of an entire fibrolytic system on an intact complex substrate. *In situ* solid state NMR, ^{13}C CP MAS (cross polarisation magic angle spinning) was used to monitor the modification of the composition and the structure of lignocellulosic fibres (^{13}C-enriched wheat straw) during growth of the bacteria on this substrate. There was no preferential degradation either of amorphous regions of cellulose versus crystalline ones, or of cellulose versus hemicelluloses in wheat straw. This suggests either simultaneous degradation of the amorphous and crystalline parts of cellulose and of cellulose and hemicelluloses by the enzymes, or degradation at the surface, in the molecular scale, that cannot be detected by NMR. Liquid state 2D NMR experiments and chemolytic methods were used to analyse in detail the various sugars released in the culture medium. Integration of NMR signals enabled the quantification of oligosaccharides produced from wheat straw at various times of culture and showed the sequential activity of some of the fibrolytic enzymes of *F. succinogenes* S85 on wheat straw. In particular, acetylxylan esterase appeared more active than arabinofuranosidase, which was more active than alpha-glucuronidase. Finally, cellodextrins did not accumulate to a great extent into the culture medium.

#3 CONTRIBUTION OF *SELENOMONAS RUMINANTIUM* TO FIBER DIGESTION IN THE RUMEN. S. Sawanon, S. Koike, and Y. Kobayashi. Graduate School of Agric., Hokkaido Univ., Sapporo, 060-8589, Japan.

Some strains of Selenomonas ruminantium possess endoglucanase and xylanase activities, implying partial contribution to fiber digestion. Active flagella make this species highly motile and help the bacterium go inside plant tissues. In fact, S. ruminantium is often detected as a member of fiber-associated bacterial community by 16SrDNA analysis. We describe here new phylogenetic groups of S. ruminantium, one of which significantly contributes to rumen fiber digestion. Nineteen strains of S. ruminantium were isolated from sheep and characterized in phylogeny and ecology by molecular approaches (sequencing and real-time PCR). Physiological evaluation was made by traditional approaches. New isolates were classified into 3 clusters (Ia, Ib and II). Cluster Ia containing type strain and all known strains exhibited a wide range of activities in fiber attachment and fiber-digesting enzymes. Strains in new cluster Ib showed high levels of CMCase production and fiber attachment. Another new cluster II comprised strains with low CMCase activity but high fiber attachment. Ruminally incubated grass hay showed that adherent cell numbers is largest in Ia followed by Ib and II. Cluster Ib showed max population size on the hay at 24 h and its time course was well synchronized with that of Fibrobacter succinogenes, suggesting that this group develops in close relationship with F. succinogenes. Addition of S. ruminantium to F.succinogenes (co-culture) improved digestibility of grass hay. This improvement was greater for Ib and Ia strains than II strains. Such improvement was not seen for legume hay. The new cluster Ib might act as potent symbiotic group with F. succinogenes in grass fiber digestion. Two new clusters from sheep rumen were detected at similar levels in cattle and deer rumens.

#4 QUANTIFICATION OF *FIBROBACTER SUCCINOGENES* CELLULASE AND XYLANASE GENE EXPRESSION IN THE RUMEN OF A GNOTOBIOTIC LAMB BY REAL-TIME RT-PCR. C. Béra-Maillet, A. Kwasiborski, P. Mosoni and E. Forano, Unité de Microbiologie, Institut National de la Recherche Agronomique, CR de Clermont-Fd/Theix, 63122 St-Genès-Champanelle, France.

Cellulolytic bacteria like *Fibrobacter succinogenes* play a major role in the degradation of plant cell wall polysaccharides in the ruminants by producing several glycoside hydrolases (GH). More than 35 different GH genes have been identified in the genome of *F. succinogenes* S85 but the role and importance of each enzyme in the fibrolytic system of this bacterium is still unknown. We have previously shown, using real-time RT-PCR, that several fibrolytic genes were more expressed *in vivo* in the rumen content of gnotobiotic lambs, than *in vitro* in pure cultures.

The relative importance of each GH produced by *F. succinogenes in vivo* can be estimated by quantifying and comparing the expression rates of the corresponding GH genes. In this study, the transcript abundance of 4 cellulase genes (*cel3, endAFS, celF*, *endB*), a cellodextrinase gene (*cedA*), and two xylanase genes (*xynC, xynD*) were quantified using RT-PCR in the rumen content of a gnotobiotic lamb fed with a lucerne diet and inoculated with S85 *F. succinogenes* as the sole cellulolytic bacterial strain. *tuf* (transcriptional unit factor) mRNAs were also quantified to standardize and compare the expression levels of target genes in several total RNA extracts. The *cel3* cellulase gene and the *xynC* and *xynD* xylanase genes were the most expressed (2.10^6 copies per µg of cDNA, $4.9.10^6$ copies and $2.4.10^6$ copies, respectively). The *cedA* cellodextrinase gene was expressed at a lower level ($1.3.10^6$ copies per µg of cDNA) and the other genes were 10 times less expressed. These results confirmed the cooperation of *F. succinogenes* cellulases and xylanases in the degradation of plant cell walls.

#5 DETECTING CHANGES IN BACTERIAL AND PROTOZOAL POPULATIONS IN RUMINAL AND OMASAL SAMPLES FROM COWS FED SUPPLEMENTAL METHIONINE. S.K.R. Karnati, J.T. Sylvester, Z. Yu, N.R. St-Pierre and J.L. Firkins. Department of Animal Sciences, The Ohio State University, Columbus, Ohio 43210.

Methionine supplemented as 2-hydroxy-4-methylthiobutanoic acid (HMB) has been suggested to alter ruminal bacterial and protozoal populations in the rumen. Our objective was to determine if source of Met induces changes in microbial populations in the rumen and to compare those results to samples from the omasum of cattle fed control, dl-Met, HMB, or the isopropyl ester of HMB (HMBi; estimated 50% rumen protection) in a 4 x 4 Latin square design using denaturing gradient gel electrophoresis (DGGE) and ribosomal intergenic spacer length polymorphism (RIS-LP). For DGGE, a hypervariable region of the rRNA gene was amplified using PCR with primers specific for protozoa and bacteria. Amplicons were separated on an 8% polyacrylamide gel with a 30-36% denaturing gradient for protozoa and 7.5% polyacrylamide gels with a 40-70% gradient for bacteria. Neither the protozoal counts nor banding patterns were significantly different among treatments or for ruminal vs. omasal samples. The DGGE profiles of bacteria clustered together by treatment in both rumen and omasal samples. For RIS-LP of bacterial populations, PCR amplicons were separated on a 4% polyacrylamide gel. Similar to DGGE, bacterial RISA banding profiles clustered together by treatments in both rumen and omasal samples. Thus, source of Met appeared to change ruminal bacterial but not protozoal populations. The similar results in the rumen vs. omasum support our previous findings using a protozoal rRNA-based assay that there is minimal selective retention of protozoal genera between the rumen and omasum (i.e., the reticulum) in dairy cattle and supports ruminal or omasal sampling for assessing bacterial populations or for collection of bacterial standards to quantify ruminal outflow of bacterial N.

#6 BOVICIN HC5, A NOVEL BACTERIOCIN FROM *STREPTOCOCCUS BOVIS.* J.B. Russell, H.C. Mantovani, A.J. Houlihan, M.D. Flythe and B.M. Xavier, ARS/USDA and Dept. of Microbiology, Cornell University, Ithaca, NY 14853.

Many gram-positive bacteria produce small peptides that have antimicrobial activity, and bacteriocins have been used for a variety of applications. Several species of ruminal bacteria are known to produce bacteriocins. Because the bacteriocin, nisin, and monensin had similar effects on ruminal fermentation, bacteriocins have been proposed as an alternative to this antibiotic. However, the quest for suitable bacteriocins has been stymied by at least 3 factors: 1) some bacteriocins are highly specific and only have a narrow spectrum of activity, 2) many bacteria can become bacteriocin-resistant and 3) some bacteriocins are highly unstable or difficult to purify. *Streptococcus bovis* HC5 produces a bacteriocin (bovicin HC5) that circumvents all of these problems. Bovicin HC5 is a pore forming lantibiotic that has only 23 amino acids, is stable at high temperatures (121ºC) and is not degraded by some proteinases. Bacteria repeatedly exposed to sub-lethal doses did not become significantly more resistant. Bovicin HC5 has maximal activity at acidic pH values, but bacterial competition experiments indicated that it could have significant effect at pH values typical of the rumen. Bovicin HC5 is a cell-associated bacteriocin, but it can be liberated from the cell by acidic NaCl. This treatment does not cause significant cell lysis and gives a nearly purified product. Bovicin HC5 appears to be chromosomally encoded. Mutants in bovicin HC5 were obtained with the insertion sequence ISS1, but the antibiotic markers did not remain in the chromosome and this approach could not be used to locate the gene. The amino acid sequence of bovicin HC5 is similar to a bacteriocin produced by *S. pyogenes*, and primers to a *S. pyogenes* immunity protein amplified an immunity protein from *S. bovis* HC5. Because immunity proteins and bacteriocin structural genes are typically contiguous, we are confident that the structural gene of bovicin HC5 will be identified.

#7 LIVE RECOMBINANT LACTIC ACID BACTERIA FOR USE IN HUMAN HEALTH. Annick Mercenier, Nestlé Research Center, Department of Nutrition and Health, Lausanne, Switzerland.

Dietary lactic acid bacteria (LAB) are mostly known for their widespread use in the production of fermented foods. As such, they have been consumed by humans since time immemorial, and they have obtained the "Generally Recognized As Safe" status. Some members of this diverse family of microorganisms are part of the natural indigenous microflora that populates the different mucosal cavities in healthy humans and animals. Specific strains have been studied for their health-promoting properties with a special interest in their beneficial effect on relieving gastro-intestinal disorders and on the host immune system. Over the last 15 years, LAB have been developed as live carriers for the mucosal delivery of antigens, allergens, interleukins, enzymes or bioactive biopeptides. Targeted diseases included intestinal, vaginal or respiratory infections, allergy, chronic intestinal inflammation and enzymatic deficiencies. Most of the genetic work has been achieved in *Lactococcus lactis*, *Lactobacillus* spp. and *Streptococcus gordonii*. The biological impact of recombinant strains producing a molecule of medical interest, or mutated in a specific gene, is increasingly evaluated in mouse models mimicking the targeted disease. Notable recent developments in the area include the construction of safe biologically-contained strains such as the recombinant *L. lactis* strain secreting human IL-10 that has been accepted by a Dutch ethical committee for use in a small human clinical trial in Crohn's Disease patients. Genome sequences are now available for more than 20 LAB and post-genomic analyses, which are no longer restricted to the study of pathogenic microorganisms, are accelerating the investigation of the mechanisms underlying the host-microbe interactions.

#8 OXALATE CONSUMPTION BY COMMERCIAL PROBIOTICS. R. Cox and S. L. Daniel, Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920.

The intestinal microbiota plays a major role in the regulation of oxalate metabolism in humans and animals. To date, most studies have focused on *Oxalobacter formigenes*, an anaerobic oxalate-degrading bacterium common to the mammalian gut. While much less is known about the oxalate-degrading abilities of other intestinal microbes, recent evidence suggests that some species of *Lactobacillus* and *Bifidobacterium* can consume oxalate and that the oral administration of these probiotic microbes to humans can impact urinary oxalate levels. Based on these findings, the goal of the present study was to determine if commercially available probiotics are capable of oxalate consumption. Seven different commercial probiotic products for humans (*Saccharomyces boulardii*, Jarro-Dophilus, Acidophilus Plus, Super Potent Acidophilus, Kyo-Dophilus, 4x6 Acidophilus, and VSL#3) and two different commercial probiotic products for pets (Pet Inoculant and Fastrack) were screened by transferring the manufacturer's recommended dose of the probiotic product (usually two capsules) to 25 ml of anaerobic culture medium which contained oxalate (10 mM), minerals, metals, yeast extract (0.1%), and a bicarbonate/CO_2-buffer system. Cultures were mixed for 1 hr to dissolve the probiotic product, incubated at 37°C for 48 hrs, and then analyzed by HPLC for the disappearance of oxalate. Jarro-Dophilus, Acidophilus Plus, Super Potent Acidophilus, Kyo-Dophilus, 4x6 Acidophilus, and Pet Inoculant cultures consumed very little of the oxalate (0-1%); *S. boulardii* and Fastrack cultures consumed 4.5 and 8% of the oxalate, respectively. In contract, VSL#3 cultures consumed all (100%) of the oxalate; furthermore, VSL#3 cultures actively consumed oxalate under both anaerobic and aerobic conditions. The exact nature of the oxalate-consuming activities of VSL#3 is presently unknown.

#9 PROBIOTICS FOR ARCTIC RUMINANTS. K.E. Præsteng[1], S.D. Mathiesen[2], R.I. Mackie[3], I.K.O. Cann[3] and M.A. Sundset[1], [1]Dept. of Arctic Biology, University of Tromsø, 9037 Tromsø, Norway, [2]Section of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, 9292 Tromsø, Norway, [3]Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL, USA.

Reindeer (*Rangifer tarandus tarandus*) in northern Norway are semi-domesticated, free-ranging animals that feed on natural pastures and are exposed to large seasonal variations in abundance and quality of forage. Supplementary feed is used to avoid starvation in winter when ice and snow occasionally cover the pastures. This may cause digestive problems. Probiotic treatment of reindeer during transition from starvation to supplementary feeding is likely to help in such situations, and may also have a positive impact in early weaning of calves. The bacterial composition of the reindeer rumen is influenced by its natural diet, and is different from that of domesticated ruminants. It is not desirable to introduce new bacteria to this unique system of which much is still unknown. We want to develop a probiotic based on bacteria naturally occurring in the reindeer rumen, and are currently screening rumen bacteria isolated from healthy reindeer. Sequencing and phylogenetic analysis of 16S rDNA sequences from 14 rumen bacterial isolates revealed that three of these isolates were not significantly related at the species level to known bacteria (>97%), suggesting that they may represent novel species. Their closest relatives were *Eubacterium ventriosum* (94.2% identity to R2-25), *Actinomyces oricola* (94.4% identity to R6-1) and *Butyrivibrio hungatei* (95.6% identity to R7-26). Three fibrolytic strains are presented as potential candidates for a reindeer probiotic. Strain R8-9 and R8-30 were both CMC-active and cellulolytic on isolation. Sequencing and phylogenetic analysis of 16S rDNA confirmed a 99% identity to the 16S rDNA of *Pseudobutyrivibrio ruminis* and 90% identity to *Butyrivibrio fibrisolvens*. Strain R8-32 was cellulolytic on isolation, and its closest relative is *Ruminococcus flavefaciens* (98% 16S rDNA sequence identity). The functional traits of these isolates are currently under evaluation.

#10 FIBROLYTIC BACTERIA FROM THE FORESTOMACH OF KANGAROOS. D. Ouwerkerk[1], A. V. Klieve[1], A. J. Maguire[1] and R. J. Forster[2], [1]Department of Primary Industries and Fisheries, Animal Research Institute, Yeerongpilly Qld Australia and [2]Lethbridge Research Centre, Agriculture and Agri-Food, Lethbridge, Alberta, Canada.

The geographic isolation of Australia has meant that large domestic herbivores were not present on the continent when Europeans arrived approx 200 years ago. Marsupials evolved to fill the niche occupied predominantly by ruminants elsewhere, and like ruminants; the macropodid marsupials (kangaroos) developed an enlarged forestomach for fermentation of cellulosic and other complex plant materials prior to further digestion. We are interested in the fibrolytic bacteria associated with the kangaroo foregut as they may have a greater affinity for native pasture grass fibre and a large proportion of Australia's sheep meat and beef industries are reliant upon the use of native pastures as the primary feed source.

Forestomach contents were collected from kangaroos feeding on native Mitchell grass pastures in Western Queensland. Initially 67 fibrolytic bacteria were isolated on a range of substrates including ball milled cellulose, birchwood xylan, oatspelt xylan and the neutral detergent fibre fraction (NDF) of Mitchell grass (a native pasture). The number of isolates investigated was reduced to 37 based on their ability to digest the various substrates. These isolates had their 16S gene PCR amplified, restriction enzyme digested and grouped based on the resulting patterns obtained. The 16S gene of a number of these isolates has been sequenced to determine their identity. An experiment was conducted to compare selected isolates with *Ruminococcus albus* AR67 for their ability to digest pasture grasses including Mitchell grass, Spear grass and Rhodes grass.

Amongst the isolates there are a number with a very high affinity for cellulose and hemi-cellulose that digested native pastures at least as well as *R. albus*. Of the two groups of kangaroo strains with the highest affinity for crystalline cellulose 16S sequencing revealed that one was related to *R. flavefaciens*. The other group was more distantly related but still within the genus *Ruminococcus*.

#11 ANALYSIS OF NOVEL ARCHAEAL AND BACTERIAL DIVERSITY ASSOCIATED WITH GALAPAGOS LAND AND MARINE IGUANAS. **M. Mori[1], B. Merchen[1], M. C. Wikelski[2], I. K. O. Cann[1] and R. I. Mackie[1], [1]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801; [2]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544609.**

The broad objective of this study is to characterize the microbial diversity in the gastrointestinal tract of two herbivorous iguanas endemic to the Galapagos archipelago, land iguanas (*Conolophus pallidus*) that feed on terrestrial plants and marine iguanas (*Amblyrynchus cristatus*) that consume macrophytic algae growing in the tidal zone. *Archaeal diversity:* Comparative DNA sequence analysis of 16S rDNA was undertaken to determine archaeal diversity. A total of 57 (land iguana) and 149 (marine iguana) clones were screened by RFLP analysis. Phylogenetic analysis of representative 16S rDNA groups revealed that all sequenced clones fell into two new clusters related to methanogenic archaea. The first cluster segregated with *Methanocorpusculum* spp., originally described in environmental samples. The second cluster was related to an, as yet uncultured, archaeal group retrieved from an anaerobic digester and hydrocarbon- and chlorinated-solvent-contaminated aquifer. This study demonstrates the presence of an unusual archaeal community associated with herbivorous iguanas in this unique evolutionary location. Despite the vast difference in habitat and diet, the same two clusters of methanogens were detected in both species suggesting that these methanogens are indeed iguana specific. *Agar-degrading bacteria*: Genotypic analysis of a range of cultivable, agar degrading bacteria was carried out. Phylogenomic analysis of the 16S rDNA revealed three distinct clusters of Clostridia. The first group clustered within Clostridium Group IV (92% similarity to *Faecalibacterium prausnitzii/Anaerofilum pentosovorans* branch) and the remaining two groups clustered within Clostridium Group XIVa (*C. herbivorans* and *C. phytofermentans* groups). These are the first representatives of the LGCGP bacteria with agarase activity. *Sulfate-reducing bacteria*: In order to verify the presence of SRB we have used a PCR approach that targets the APS reductase (*aps*A) gene, a useful phylogenetic marker for SRB. All sequences recovered were associated with classical gram-negative, lactate-utilizing SRB, *Desulfovibrio termitidis*, *D. vulgaris*, *D. desulfuricans* and *Desulfomonas pigra*. Attempts to cultivate representative strains are underway.

#12 REASSESSMENT OF THE HUMAN INTESTINAL MICROBIOTA - FROM PHYLOGENETICS TO METAGENOMICS. **Joël M. Doré and Marion Leclerc, INRA, Unité d'Ecologie et Physiologie du Système Digestif. 78352 Jouy-en-Josas Cedex, France.**

During the past century, recognition of anaerobiosis and the development of anaerobic culture techniques allowed the isolation and characterization of a large bacterial diversity from the dominant human fecal microbiota. It is commonly reported that over 400 species compose this microbiota.

Nevertheless, a large fraction of the dominant gut microbes remains unculturable and most recent evaluations converge towards 70% uncultured cells from fecal dilutions. Today, the long awaited culture independent tools that dramatically developed during the past decades have been allowing a complete reassessment of human gut microbial diversity. Molecular tools have their own biases and limitations, yet they allow the recognition of a myriad of species bearing no cultured representative in currently available strain collections. Comparative sequence analysis of cloned 16S rDNA has proven especially adapted. We will review the information derived from this approach that sets the stage for future perspectives.

On a molecular basis, 80% of the phylotypes observed in the fecal microbiota of healthy young adults belong to four phylogenetic groups: the Gram negative *Bacteroides-Porphyromonas-Prevotella* cluster, low-GC Gram positives of the phylum Firmicutes, belonging to the *Eubacterium rectale-Clostridium coccoides* (cluster XIV) phylogenetic group and to the *Fusobacterium prausnitzii-Clostridium leptum* (cluster IV) and high-GC Gram positives of the *Bifidobacterium* and *Collinsella-Atopobium* groups. Other approaches such as fluorescent in situ hybridization have confirmed this observation.

Further, 80% of the phylotypes (60% of the cloned rDNA sequences) derive from microorganisms that have no culturable representative. This is especially true for the Firmicutes. The proportion of yet un-recognized species present in an individual's gut microbiota increases from birth to the old age. Accordingly, specificities of the gut microbiota associated with ageing or intestinal disorders such as inflammatory diseases can be outlined.

Comparison of several 16S rDNA based molecular inventories indicates that the dominant fecal microbiota is essentially specific to its host at the species level. High throughput methods also inform us of its resistance to modification and its marked resilience following stress.

Considering the expected functional homogeneity of the human intestinal ecosystem, it may be speculated that a true functional redundancy will be evidenced between individuals. Yet it is not clear at this stage whether this is to be found at the level of the gut microbiota genome, proteome or metabolites. We will review the preliminary results derived from the investigation of the metagenome of the human faecal microbiota.

#13 HOST AND INTESTINAL MICROBIOTA NEGOTIATIONS IN THE CONTEXT OF ANIMAL GROWTH EFFICIENCY. H. Rex Gaskins, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.

The gastrointestinal tract is a dynamic ecosystem composed of an organized matrix of host eukaryotic cells, including a fully functional immune system, and numerous microbial habitats colonized by a diverse array of commensal bacterial species. Indeed, all animals have, and seemingly require, long-term cooperative associations with indigenous bacteria in the GI tract. As the first tier of defense, the normal microbiota interferes with intestinal colonization by pathogens. Proposed mechanisms include direct competition for nutrients or mucosal attachment sites, or through alteration of the local growth environment via the production of antimicrobial compounds, volatile fatty acids, and chemically modified bile acids. Commensal gut bacteria also indirectly benefit the host by stimulating development of mucosal defenses including the mucus layer, the epithelial monolayer, and the underlying lamina propria, home of multiple immune cell types. This is best exemplified by the alterations of both innate and acquired components of mucosal defense in germfree animals including reduced epithelial and mucous cell development, decreased lymphocyte numbers, skewed intestinal lymphocyte and antibody profiles, and underdeveloped Peyer's patches and mesenteric lymph nodes lacking germinal centers and plasma cells. These alterations are quickly reversed when germfree animals are colonized with indigenous bacteria. Similarly, successional changes in the intestinal microbiota during the neonatal period correlate with the ontogenic development of innate and acquired components of mucosal defense. Such interplay generally ensures intestinal homeostasis. However, two examples will be described in which the negotiated balance between the host and its intestinal microbiota creates inefficiencies in the context of productive growth of piglets and broiler chicks.

#14 RACTOPAMINE SUPPLEMENTATION DECREASED FECAL SHEDDING OF *E. COLI* O157:H7 IN NATURALLY-INFECTED BEEF CATTLE. T.S. Edrington[1], T.R. Callaway[1], K.J. Genovese[1], C.L. Schultz[1], T.H. Welsh[2], L.A. Soliz[2], S.B. Schroeder[2], R.C. Anderson[1] and D.J. Nisbet[1], [1]USDA-ARS-FFSRU and [2]Texas A&M University, College Station, TX 77845.

The synthetic β-agonist ractopamine (RAC) improves carcass quality and animal performance. The physiological counterparts to synthetic β-agonists are the catecholamines, hormones involved in a bacterial quorum-sensing system that regulates virulence gene expression and stimulates growth of *E. coli* O157:H7 (EHEC). We conducted a study in which yearling beef cattle, naturally-infected with EHEC received RAC, based on our hypothesis that RAC may either directly, or via elevated catecholamines, increase gut populations and fecal shedding of EHEC. Twenty animals were grouped in an outdoor pen, fed a 80% concentrate ration, and randomly assigned to one of two treatments (10 hd/trt): Control (empty capsule) or RAC (20 mg/hd) dosed daily for 28 days. Animals were restrained in a squeeze chute for administration of treatments, fecal collection and blood sampling. Intermittent shedding patterns of EHEC were observed throughout the study regardless of treatment, with a gradual decline in the number of shedders occurring as the study progressed. Overall, the percentage of cattle shedding EHEC over the 28-d period was lower ($P = 0.006$) in the RAC (41.8%) compared to Control (58.2%) treatment. No differences ($P = 0.87$) were observed during the first week of the study, however during the second ($P = 0.002$) and third ($P = 0.006$) weeks, the percentage of cattle shedding EHEC was lower in the RAC treatment. This same tendency ($P = 0.08$) was observed during the fourth week, and while the same percentage of shedders vs non-shedders was observed as in week 2, the difference was not significant due to the decrease in the overall number of animals shedding during week 4. Plasma catecholamine concentrations were similar ($P > 0.10$) among treatments on days 0, 14, and 28. While results of this preliminary study have exciting food safety implications, considering RAC is designed to be fed the last 28 days prior to slaughter, further research is needed to confirm the observations reported herein.

#15 THE EFFECTS OF LARCH EXTRACT ON PERFORMANCE AND FECAL FLORA IN NURSERY PIGS.
J.E. Wells and J.T. Yen, USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933.

The swine industry is under pressure to minimize synthetic antimicrobial growth promoters in diets. Larch extract is predominantly a polymer of arabinogalactan containing up to 20% taxifolin, which exhibits antibacterial properties. To determine the potential benefit of larch extract in young swine diets, 192 nursery piglets were sorted by weaning weight into one of four treatment diets: 1) no growth promoters (negative control, NC); 2) Mecadox + copper sulfate (positive control, PC); 3) 0.1% larch extract (LE); or 4) 0.2% larch extract and were fed for a 4-week period. The PC group consumed significantly more feed over the 4-week period relative to the other three diet groups (200 g vs 183 g per animal, $P < 0.05$), but did not gain significantly more weight (110 g vs 105 g per animal, $P > 0.1$). Gain to feed was significantly lower at week 2 for the PC group relative to all treatments, but 0.1% LE was significantly higher (0.64 g/g) than the PC or NC groups (0.58 and 0.61 g/g, respectively). No effect on gain to feed was observed from week 2 to week 4. At week 4, fecal *Lactobacillus* spp., coliforms, and *Escherichia coli* were similar (approximately 8.56, 4.99, and 4.87 $\log_{10}$ CFUs per g feces, respectively) for NC and both LE groups. However, for the PC diet group, the *Lactobacillus* spp. CFUs (7.59 $\log_{10}$) were nearly 1 log lower, and the coliform and *Escherichia coli* CFUs (6.02 and 6.02 $\log_{10}$, respectively) were more than 1 log greater than the other three treatments. Fecal shedding of pathogens (*Salmonella* spp. and *Campylobacter* spp.) was lowest for the LE groups (15%) and highest for the NC group (25%). From this study, larch extract may be a beneficial feed additive to nursery swine at less than 0.2% of diet.

#16 COCCIDIA-INDUCED MUCOGENESIS PROMOTES *CLOSTRIDIUM PERFRINGENS* GROWTH AND SEVERITY OF DISEASE IN A CHICK MODEL OF NECROTIC ENTERITIS.
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This study tested the hypothesis that the host-derived mucogenic response to intestinal coccidial infection predisposes to the onset of necrotic enteritis (NE) due to *Clostridium perfringens*' ability to utilize mucus for growth. A chick NE infection model was used in which birds (0 – 28 days of age; $n = 7$ birds/treatment/day) were inoculated with *Eimeria acervulina*, *E. maxima*, and *C. perfringens* (EAM/CP). A second group was comprised of EAM/CP-infected birds treated with the ionophore narasin (NAR) from days 1 - 28. These treatment groups were compared to birds that were: 1) noninfected (NIF); 2) infected with *C. perfringens* (CP) or 3) *E. acervulina* and *E. maxima* (EAM). *Clostridium perfringens*-specific qPCR and V3-16S rDNA PCR-DGGE data demonstrate that NAR reduced ($P < 0.05$) *C. perfringens* growth and toxin A production at days 20 and 22 compared to EAM/CP-infected birds. These results correlate with an absence of necrotic lesions and decreased mortality in NAR-treated birds relative to the other infection groups. Culture-based techniques, in which ileal mucosal scrapings were grown on mucin-limiting, habitat-simulating medium, demonstrated that NAR reduced ($P < 0.05$) the density of mucolytic bacteria in general and *C. perfringens* specifically at days 20 and 22, and this response correlated temporally with decreased ($P < 0.05$) IL-10 and IFN-γ rDNA expression as determined by qPCR. Moreover, histochemical analysis indicates that goblet cells are larger ($P < 0.05$) in size in EAM/CP-infected relative to NIF birds. These data provide evidence that overt coccidial infection may increase mucus production, promoting *C. perfringens* growth via its mucolytic properties and thereby contribute to the onset of NE.

#17 EVALUATION OF DIETARY LACTOSE ON THE MICROBIAL ECOLOGY IN POULTRY WITH NECROTIC ENTERITIS. J. L. McReynolds, J. A. Byrd, S. E. Duke, L. F. Kubena, and D. J. Nisbet. USDA/ARS/SPARC, Food & Feed Safety Research Unit, College Station, TX 77845.

The commercial poultry industry uses a wide variety of management tools to control pathogens including antibiotics, vaccines, and competitive exclusion cultures. The industry has used growth promoting antibiotics (GPA) to target gram-positive organisms which are associated with lower levels of performance and health. One target organism controlled with GPA is *Clostridium perfringens,* the etiologic agent of Necrotic enteritis (NE). Due to increasing consumer pressure to remove GPA from the market, our laboratory is currently evaluating the effects of dietary lactose on the microbial populations of the GI tract in the disease condition of NE. Four populations of bacteria along with clinical signs were evaluated to determine the effects of the dietary lactose. Day-of-hatch broilers were assigned to one of the following groups containing 45 birds / treatment: negative control (0%), 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5% dietary lactose; fed from day one until termination of the experiment (d 21). *Clostridium perfringens* (10^7 cfu / mL) was administered once daily via oral gavage to the birds for three consecutive days starting on day seventeen. In the control group, 100% of the birds showed clinical signs of NE compared to the 1, 1.5, 2.0, and 2.5% lactose treatment groups whose clinical signs were significantly reduced ($P < 0.05$, N=10) to 66, 66, 50, 22% for the respective groups. It is our conclusion that a 2.5% lactose dietary additive is optimal in reducing the clinical signs of NE. When comparing the chance of a bird developing a clinical infection at 1, 2, or 4% lactose relative to 2.5%, it is estimated that there was a 13, 4.5, 49 greater chance of developing a clinical infection respectively. Lactobacilli, *E. coli,* Clostridia, and *Enterococcus* were monitored, but there was no significant association between these bacteria and NE in this model. With further research, this technology could provide the industry with a viable alternative that can immediately be used in current commercial production operations in reducing the clinical infection of Necrotic enteritis.

#18 EVALUATION OF PERFORMANCE AND PCR-DGGE ANALYSIS OF CECAL MICROFLORA OF NATUSTAT-SUPPLEMENTED TURKEYS CHALLENGED WITH *HISTOMONAS MELEAGRIDIS*. Sinéad M. Waters[1], Cepta F. Duffy[1], Michael D. Sims[2] and Ronan F.G. Power[3]. [1]Alltech Ireland Ltd., Sarney, Summerhill Rd., Dunboyne, Co. Meath, Ireland. [2]Virginia Diversified Research Corporation, Harrisonburg, VA 22801, USA. [3]Alltech, Inc., 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA.

Histomoniasis is a disease of turkeys on litter or range caused by the fragile protozoan *Histomonas meleagridis*, a parasite of worms, primarily spread in feces, in *Heterakis gallinarum* (cecal worm) eggs or in *Eisenia foetita* (earthworms). Symptoms include poor feed conversion ratio (FCR) and body weight (BW), diarrhea, cecal and liver lesions, darkening of the facial regions and sometimes death.

Nitarsone is used worldwide as an aid in the prevention of histomoniasis. Natustat™ (Alltech, Inc., Nicholasville, KY), a proprietary plant derived product is a natural alternative for the prevention of histomoniasis disease in poultry. In this trial, Natustat™, was used at 1.925kg/Tonne and compared with nitarsone in male Hybrid turkey diets to 42 d of age on histomad infected litter (d 7) from broiler breeders. Infected and uninfected, non-supplemented control groups were also included.

On d 28, 35 and 42 of the trial, 16 birds from each group were euthanized. Cecal contents were removed, lyophilised and pooled by group for each sampling day. These samples were subjected to a molecular culture-independent methodology; polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE), to identify shifts in cecal microbial populations between the different groups. In this technique, DNA was extracted from the pooled lyophilised cecal samples and a hypervariable V3 region of the 16S rRNA gene was amplified by PCR. Resultant amplicons were subjected to DGGE, from which a profile was generated due to differences in nucleotide base composition within the 16S rRNA gene fragments. Sequence analysis was performed on subsequent bands using the Basic Local Alignment Search Tool (BLAST) sequence analysis package online at the National Centre for Biotechnology Information (NCBI) homepage (http://www.ncbi.nlm.nih.gov).

Notable shift in the DGGE profiles were observed between the different groups. It was concluded that DGGE profiles could be used to provide an extensive ecological view of shifts in gut microbial communities such as cecal contents, circumventing biases imposed by culturing.

#19 FERMENTATION PRODUCTS IN COLON HEALTH: MEDIATORS OF CELL KINETICS AND GENE EXPRESSION. N.D. Turner, C.A. Warren, K.J. Paulhill, L.M. Sanders, M.Y. Hong, K.L. Covert, L.A. Davidson, R.S. Chapkin, and J.R. Lupton, Faculty of Nutrition, Texas A&M University, College Station, TX 77843.

Colonic epithelial cells derive nutrients and signaling molecules from both the external (luminal) and internal sources. The relative abundance and distribution within the population of microflora in the colon lumen are determined by the fermentable substrates available. The continuing dynamic between presentation of various dietary residues and microflora changes influences colonic health through the fermentation products. The most routinely studied fermentation product is butyrate because it is the preferred metabolic substrate of colonocytes. However, butyrate directly influences cell cycle progression by altering histone deacetylase activity and recruitment of transcription factors to Sp1 and Sp3 DNA binding sites. Butyrate thereby regulates gene expression in a manner dependent upon concentration and the normal/neoplastic status of colonocytes. The ability of butyrate to regulate cell cycle activity and/or apoptosis induction through changes in gene expression has warranted the extensive amount of research conducted to date. Yet, there are other fermentation products that may influence colonocyte health, and we are only now starting to appreciate their presence and potential impact. Many of these "bioactive compounds" become available because the microflora are capable of cleaving side-chains that otherwise inhibit their absorption. Bioactive compounds are capable of influencing a variety of signaling cascades in addition to their routinely reported antioxidant potential. It will be necessary to apply the same level of effort that developed our current understanding of the role of butyrate in colon health before we are able to fully appreciate the potential impact of these compounds individually and more importantly in concert with the other compounds present in the luminal milieu. Supported by USDA (2003-34402-13647), NIH (CA61750, CA82907, CA59034), NSBRI (NASA NCC 9-58), NIEHS P30ES09106.

#20 FATTY ACID METABOLISM, SENSITIVITY AND MECHANISM OF BUTYRATE FORMATION ARE LINKED IN DIFFERENT *BUTYRIVIBRIO* ISOLATES. R. J. Wallace[a], D. Paillard[a], N. McKain[a], L. C. Chaudhary[a], N. D. Walker[a], N. R. McEwan[a], I. Koppova[b], J. Kopecny[b] and P. E. Vercoe[c], [a]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [b]Institute of Animal Physiology & Genetics, Prague, Czech Republic; [c]Animal Science Group, University of Western Australia, Crawley, WA 6009, Australia.

Butyrivibrio forms a genetically diverse group of Gram-positive bacteria isolated mainly from the rumen of cattle and sheep. *Butyrivibrio* isolates are particularly important in fatty acid biohydrogenation, as they biohydrogenate linoleic (LA) and linolenic acids much more rapidly than other ruminal species. Here, 69 different *Butyrivibrio* isolates from different countries were grouped in a phylogenetic tree derived from 16S rDNA sequence data. Their position was then compared to their metabolic activity. Two clear sub-groups were evident, both phylogenetically and metabolically. Group A typified most *B. fibrisolvens* and *Pseudobutyrivibrio* isolates, while Group B contained isolates related to *B. hungatei* and *Clostridium proteoclasticum.* All produced butyrate, but Group A bacteria had a butyrate kinase activity <40 U/mg protein, while Group B were all >600 U/mg protein. The butyrate kinase gene was present in all Group B bacteria tested but not in Group A. Lipase activity, measured by tributyrin hydrolysis, was high in Group B and low in Group A, without exception. LA isomerase activity (forming CLA), on the other hand, did not correspond with phylogenetic position. Group A bacteria all grew in the presence of 200 μg/ml LA, while Group B bacteria were sensitive to concentrations as low as 5 μg/ml. A sub-group of Group B bacteria containing *C. proteoclasticum* formed abundant stearate under specific growth conditions, whereas Group A did not. Thus, *C. proteoclasticum* is probably the same as the '*Fusocillus*' isolated more than 25 years ago – the only ruminal bacterium then known to hydrogenate linoleic acid to stearic acid.

#21 LINOLEIC ACID BIOHYDROGENATION BY HUMAN INTESTINAL MICROORGANISMS AS A MIXED FLORA AND AS ISOLATED BACTERIAL STRAINS. E. Devillard, F.M. McIntosh and R.J. Wallace, Rowett Research Institute, Bucksburn, Aberdeen, UK.

Conjugated linoleic acids (CLA) confer a broad range of health-promoting properties in experimental animals, including prevention of cancer, heart disease and stimulation of immune response. The main dietary sources of CLA in the human diet are ruminant products. CLA are formed in the rumen as intermediates in the biohydrogenation of linoleic acid (LA; C18:2). LA is isomerized to CLA, which is then hydrogenated to *trans*-vaccenic acid (TVA; C18:1) then to stearic acid (SA; C18:0). The likely importance of TVA as a precursor of CLA in man has also been demonstrated recently. Thus, CLA and TVA of ruminal origin have been the focus of much research activity. Here, we demonstrate that human fecal bacteria have a biohydrogenation activity which is comparable to that of the rumen microflora, with individual colonic species also having activities similar to related ruminal species. LA metabolism by rumen mixed flora and by colonic mixed flora were compared by incubating ruminal fluid or fecal samples for 4 h with LA. Total fatty acids were extracted every hour and analysed by gas chromatography. The results were very similar between the two types of samples. Perhaps significantly, samples from a human subject on a vegetarian diet showed a much higher concentration of biohydrogenation intermediates (TVA and oleic acid) than samples from omnivorous subjects. Some human bacterial isolates selected for their ability to produce butyrate were analysed for LA biohydrogenation. Three of the 8 strains studied converted LA extensively to TVA. Three strains of bifidobacteria were also studied: only one strain metabolised LA and produced CLA as end product. Its activity was much lower than the butyrate producers. These results suggest that, if LA can be delivered as a precursor to the large intestine in man, the endogenous flora might form CLA and TVA that could be useful in suppressing inflammatory bowel disease. Simultaneous provision of a probiotic based on human butyrate producers such as *Roseburia* spp. might enhance the formation of CLA and TVA throughout the digestive tract.

#22 QUANTITATIVE MONITORING OF DISSEMINATION OF TETRACYCLINE RESISTANCE GENES FROM MANURE LAGOONS INTO GROUNDWATER UNDERLYING THE SWINE FARMS. S. Koike[1], H. D. Oliver[1], I. G. Krapac[2], J. C. Chee-Sanford[3] and R. I. Mackie[1], [1]Dept. of Animal Science and [3]Dept. of Crop Science, University of Illinois, Urbana, IL 61801, [2]Illinois State Geological Survey, Champaign, IL 61820.

Antibiotics are routinely used in the livestock industry to treat disease and to promote growth. However, antibiotics can select for resistant microorganisms in the gastrointestinal tract of animals, providing a potential reservoir for dissemination of antibiotic resistant bacteria into other animals, humans and the environment. In this study, we carried out long term monitoring of tetracycline resistance genes (Tc^r) in groundwater underlying two swine farms to track the dissemination of resistance genes into the environment. Monitoring wells were established around the lagoon at two different Illinois swine farms: 16 wells at site A and 6 wells at site C. Quarterly samples of groundwater (n = 124) and waste lagoons (n = 12) were collected from both sites from 2000 through 2003. Total DNA was extracted and PCR used to detect seven Tc^r (*tet*MOQWCHZ). The concentration of Tc^r was quantified by real-time qPCR. Comparative analysis of *tet*(W) gene sequence was performed on groundwater and lagoon samples to confirm the *tet* gene source in groundwater. All seven Tc^r persisted in groundwater during the 3-yr monitoring period at both sites. At site A, the level of detection frequency and concentration for Tc^r was correlated with chloride concentration which is an indicator of contamination from manure waste in the lagoon. This result indicates that seepage from the waste lagoon influences the distribution of Tc^r in groundwater underlying site A. Comparative analysis for *tet*(W) sequence revealed that the impacted groundwater contained almost identical gene sequences (99.9% identity) with that found in lagoon. This result supports the dissemination of Tc^r from lagoon into groundwater. However, the concentration of *tet*(C) was markedly higher than that of *tet*(MOQWHZ) in groundwater, conversely in the lagoon *tet*(MOQW) were dominant, suggesting differential dissemination of Tc^r into the environment.

#23 QUANTIFICATION OF GENES ENCODING TETRACYCLINE RESISTANCE IN MICROBIAL COMMUNITIES BY REAL-TIME PCR ASSAYS. Zhongtang Yu[1], Frederick Michel[2], Glenn Hansen[3], Tom Wittum[3], and Mark Morrison[1]. [1]The MAPLE Research Initiative, Dept of Animal Sciences, [2]Dept of Food, Agri. and Biol. Engineering, and [3]Dept of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH 43210.

Quantitative measurements of antimicrobial resistance reservoirs in microbial communities are required to understand its ecology (such as emergence, persistence, decline and dissemination). We report here the development, validation and use of three real-time PCR assays to quantify the abundance of three groups of tetracycline resistance genes: *tet*(A) and tet(C); *tet*(G); and *tet* genes encoding ribosomal protection proteins (RPP) including *tet*(M), *tet*(O), *tet*B(P), *tet*(Q), *tet*(S), *tet*(T), and *tet*(W). The assays were validated using known numbers of sample-derived *tet* gene standards added to microbiome DNA. These assays are both precise and accurate over at least six logs of *tet* gene copies. New *tet* genes were also identified from cloned *tet* amplicons as part of this study. The utility of these real-time PCR assays was demonstrated by quantifying the three *tet* gene groups present in bovine and swine manures, composts of swine manure, lagoons of hog house effluent, and samples from an Ekokan upflow biofilter system treating hog house effluents. Bovine manures were found to contain fewer copies of all three groups of *tet* genes than swine manures. Composts of swine manures had substantially reduced *tet* gene abundance (by up to six logs), and lagoon storage or the upflow biofilter had little effect on *tet* gene abundance. These results suggest that the methods of manure storage and treatment may have a substantial impact on the persistence and decline of tetracycline resistance originating from food animal production systems, thus affecting the dissemination of such resistance to the environments. These real-time PCR assays provide a rapid, quantitative and cultivation-independent measurement of ten major classes of *tet* genes, which should be useful for ecological studies of antibiotic resistance.

#24 FODDER LEAVES RICH IN TANNIN, SAPONIN AND NON-STARCH POLYSACCHARIDE MODULATES RUMEN FERMENTATION *IN VITRO*. M. R. Amin*, N. Selje, E. M. Hoffmann and K. Becker, Institute for Aquaculture Systems and Animal Nutrition in the Tropics and Subtropics, University of Hohenheim, Fruwirthstr. 12, D-70599 Stuttgart, Germany.

Leaves of unconventional plants are increasingly recognized as high quality feed resources and are a potential source of protein supplements for ruminants. Many of them contain secondary compounds that reduce nutritional value but with a better understanding of their properties those compounds may become useful to increase the proteins and the essential amino acids available for absorption. This study is aimed at investigating fermentation patterns *in vitro* with special emphasis on protein protection in the rumen.

Fourteen plants often fed to ruminants in Bangladesh were screened and five of them were selected. Leaves from *Syzygium cumini* are rich in total tannins (TT), *Artocarpus heterophyllus* in condensed tannins (CT), *Acacia nilotica* in hydrolysable tannins (HT), *Sesbania acumulata* in saponins (SAP) and *Eichhornia crassipes* in non-starch polysaccharides (NSP). Leaves were incubated with bovine rumen fluid and buffers (75ml) using the Reading Pressure Technique. The substrate (835mg) was composed of maize silage, barley grain, soybean meal, BSA and ground leaves replacing silage (inclusion 27%). Monensin (3μM) served as an external control. Protein degradation kinetics (dot-blot, SDS-PAGE), ammonia (nitroprusside), gas and SCFA (GC) at 1, 3, 6, 9 and 12h were investigated.

TT, CT and HT protected protein from ruminal fermentation by precipitating soluble protein, while monensin conserved proteins in soluble form. Protein precipitation capacity was highly correlated with TT determined by ferric chloride assay and was highest in *S. cumini*. Ammonia and iso-SCFA decreased strongly with TT, similar to monensin, slightly with CT and HT and increased with SAP and NSP. Gas production was decreased with all the additives, the effect being highest with TT, which also caused the strongest decrease in SCFA. The efficacy of leaves to maximise protein protection with minimum inhibition of carbohydrate fermentation needs comprehensive investigation. Detailed studies are in progress.

#25 EFFECT OF INTRARUMINAL NITROETHANE ADMINISTRATION ON METHANE-PRODUCING ACTIVITY AND VOLATILE FATTY ACID PRODUCTION *IN VIVO*. R. C. Anderson[1*], G. E. Carstens[2], T. R. Callaway[1], C. L. Schultz[1], T. S. Edrington[1] and D. J. Nisbet[1], [1]USDA/ARS, SPARC, FFSRU, College Station, TX and [2]Dept. of Animal Science, Texas A&M University, College Station, TX.

Strategies are sought to reduce economic and environmental costs of ruminal methane production. The effect of nitroethane on methane-producing activity and volatile fatty acid (VFA) production was evaluated in two ruminally cannulated Holstein cows maintained on rye grass pasture. Each cow was administered 120 mg nitroethane/kg body weight per day for 8 d. Nitroethane was administered intraruminally as the sodium salt in two equal sized portions (08:00 and 16:00). Methane-producing activity was determined by *in vitro* incubation (39ºC for 3 h) of rumen fluid or feces (5 or 2 g, respectively) in anaerobic buffer (5 or 10 ml, respectively) containing 60 mM sodium formate, 0.2 g ground alfalfa and a H_2:CO_2 (1:1) atmosphere. Ruminal methane-producing activity was reduced 2.9, 2.3, 2.0, 4.7, 5.8, 7.6 6.1, 2.7 and 1.4-fold from pretreatment measurements (0.44 ± 0.01 micromol CH_4/g rumen fluid per h) at 2, 8, 16, 24, 48, 72, 96, 168 and 192 h of treatment, respectively. Thus, nitroethane markedly reduced ruminal methane-producing activity within the first 4 d of treatment but was diminished by d 8 of treatment. In contrast, fecal methane-producing activity differed little from pretreatment measurements (0.17 ± 0.10 micromol CH_4/g feces per h) at 8 and 16 h of treatment but was increased 9.9, 5.6 and 7.7-fold from pretreatment measurements by 48, 72 and 96 h of treatment, respectively. By 168 and 192 h of treatment, fecal methane-producing activity was only 0.5 and 1.1-fold higher than pretreatment measurements. These data suggest an initial but temporary displacement of methanogenic bacteria from the rumen to the lower gut. Ruminal VFA concentrations, determined by gas chromatography, fluctuated during the course of the study but molar proportions of acetate to propionate differed little throughout the study. Thus, electrons produced during fermentation were not disposed of via production of more reduced fermentation acids.

#26 MICROBIAL DEGRADATION OF OXALATE IN THE GASTROINTESTINAL TRACTS OF CATS AND DOGS. T. N. Talarico, T. Millis, and S. L. Daniel, Department of Biological Sciences, Eastern Illinois University, Charleston, IL 61920.

Urinary tract stones are a common ailment of cats and dogs. Most of these stones consist of calcium oxalate. Calcium oxalate stones form when urinary oxalate levels become elevated. Urinary oxalate is derived from the diet or is endogenously formed. However, one factor that has not been addressed in cats and dogs is the impact of intestinal oxalate-degrading microbes on urinary oxalate excretion. In humans, intestinal oxalate-degrading bacteria decrease the absorption of dietary oxalate, thereby reducing urinary oxalate levels. *Oxalobacter formigenes* is responsible for oxalate degradation in the mammalian intestinal tract. Furthermore, the absence of this anaerobic bacterium from the human gut has been linked to increased urinary oxalate excretion and an increased risk for stone formation. The goal of this study was to determine if oxalate-degrading microbes are present in the gastrointestinal tracts of cats and dogs. Fecal samples from 5 cats and 20 dogs were collected without environmental contamination and immediately processed by homogenizing 5 grams of feces in 50 ml of anaerobic dilution solution. Fecal homogenates were used to inoculate anaerobic culture media containing 10 mM oxalate; some fecal cultures were also supplemented with 10 mM nitrate or sulfate. Fecal cultures were incubated at 37°C and analyzed at regular time intervals by HPLC for the microbial consumption of oxalate. After 15 days of incubation, oxalate consumption was not detected in any of the fecal cultures tested, indicating the absence of anaerobic oxalate-degrading microbes in the gastrointestinal tracts of cats and dogs examined. In contrast, when aerobic culture media containing 10 mM oxalate was inoculated with fecal homogenates derived from three of these dogs, microbial consumption of oxalate was detected. In aerobic fecal cultures, oxalate was completely consumed within 5 days. The nature of the organisms responsible for this consumption and the potential roles that these aerobic oxalate-utilizing microbes might be playing relative to canine oxalate metabolism are presently being investigated.

#27 CALCIUM REQUIREMENTS FOR GROWTH AND CELLULOSE DEGRADATION BY RUMEN BACTERIA. Maria Sol Morales and Burk A. Dehority, Dept. Animal Sciences. OARDC, The Ohio State University, Wooster, OH.

To establish the ionized calcium (Ca^{+2}) requirement for growth (G) and cellulose degradation (CD) by rumen bacteria, the principal cellulolytic rumen bacteria were used: *F. succinogenes* (FS) A3c and S85, *R. albus* (RA) 7 and 8, and *R. flavefaciens* (RF) B34b and C94. They were incubated in complete liquid media with cellobiose for G experiments and complete media with cellulose for CD experiments; both varied in Ca^{+2} concentrations. G was measured by OD (600 nm) and CD by substrate disappearance. Results from both processes were adjusted by a logistic function, using nonlinear regression (PROC NLIN-SAS); for G: max growth (MG), rate of growth (RG) and lag time (LT), and for CD: extent of degradation (MD), rate of degradation (RD) and LT coefficients were obtained. Bacterial species responded differently to the Ca^{+2} treatments. For FS, MG did not differ ($P>0.05$), RG increased with increased Ca^{+2}, and LT tended to be longer for A3c at lower [Ca^{+2}]. Surprisingly, RF C94 showed higher MG and RG as [Ca^{+2}] decreased. G of RA 7 and RF B34b were not affected by [Ca^{+2}]. FS showed an absolute requirement of Ca^{+2} for CD, with A3c being more sensitive to this cation. MD, RD and LT were significantly affected by [Ca^{+2}] in A3c and S85 ($P<0.05$). Also, FS showed high variation of CD, perhaps due to interactions of G and CD; therefore, NH_3-free complete cellulose media were used to separate the G effect on CD; the results confirmed the higher Ca^{+2} requirement of FS for CD. RA and RF did not require Ca^{+2} for CD. A logistic function could not be fixed for RA 8, due to the slow G and CD. Due to the lack of response to Ca^{+2}, RF was tested for other specific cation requirements. These showed RF has an absolute Mg requirement for G. When different [Mg] were used in complete liquid media with cellobiose, to evaluate G requirements, C94 showed higher requirements than B34b. FS and RA also were evaluated for Mg requirements for G, showing differences among strains. Ca^{+2} and Mg requirements differ among rumen bacteria species and strains and also by function (G or CD).

#28 EFFECTS OF VARIOUS METHODS USED TO PROCESS SOYBEAN MEAL ON PROTEIN DIGESTION IN THE RUMEN AND SMALL INTESTINE. M. D. Stern[1], M. Ruiz Moreno[1] and C. A. Macgregor[2], [1]Dept. of Animal Science, University of Minnesota, St. Paul, MN 55108, [2]Grain States Soya, Inc., West Point, NE.

A three-step procedure was used to evaluate the effects of soybean meal processing on ruminal crude protein (CP) degradation and intestinal CP digestion. Residue from 16 h in situ ruminal incubation simulated ruminal undegradable protein (RUP), and was incubated for 1 h in a 1 N HCl solution with 1g/L of pepsin, simulating abomasal CP digestion. After incubation, pH was neutralized and a pH 7.8 phosphate buffer with 3 g/L of pancreatin added, then incubated for 24 h at 38 °C, simulating intestinal digestion. This procedure was used to evaluate seven soybean meal (SBM) products including solvent-extracted SBM (SE), mechanical-extracted (ME) SBM #1 with fresh soy gums (ME1G), ME SBM #2 (ME2), ME SBM #3 (ME3), ME SBM extruded (MEE), SE heat treated (SEH), and SE nonenzymatically browned (SENB). Ruminal undegradable CP (RUP) was 23.2, 49.3, 42.1, 33.4, 38.3, 52.3 and 68.3% for SE, ME1G, ME2, ME3, MEE, SEH and SENB, respectively. Intestinal CP digestion (ID) was 67.5, 83.8, 78.9, 75.7, 76.5, 65.4 and 57.7% for SE, ME1G, ME2, ME3, MEE, SEH and SENB, respectively; indicating that processing can overprotect protein from digestion in the small intestine. Intestinally absorbable dietary protein (IADP), calculated as RUP x ID was 15.7, 41.3, 33.2, 25.2, 29.3, 34.2 and 39.4% for SE, ME1G, ME2, ME3, MEE, SEH and SENB, respectively. The range of IADP was 15.5% for SE to 41.3% for ME1G, however it is interesting to note that SENB was next highest at 39.4% because of a high RUP value that compensated for lower intestinal digestion. These results demonstrate that it is important to account for RUP and intestinal CP digestion when selecting a SBM source to include in the ruminant diet.

#29 THE EFFECT OF DIET ON THE POPULATIONS OF RUMEN METHANOGENS AND CILIATE PROTOZOA IN RED DEER. M. J. Nicholson, N. D. Walker, P. Evans and K. N. Joblin, Rumen Microbiology, AgResearch Grasslands, Private Bag 11008, Palmerston North, New Zealand.

Methane production in the rumen contributes to greenhouse gas emissions and represents a loss of dietary energy to the animal. Methanogenic archaea are directly responsible for methane production in this environment and ciliate protozoa are indirectly involved because of the symbiotic associations which some rumen ciliates form with methanogens. We have analysed changes in ciliate and methanogen populations in farmed red deer before and after dietary transition between ryegrass and plantain. Five rumen-fistulated deer were allowed to adapt to specific forages by grazing on appropriate pasture and were then maintained indoors for rumen sampling over five consecutive days. In the first sampling period, two animals received plantain whereas the other three received ryegrass. The diets were then reversed and after re-adaptation the animals were returned indoors for a second sampling period.

Digesta contents were serially diluted and methanogens enumerated in selective growth medium. In all five animals, the number of culturable methanogens was approximately ten-fold lower whist eating plantain as compared with ryegrass. Agar roll tubes were inoculated from dilution tubes and single colonies of methanogens were picked, further purified and identified using SSU rDNA sequence data. Methanogens of the *Methanobrevibacter* genus were the most predominant for both diets. Examination of ciliate cells by epifluorescence microscopy revealed that both endo- and ecto-symbiotic methanogens were associated with them. After fixation of the rumen samples detailed counts were made of the ciliate species and genera present. Ciliates belonging to eight different genera were identified and distinct population shifts were seen after dietary transition with some genera which were prevalent on one diet having population sizes at least ten-fold lower on the other. This study suggests that forages may have the potential to influence ruminal ciliate and methanogen populations and could therefore lead to grazing options for controlling ruminant methane emissions.

#30 EFFECT OF PARTICLE SIZE ON PASSAGE RATES FROM THE RUMEN TO THE DUODENUM OF LACTATING DAIRY COWS. A. B. Peterson[1], R. L. Baldwin, VI[2] and R. A. Kohn[1]. [1]Dept. of Animal and Avian Sciences, College Park, MD 20742. [2]Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville, MD 20705.

The objectives were to determine the effect of particle size and sampling sites (reticulum and duodenum) on passage rates from the rumen. Samples were collected from 8 ruminally and duodenally cannulated lactating Holstein cows every 4 h for 48 h over 4 periods (n=32). Samples were poured through sieves measuring 2.0, 1.0, 0.5, and 0.25 mm. Fractions were composited over 48 h for each cow. The average DM of duodenal samples was 4.89% (SEM=0.067) as compared to 3.21% (SEM=0.046) for the reticulorumen sample ($P < 0.01$) indicating water and VFA removal between the two sampling sites. For duodenal samples, 48% of DM was in the liquid fraction while 5, 11, 14, and 26% of DM was on the 0.25, 0.5, 1.0 and 2.0 mm sieves respectively. For reticulum samples, the particles contained a lower fraction of DM while the liquid fraction contained 76% of DM. On a DM basis it appears that large particles were under-sampled in the reticulum and/or over-sampled in the duodenum. The NDF% of the duodenal sample was less than that of the reticulum ($P < 0.01$; 60 vs. 75%) suggesting that at least one of the sampling sites was not representative of total flow. When NDF for each sieve was expressed as a fraction of total sample NDF no difference was found between reticulum and duodenal samples for each sieve. These results suggest that although samples were not representative of flow on a DM basis, reticulum and duodenal samples were similar after removal of cell contents. Based on duodenal samples, 48% of DM flow was in the liquid phase and 26% was retained on the 2 mm sieve. There is a potential to estimate flow using reticulum samples in place of duodenal samples.

#31 AN ANALYSIS OF THE CONTROL OF THE VOLATILE FATTY ACID PROFILE IN THE RUMEN. E. M. Ungerfeld and R. A. Kohn, Dept. of Animal & Avian Sciences, University of Maryland, College Park, MD 20742.

The ruminal volatile fatty acid (VFA) profile affects the efficiency of energy and carbon capture in end products useful to the host animal, energy capture in microbial ATP, and the host's metabolism. Carbon in pyruvate or phosphoenolpyruvate originated in glycolysis is diverted towards acetate, propionate or butyrate. The present analysis deals with the question of why VFA ratios are held within a relatively narrow range. A kinetic control of the VFA profile would be dictated by the microbial species present and their enzymatic activities. Thermodynamics would play a role in controlling the VFA profile if the VFA are in equilibrium. As acetate, propionate and butyrate have common intermediates, there is thermodynamic interconnection among end products. Interconversion among VFA in the rumen has been documented and measured in vitro and in vivo. We summarized results of published experiments that measured interconversion flows between VFA. Fourteen out of 17 ratios of interconversion flows between acetate and propionate, and 15 out of 16 between acetate and butyrate were within one order of magnitude of equilibrium. There was generally some net flow of acetate towards butyrate. There was no unequivocal evidence of the type of diet or animal species influencing the equilibria. Fractional rates of conversion of acetate into propionate and viceversa varied amply, but somewhat less so for acetate and butyrate. Even though this hypothesis still cannot explain the VFA shifts that occur when changing types of diets, these results suggest that commonly observed VFA proportions in the rumen could be a result of thermodynamic equilibria controlling the diversion of carbon towards the different end products.

#32 CHARACTERIZATION OF MICROBIAL POPULATIONS OF IN VITRO RUMINAL PROTOZOAL CULTURES BY 16S rDNA SEQUENCE ANALYSES. T. R. Whitehead[1] and B. A. Dehority[2], [1]USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL 61604, and [2]Dept. of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691.

Previous reports have suggested that in vitro maintenance of ruminal protozoa requires the presence of a bacterial population. Whether the bacteria are used as a source of food or alternatively provide a nutritional factor is as yet unclear. Little is known about the bacterial populations that are present in single species or clone cultures of ruminal protozoa, and if these populations vary between different protozoal species. Antibiotic treatment has been used previously by a number of researchers to reduce or eliminate the bacterial populations of in vitro cultures to determine the effects on the protozoa. For both species used in the present study, incubation with antibiotics had no immediate effect on protozoal concentrations. However, washing the cells and transferring half the culture to fresh medium several times did not prevent death of the protozoa by 8-12 days. We have initiated a study of the bacterial flora present in cultures of *Entodinium caudatum* and *Epidinium caudatum* with and without antibiotic treatment (penicillin and streptomycin). Bacteria from cultures were recovered and total DNA prepared. DNA sequence analyses of PCR amplified 16S rDNA genes derived from eubacterial primers were carried out (40 sequences/culture). Similarity analyses of the 16S rDNA sequences indicated the presence of both Gram-negative and Gram-positive anaerobic bacteria from all cultures. Bacterial diversity, as measured by the number of Operational Taxonomic Units (OTUs), was much less in the *Entodinium caudatum* cultures as compared to the *Epidinium caudatum* cultures. Almost all of the OTUs could not be identified to known bacteria when compared to 16S rDNA sequences in the GenBank database. Antibiotic treatments resulted in a shift towards more Gram-positive OTUs with both cultures. These results indicate that the bacterial flora varies between protozoal species, and antibiotic treatment results in a shift in the bacterial populations.

#33 EVALUATION OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* SURVIVAL IN THE ENVIRONMENT. K. Cook[1], J. Britt[2], and A. Pike[2], [1]USDA-ARS and [2]Western Kentucky University, Bowling Green, KY (270)781-2579.

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease, a chronic, enteric infection that is passed insidiously from adults to calves via the fecal-oral route. MAP has become the focus of unwanted attention due to its increased prevalence and the economic impact resulting from decreased milk production and the need to replace culled animals. MAP awareness has also risen due to its possible association with Crohn's disease in humans. Johne's disease has been detected on the agricultural research farm at Western Kentucky University, and although eradication plans have been in place for the past 4 years, there has been no reduction in the number of clinical cases. There is now a cooperative effort underway to attempt to eradicate the disease through optimization of best management practices and determination of site-sources of the organism. The goal of this study was to evaluate MAP incidence in dairy cattle as well as in potentially infected areas on the farm (e.g. cattle alleyways, barns, storage facilities). A PCR assay was developed to target the IS900 insertion sequence, a sequence specific for MAP. Molecular analysis of fecal and agricultural samples was conducted and positive results were obtained for three different cattle samples and from bedding in a contaminated birthing stall. Amplification of the correct product was confirmed by DNA sequence analysis of cloned PCR products. Standards were established for use in quantitative real-time PCR analysis. Further cattle fecal and farm-source samples will be obtained and quantified to detect MAP. Results to date suggest that molecular analysis should permit detection of key sources of the pathogen and provide fundamental new information that will aid in eradication of the disease.

#34 PHYLOGENETIC ANALYSIS OF RUMEN BACTERIAL POPULATIONS IN REINDEER. M. A. Sundset[1], I.K.O. Cann[2], S.D. Mathiesen[3] and R.I. Mackie[2], [1]Dept. of Arctic Biology, University of Tromsø, 9037 Tromsø, Norway, [2]Department of Animal Sciences, University of Illinois at Urbana-Champaign, IL, USA, [3]Section of Arctic Veterinary Medicine, The Norwegian School of Veterinary Science, 9292 Tromsø, Norway.

Little has been published on the phylogenetic diversity of the gut microflora of wild herbivores, particularly those few inhabiting the pristine areas of the Arctic. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) have not been domesticated and are isolated under austere nutritional conditions on the high-arctic archipelago of Svalbard (74-81°N), while the semi-domesticated Norwegian reindeer (*R. t. tarandus*) in northern Norway, migrate between lush coastal summer pastures and inland winter pastures on mainland Norway including large amounts of lichens in their diet. We suggest that interactions between reindeer and arctic plants such as lichens, shrubs, herbs, sedges and mosses through 15 million years of evolution have resulted in a unique rumen microbial ecosystem. Phylogenetic analysis on rumen bacterial diversity based on PCR amplification and sequencing of 16S rDNA clone libraries demonstrate the presence of novel sequences not previously described and highlights the large diversity that exists. From Svalbard reindeer 31 16S rDNA sequences (1.5 kb) were obtained, including representatives of three bacterial phyla: *Firmicutes* (*Clostridiales*, n=17), *Bacteriodetes* (*Bacteroidales*, n=13) and *Cyanobacteria* (*Deferribacteriales*, n=1). None of these sequences showed >97% identity to any previously cultivated species, but a 98% identity to the 16S rDNA of unclassified rumen bacteria from domestic cattle were shown for 2 of the clones. In the 16S rDNA clone library from Norwegian reindeer, 34 clones were obtained from animals on natural summer pasture and 56 clones from animals fed a concentrate diet. In the library from free-ranging animals 67.6% of the sequences belonged to the low G+C Gram-positive bacteria, compared to 91.1% in the artificially fed reindeer. Likewise, 32.4% belonged to *Cytophaga-Flavobacter-Bacteroides* bacteria in the first library compared to only 7.1% in the second library.

#35 PHAGE IN THE GUT ECOSYSTEMS OF GRAZING LIVESTOCK. N.D. Walker and K.N. Joblin. Rumen Microbiology Group, AgResearch Grasslands, Palmerston North, New Zealand.

Bacteria, archaea, protozoa, fungi, mycoplasmas and bacteriophages all interact in a complex manner to breakdown ingested plant material in the gut of grazing animals. Phage have the potential to significantly impact on this fermentation process through the attack and lysis of bacteria. Consequently the possibility of using phage therapy as a natural alternative to antibiotics to manipulate gut fermentation is under investigation. However, surprisingly little is known about the phage populations in the rumen or hindgut, or about the factors affecting their population densities. The aim of the present work was to obtain information on phage populations in the gut of animals grazing pasture.

Rumen samples were removed in the morning and afternoon from 3 rumen-fistulated sheep and 2 rumen-fistulated cows grazing ryegrass/clover pastures on 2 days, 1 week apart. Caecal (hindgut) samples were removed from 2 euthanased horses which had previously been grazing pasture. Phage were extracted and examined by transmission electron microscopy. Analysis showed the presence of very diverse populations which contained phage morphologically similar to previously described bacteriophage. PFGE fingerprints were generated from ruminal phage DNA extracts. Most phage genomes were found to be between 20 and 200 kb in size. In some instances, fingerprint patterns changed significantly within a period of a few hours and intense bands indicative of lytic or lysogenic phage blooms were observed. These differences in banding patterns demonstrated a temporal effect, with the phage population composition changing over time, thus having the potential to impact upon the populations of their bacterial hosts. Gel densitometry analysis showed that all the gut samples examined contained in excess of 10^9 viral particles/gm contents. The phage parameters found in this study are similar to those previously found for ruminal phage in concentrate-fed ruminants.

#36 PLANT OILS AND UREASE INHIBITORS AS SOLUTIONS FOR ENVIRONMENTAL ISSUES ASSOCIATED WITH CONFINED ANIMAL FEEDING OPERATIONS. V.H. Varel and J.E. Wells, USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE 68933.

Air quality and transmission of pathogens are concerning issues for confined animal feeding operations (CAFOs). The objectives of our work are to evaluate urea hydrolysis, VFA production (odor), and fecal coliforms in cattle waste slurries after a urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) and a plant oil of lower cost than thymol, i.e., alpha-terpineol or plinol, are added. Feces from cattle fed a diet of 83% corn were blended with urine and distilled water (50:35:15). Triplicate aliquots were amended with additives, reblended, and poured into 1.6 L wide-mouth jars covered 90% with a lid. Treatments were sampled periodically out to 56 days. Thymol, terpineol, or plinol (2000 mg/kg waste) in combination with NBPT (80 mg/kg waste) did not prevent hydrolysis of urea beyond that of NBPT by itself. Essentially all urea (4.0 g/kg waste) was converted to ammonium after 21 days; whereas, with no NBPT this usually occurs after one day. Fecal coliforms (6.41 $\log_{10}$ CFUs per g, day 0) were immediately eliminated with thymol, and by day 4 with terpineol and plinol. No lactate accumulated in the waste treated with thymol; however, 250 mM lactate accumulated in the terpineol and plinol treatments, and 230 mM in the untreated waste. Minimal VFA (60 mM) accumulated in the thymol treatment, compared to 200 mM in the untreated waste and 80 to 120 mM in the terpineol and plinol treatments. It is concluded that terpineol and plinol may offer lower cost amendments for cattle waste emissions than thymol; and, they allow lactate to accumulate in the waste which keeps the pH lower and further inhibits microbial activity.

#37 USE OF ACIDIFIERS IN FEEDING OF BROILERS AND COMPARISON WITH USE OF VIRGINIAMYCIN. Seyed Mohammad Hashemi, Morteza Beiki. Scientific Member of Qom Agriculture and Natural Resources Research Center Qom, P.O. Box 37185-779, Qom, Iran.

In order to study acidifier usage in broiler diet as growth promotor and comparison them with virginiamycin an experiment was conducted with 4 treatments and 4 replicates with randomized design.Treatments including Galliacid (a commercial acidifier), lactic acid, phosphoric acid and virginiamycin were added to a basal diet (control groupe). 160 one week old chicks were divided to 20 experimental field containing 8 for each pen with the same mass chick weights. Traits measured included feed intake (g/chick/d) and daily gain (g/chick/d) weekly and mortality rate daily. At the end of experiment 2 chicks in each pen were selected for determining intestin microbial total count and PH. Results showed that treatments in weeks 2-3 had better effect on performance. Lactic acid and virginiamycin significantly ($P< 0.05$) increased feed intake in weeks 2-3 and phosphoric acid and virginiamycin significantly ($P< 0.05$) increased daily gain and lactic acid improved feed conversion ratio significantly ($P< 0.05$) compared with control group. In weeks 4, 5, 6 and 7, daily gain and feed conversion ration were not affected by treatments. In weeks 6-7, control groupe and Galliacid had significantly ($P< 0.05$) better feed intake. In whole production period only daily gain was influenced by treatments and virginiamycin had better effects on traits. Intestine PH and microbial count were not affected by treatments. It is concluded that acids used in this experiment were of beneficial in weeks 2 and 3 more than other weeks. Among treatments, virginiamycin had better effects on weight gain and phosphoric acid is the best for feed conversion ratio and from economic viewpoint.

#38 PREVALENCE AND GENETIC TYPING OF *ESCHERICHIA COLI* O157 IN THE RECTOANAL MUCOSAL REGION OF BEEF CATTLE. J. T. Fox, X. Shi and T. G. Nagaraja. Dept. of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS 66506.

Lymphoid follicle-dense mucosa near the rectoanal junction (RAJ) has been implicated as the principle site of colonization of *E*. coli 0157 in cattle. This study was conducted to evaluate the prevalence of *E. coli* 0157 in the mucosal region of the RAJ in cattle and to determine their genetic relatedness to isolates from colonic contents or feces. Colonic contents (n=291) and rectums (n=377) approximately 8 cm in length from the anus, were collected from cattle at slaughter. Rectums were cut open and the feces was sampled with a sterile cotton swab. The mucosal surface of the rectum was cleansed free of visible feces with water and rinsed with saline. The region, 2 to 5 cm proximal to the RAJ, was swabbed with a foam-tipped applicator. Incisions of the mucosa in the same region were made with a sterile scalpel and the submucosa was then swabbed with a foam-tipped applicator. Isolation and identification of *E. coli* O157 from colonic contents, feces and mucosal swabs was carried out by selective enrichment, followed by IMS and plating on selective agar. Prevalence of *E. coli* 0157 in the colonic contents, feces, rectal mucosal surface, and rectal submucosal region was 21, 28, 53, and 34%, respectively. Sixty-one percent of the animals were positive for *E. coli* 0157 in at least one sampling site and was higher ($P < 0.05$) than prevalence in any single site. Pulse-field gel electrophoresis was used to compare clonal similarity among O157 positive isolates from the different sampling regions within animal. Sixty-five animals had O157 positive isolates in the rectal mucosa and the feces of which 54 (83%) were identical (clonal similarity > 95%); whereas 45 animals had isolates in the rectal mucosa and the colonic contents and 28 (62%) were identical. Our results suggest that rectal mucosal swabs were more sensitive in detecting *E. coli* 0157 than the conventional fecal samples and the majority of the RAJ isolates were clonally identical to fecal or colonic content isolates.

#39 IN VITRO GROWTH AND GAS PRODUCTION OF RUMEN BACTERIA AND POTENTIAL BLOAT MITIGATION WITH CONDENSED TANNINS IN WINTER WHEAT. B.R. Min[1], R.A. Anderson[2], and W.E. Pinchak[1]. [1]Texas Agri. Exp. Station, Vernon, TX 76385. [2]USDA-ARS, College Station, TX 77845.

Six strains of ruminal bacteria (*Streptococcus bovis* No 26, *Prevotella ruminicola* ssp. 23, *Eubacterium ruminantium* B1C23, *Ruminococcus albus* SY3, *Fibrobacter succinogenes* ssp. S85, *R. flavefaciens* C94) were used to determine the effect of soluble plant protein from winter wheat forage on specific bacterial growth rate (SGR), bio-film complexes, and gas production (H_2 and CH_4) in pure and mixed cultures with *Methanobrevibacter smittii*. The SGR in GM medium containing soluble plant protein (3.27% N/ml) was measured during 24 h incubation (39 ^oC) in Hungate tubes under a CO_2 gas phase. A pure culture of *M. smittii* was grown similarly, except under H_2:CO_2 (1:1), in a basal methanogen medium supplemented likewise with soluble plant protein. The effect of Quebracho tannins (QT) on the SGR of two proteolytic (*P. ruminicola* and *S. bovis*) strains cultured with or without *M. smittii* was also evaluated. Cultures of *R. albus*, *R. albus*+*M. smittii*, *S. bovis*, and *S. bovis*+*M. smittii* produced the most total gas among strains (10 to 15 cc/24h). As expected, *R. albus* and *R. flavefaciens* produced the most H_2 among strains and supported CH_4 production when cocultured with *M. smitti*. Cultures of *S. bovis* and *E. ruminantium*+*M. smittii* produced the most bio-film complexes among strains. Addition of QT reduced SGR up to 1.8%/h compared to cultures grown without QT (up to 7.6%/h). Specific growth rate of *P. ruminicola* and *P. ruminicola*+*M. smittii* appeared to be more resistant to QT addition than *S. bovis*, *S. bovis*+*M. smittii*, and *M. smittii* alone. Results indicate that *S. bovis* and *E. ruminantium*+*M. smittii* may be major contributors to frothy bloat and the addition of QT reduces SGR, including that of methanogenic bacteria.

#40 MOLECULAR MONITORING OF TYLOSIN- AND ERYTHROMYCIN- RESISTANCE-GENES IN LAGOONS AND GROUNDWATER UNDERLYING TWO SWINE PRODUCTION FACILITIES. H. D. Oliver[1], S. Koike[1], I. G. Krapac[2], J. C. Chee-Sanford[3] and R. I. Mackie[1], [1]Dept. of Animal Science and [3]Dept. of Crop Science, University of Illinois, Urbana, IL 61801, [2]Illinois State Geological Survey, Champaign, IL 61820.

Tylosin and erythromycin are both macrolide antibiotics. Tylosin is commonly used in therapy, prophylaxis and growth promotion on swine farms, whereas erythromycin is reserved for human use. Since these two antibiotics have similar modes of action by binding to the 50S ribosomal subunit, bacterial resistance mechanisms against tylosin and erythromycin are similar. In fact, erythromycin resistance genes are known to confer resistance against tylosin. Therefore, there is a possibility that tylosin usage on swine farms selects for cross-resistance against macrolide antibiotics. In this study, distribution of tylosin- and erythromycin- resistance genes was monitored by PCR to assess the presence of macrolide resistance determinants in the waste lagoons and in groundwater underlying two swine farms. Between 2001 and 2003, lagoon and groundwater samples were collected from two swine farms where tylosin has been used and total DNA was extracted. PCR detection was performed using the primer sets specific for two tylosin resistance genes (*tlrB* and *tlrD*) and six clinically relevant erythromycin resistance genes (*erm*A, *erm*B, *erm*C, *erm*F, *erm*G and *erm*Q). Neither tylosin resistance gene was detected in waste lagoons or groundwater samples from either farm. All six *erm* genes were found in lagoon samples at both farms. Of the six classes of *erm* genes, *erm*B and *erm*C were frequently detected in the total DNA extracted from groundwater. In addition, *erm* genes have also been detected in some of the background control wells located up gradient and distant from the lagoons. However, compared to the control wells, the incidence of these genes was much higher in the lagoons and groundwater from wells that were highly impacted by inorganic compounds from the lagoons. These results support the hypothesis that tylosin usage on swine farms results in the selection of *erm* genes as a means of resistance against tylosin.

#41 EFFECT OF DIETARY SOURCES ON RUMEN ECOLOGY OF SWAMP BUFFALOES (BUBALUS BUBALIS). M. Wanapat, S. Wora-anu, C. Yuangklang, P. Chanjula and O. Puongchompu, Dept. of Anim. Sci., Fac. of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand.

Three trials were conducted to study the effect of various roughage sources on rumen ecology of swamp buffaloes. Trial I, four different roughages; rice straw (RS), urea-treated rice straw (UTS), ruzi grass hay (RGH) and cassava hay (CH, containing condensed tannins) were randomly allotted to four mature male rumen fistulated swamp buffaloes in a 4 x 4 Latin square design. It was found that ruminal bacterial population in UTS (9.2 x 10^{10}cells/ml) was higher ($p<0.05$) than those in RS, RH and CH (8.4, 7.2 and 8.1 x 10^{10}cells/ml, respectively). Protozoal population were lowest in CH treatment ($p<0.05$). Cellulolytic and proteolytic bacteria in UTS (8.73 x 10^{8} CFU/ml and 22.3 x 10^{6} CFU/ml) were higher ($p<0.05$) than those in RS, RH and CH treatments, while total viable and amylolytic bacteria were similar among treatments. Trial II, four, mature rumen fistulated male swamp buffaloes were randomly allocated to receive dietary treatments according to a 4 x 4 Latin square design. Four dietary treatments with varying proportions of urea-treated rice straw (UTS) and cassava hay (CH) were offered (100:0, 75:25, 50:50, 0:100). Ruminal NH_3-N concentrations were significantly ($p<0.05$) raised as higher levels of CH were incorporated into the diets. Moreover, cellulolytic and proteolytic bacterial populations were enhanced while total protozoal counts were decreased ($p<0.05$). The results suggest a favorable effect of CH use with UTS to improve rumen ecology. Triall III, this experiment was to study the effect of roughage and concentrate ratio on voluntary feed intake, digestibility, ruminal fermentation and ruminal microorganisms in cattle and swamp buffaloes. Three, rumen fistulated cattle and swamp buffalo steers, about 3 years of age, were randomly allotted into a 3 x 3 Latin square design. Three dietary treatments were used; treatment I, urea-treated rice straw (100%UTS, T1), treatment II, 60% urea-treated rice straw and 40% extracted rice bran (60%UTS:40%ERB, T2) and treatment III, 40%UTS:60ERB (T3). Ruminal bacterial population in swamp buffaloes fed on 100%UTS (3.2 x 10^{12}cells/ml) was higher ($p<0.05$) than those in other treatments, while protozoal population were lowest ($p<0.05$). Fungal zoospores were similar among treatments. Cellulolytic bacteria in swamp buffaloes fed on T1 (6.8 x 10^{10}CF/ml) was higher ($p<0.05$) than those of T2 and T3 (3.7, 5.2 x 10^{10}CFU/ml), respectively, and was higher than in cattle fed on T1, T2 and T3. Proteolytic bacteria of swamp buffaloes fed on T3 was higher ($p<0.05$) than those in T1 and T2 and for cattle fed on T1, T2 and T3, respectively. Amylolytic bacteria were higher in swamp buffaloes fed on T3 than those in other treatments. Total volatile fatty acids (TVFA); acetic acid and propionic acid were higher in swamp buffaloes than in cattle, while butyric acid was similar among treatments. Based on the above experiments, rumen ecology of swamp buffaloes could be enhanced by dietary manipulations particularly those fed local resources containing condensed tannins.

#42 AMYLASE AND CALCIUM CHELATOR INTERACT DURING NEUTRAL DETERGENT FIBER ANALYSIS. R. A. Kohn, D. S. Brougher, and T. B. Oleas, Dept. of Animal and Avian Sciences, University of Maryland, College Park, Maryland 20742.

Amylase and calcium chelators such as disodium ethylene diaminotetraacetate (EDTA) are used in analysis of neutral detergent fiber (NDF) to dissolve starch and pectin respectively. However, these reagents may interfere with each other's activity. The EDTA binds calcium ions of the calcium-pectin matrix, but calcium is required for activity of amylase. Therefore, the objectives of this study were to test various combinations of amylase and EDTA additions on NDF analysis of selected feed ingredients, and in so doing, propose the best methods for NDF analysis for different types of feeds. Six combinations of alpha-amylase and EDTA were examined for determining NDF values of beet pulp, ground corn, soybean meal, and timothy hay. For treatment A, 2.5 ml amylase were added 5 min after boiling. Other treatments differed as follows: B) 4.5 ml amylase, C) 4.5 ml amylase added 30 min after boiling, D) delayed addition of EDTA to 30 min after boiling, E) no EDTA, and F) no amylase. NDF was determined on ten replicates for each treatment and feed. Ash and N were determined on NDF for 5 replicates of each treatment and feed. Inclusion of EDTA interfered with amylase activity in corn grain samples, and addition of amylase to beet pulp and soybean meal samples reduced effectiveness of EDTA and increased ash in the NDF residue. There was minimal effect of treatments on NDF-N. When using amylase for samples that contain starch, enzyme should be added early during reflux at a high enough concentration to compensate for effects of EDTA, and NDF should be calculated on an ash-free basis to correct for incomplete removal of ash. Amylase should not be used for feeds that do not have much starch, particularly if pectin is present.

#43 BIPLOT ANALYSIS TO DESCRIBE THE RELATIONSHIPS BETWEEN PLANT AND MICROBIAL FATTY ACIDS IN INGESTED HERBAGE. E. J. Kim, R. Sanderson, M. S. Dhanoa and R. J. Dewhurst, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK.

Dacron bags are widely used to estimate rumen degradation. Problems with rinsing losses and microbial contamination are well known. However, these effects also suggest potential to use the technique to study microbial colonisation. This work used multivariate statistical analysis to explore the relationships between plant and microbial fatty acids in ingested herbage. Two *in situ* experiments were conducted using two dry fistulated Holstein-Friesian cows, grazing perennial ryegrass. In experiment 1, the effects of sample preparation method were investigated with perennial ryegrass: (M1) folded and placed into Dacron bags; (M2) chopping into approximately 1 cm lengths using scissors; (M3) crushing with a metal roller, but not chopping; (M4) chopping and crushing; (M5) mechanical chopping for 30 sec; (M6) ingested bolus material, and (M7) freeze-dried and ground. Duplicate bags were incubated in each of two cows for both 2 and 7 h periods. Experiment 2 investigated the effects of different washing procedures after removal of Dacron bags from cows. Approximately 7 g of DM was incubated in each of two cows for 2, 8 and 24 h. On removal, duplicate bags from each cow were washed according to one of four washing procedures: (W1) squeezing of the bag until no more liquid ran out; (W2) gentle agitation in a sink of cold water until there was no further visible loss; (W3) hand washing under a continuous stream of cold water: until the water ran clear; and (W4) machine washing in cold water for 50 min. Fatty acid methyl esters were prepared (methanolic HCl, 5%) and determined by GC. Biplot analysis (GenStat® 7) was used to examine the variation in the major plant and microbial fatty acids in these samples. In both experiments, the concentration of odd-chain fatty acids increased with time of incubation while that of C18:2 and C18:3 decreased. The biplot procedure simultaneously showed variation in fatty acids (86.1 and 86.8% for experiments 1 and 2) and the effects of treatments on that variation. Generally, C18:2 and C18:3 behaved similarly, whilst the odd-chain fatty acids varied in a similar but opposite direction. The effect of the incubation periods, sample processing methods and washing procedures were clearly separated, indicating different degrees of microbial colonisation/contamination.

#44 PHYLOGENETIC ANALYSIS OF A CONSORTIUM OF OVINE RUMINAL MICROBES THAT BIODEGRADES PYRROLIZIDINE ALKALOIDS. R. Rattray, S. Ivey-Lodge, and A.M. Craig, Dept. of Biomedical Science, Oregon State University, Corvallis, OR 97331.

Members of a consortium of sheep ruminal bacteria (designated as L4M2) which degrade pyrrolizidine alkaloids (PAs) were characterized. An enrichment of ruminal bacteria was isolated from a sample of rumen fluid using standard anaerobic techniques. The PA degradation capacity of the enrichment was tested by spiking purified PA extract from tansy ragwort. LH-PCR and RFLP analysis as well as cloning and sequencing analysis were used to identify members of the consortium. Analysis of 16S rDNA revealed differing results based on the molecular method used. LH-PCR identified seven clones of different fragment lengths. RFLP analysis of plated isolates of the consortia revealed five ribotypes. Phylogenetic analysis of sequences from both methods yielded six distinct groups of organisms within three classes of bacteria. The majority of the clone and isolate sequences had less than 90% homology to sequences of known organisms that were used to make phylogenetic comparisons. These data would indicate that this consortium may contain novel organisms which participate in the degradation of PAs.

#45 ENZYMES OF AMMONIA ASSIMILATION IN *RUMINOCOCCUS FLAVEFACIENS* FD-1 AND *RUMINOCOCCUS ALBUS* 8. S.A. Kocherginskaya[1], K.R. Amaya[1], K.E. Nelson[2], M. Morrison[3], I.K.O. Cann[1], and R.I. Mackie[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL 61801, [2]The Institute for Genomic Research, Rockville, MD 20850, [3]The Ohio State University, Columbus, OH 43210.

Ruminococcus flavefaciens FD-1 and *Ruminococcus albus* 8 are closely related bacteria that play key roles in fiber digestion in the rumen. Using a genome walking approach, we sequenced the genes coding for *gltB* and *gltD,* the subunits of *R. flavefaciens* FD-1 glutamate synthase (GOGAT). Interestingly, the genes were located upstream of *gln*A, encoding a glutamine synthetase type I (GSI). The GOGAT and its individual subunits were expressed, but functional activities have not been detected. In the *R. albus* 8 genome, we identified the key ammonia assimilation genes encoding glutamate dehydrogenase (GDH), GOGAT, glutamine synthetase type I and III (GSI and GSIII). Surprisingly, we found downstream of the *R. albus* 8 *glnN* gene (encoding the GSIII) a single gene coding for both an ammonium transporter (AmtB) and a PII-like (GlnK) protein. *glnA* and *glnN* from *R. albus* 8 were cloned, expressed, and assayed for activity. Using both the biosynthetic and the γ-transferase assays, we did not detect enzymatic activity for recombinant GSI, while recombinant GSIII was functional in each assay. Kinetic studies were performed for GSIII using both assays. The apparent K_m for glutamine in the γ-transferase assay was 6.8 mM. Results for the kinetics based on glutamate in the biosynthetic assay, in contrast, did not follow Michaelis-Menten kinetics. Mutations that changed conserved glutamic acid to alanine in four unique GSIII motifs suggested a contribution to structure/function. Unexpectedly, an E380A mutation in motif IV exhibited increased biosynthetic activity over wild type protein. Mutations in a putative nucleotide-binding motif (K308A and K318A) drastically reduced GSIII activity. Northern blot analysis detected GSIII mRNA transcripts in *R. albus* 8 cells grown under ammonia-limiting conditions, while the transcript was almost absent under ammonia sufficient conditions. Of much significance is our finding that *R. flavefaciens* FD-1 and *R. albus* 8 contain bacterial and archaeal type GOGAT, respectively. We hypothesize that *R. albus* 8 acquired its GOGAT through lateral gene transfer in the rumen, a diverse habitat supporting all three domains of life.

#46 EFFECT OF FEED ADDITIVES ON RUMEN FERMENTATION CHARACTERISTICS AND FROTHY BLOAT IN STEERS GRAZING WHEAT PASTURE. B.R. Min[1], W.E. Pinchak[1], J.D. Fulford[1], and R. Puchala[2]. [1]Texas Agri. Exp. Station, Vernon, TX 76385, [2]Langston University, Langston, OK.

Frothy bloat is the major non-pathogenic source of death loss and decreased animal performance in wheat pasture. The potential benefit of monensin (M), poloxalene (P) and Quebracho tannins (QT) as feed additives (FA) to mitigate bloat impacts were determined in a series of in vitro and in vivo grazing Exp. Eight ruminally cannulated steers (386±36 kg/steer) grazed a wheat pasture from Mar. 5 through Apr. 12 and were randomly allocated to four treatments: control (C; no FA), M (170 mg), P (19 g) and QT (150 g/d) added via rumen cannula premixed with textured feed (300 mg/d). Rumen contents were collected 2 h post FA administrated on d – 5, 0, 5, 15, and 22 to measure ruminal protein characteristics. Visual assessments of bloat score (BS) on a scale of 0 (no bloat) to 3 (severe) were made twice a wk. In vitro gas and CH_4 production were measured. The BS was lower for P and QT than for M and C treatments. Among six-rumen protein fractions assayed, steers receiving QT after 10, 15, and 22 d had greater (P<0.05) protein concentration in whole rumen content (WRC), particulate matter, cheese-cloth filtrate (CCF), and protozoa and plant particle fractions than steers fed other treatments. Bacterial and cell-free supernatant (CFS) fractions were similar among treatments. Ruminal DM content, CFS, protozoal and bacterial fractions were similar between the bloated (BS=>1) and non-bloated (BS=0) steers. In bloated animals, ruminal pH was lower (P<0.05), and WRC and CCF protein fractions tended to be greater (P=0.08) than non-bloated animals. Ruminal gas and CH_4 production were similar between C and P, but were lower for M and QT (P<0.05) treatments. The results of this trial suggest that wheat pasture bloat was probably associated with dietary protein and low ruminal pH.

#47 THE *RUMINOCOCCUS ALBUS pilA1-pilA2* LOCUS: EXPRESSION AND PUTATIVE ROLE OF TWO ADJACENT *pil* GENES IN PILUS FORMATION AND BACTERIAL ADHESION TO CELLULOSE. **Harivony Rakotoarivonina[a], Marilynn A. Larson[b], Mark Morrison[c], Jean-Pierre Girardeau[a], Brigitte Gaillard-Martinie[a], Evelyne Forano[a] and Pascale Mosoni[a]. [a]Unité de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France; [b]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, 68198, USA; [c]The MAPLE Research Program, Department of Animal Sciences, The Ohio State University, 2027 Coffey Road, Columbus, OH 43210, USA.**

Ruminococcus albus produces fimbrial-like structures that are involved with the bacterium's adhesion to cellulose. The subunit protein has been identified in strain 8 (CbpC) and strain 20 (GP25) and both are type 4 fimbrial (Pil) proteins. We report here the presence of a *pil* locus that is organized similarly in both strains and our initial examination of a second Pil-protein. Downstream of the *cbpC/gp25* gene (hereafter referred to as *pilA1)*, is a second pilin gene *(pilA2).* Northern blot analysis of *pilA1* and *pilA2* transcripts showed that *pilA1* transcript abundance is much greater in *R. albus* 8, and real-time PCR was used to measure *pilA1* and *pilA2* transcript abundance in *R. albus* 20, and its adhesion-defective mutant D5. Similar to the findings with *R. albus* 8, the relative expression of *pilA1* in the wild-type strain was 73-fold higher than that of *pilA2* following growth with cellobiose, and there were only slight differences between the wild-type and mutant strain in *pilA1* and *pilA2* transcript abundances, indicating that neither *pilA1* nor *pilA2* transcription is adversely affected in the mutant strain. Western immunoblots showed that the PilA2 protein is localized primarily to the membrane fraction, and the anti-PilA2 antiserum does not inhibit bacterial adhesion to cellulose. We suggest that the PilA2 protein plays a role in the synthesis and assembly of type 4 fimbrial-like structures by *R. albus,* but its role is restricted to cell associated functions, rather than as part of the externalised fimbrial structure.

#48 TETRACYCLINE RESISTANCE RESERVOIRS IN DIFFERENT SWINE FARMS AND AT DIFFERENT LOCATIONS WITHIN EACH FARM AS DETERMINED BY REAL-TIME PCR. **Jing Chen[1], Z. Yu[1], Qing Xia[1], Svetlana Kocherginskaya[2], Asma Mehboob[2], R.I. Mackie[2], and M. Morrison[1]. [1]The MAPLE Research Initiative, Dept. of Animal Science, The Ohio State University, Columbus, OH 43210; [2]Dept. of Animal Science, University of Illinois at Urbana-Champaign, IL 61801.**

The use of antibiotics in animal production systems is believed to impact antimicrobial resistance in the environment. The magnitude of such impact was quantitatively assessed using tetracycline resistance as a model and real-time PCR as a tool. The abundance of tetracycline resistance genes (*tet*) was determined in five different swine farms: one organic farm and four conventional farms where chlortetracycline was used for growth promotion. Samples were collected throughout the farms from feeds, swine manures, lagoons and soils amended with the swine manures. The following three groups of *tet* genes were quantified using real-time PCR employing specific primers: *tet*(A,C), *tet*(G) and seven classes of *tet* genes encoding ribosomal protection proteins (RPP) that include *tet*(M, O, P, Q, S, T, W). The RPP *tet* genes were not detectable in any of the feed samples, while *tet*(A,C) and *tet*(G) were found present in four of the five feed samples. More *tet*(A,C) genes (10^5-10^6 copies/g) than *tet*(G) genes (10^3-10^4 copies/g) were found in these feeds. All the conventional farms employ solids separation and lagoons to manage their manures. No significant reduction of *tet* gene abundance was evident in any of the lagoons. The RPP genes were detected in only one soil sample, while *tet*(A,C) and *tet*(G) genes were detected in all the soil samples. Relative to the samples collected from the barns, solids separators, and lagoons, manure-amended soil had a lesser abundance of *tet* genes, which we attribute to dilution effects when manure or lagoon effluents are applied to land. However, relatively greater abundances of *tet* genes were detected in manure-amended soils than in control soils and the organic farm had significantly lesser *tet* gene abundances than the conventional farms. Collectively, these results suggest that: (i) *tet*(A,C) and *tet*(G) genes originating from swine farms can be more readily disseminated into manure-amended soil, and then possibly to a wider environment, than the RPP genes; (ii) swine manure management through solid separation and lagoon storage does not result in effective containment or reduction of antimicrobial resistance originating from animal farms; and (iii) reduced use of antibiotics would reduce the amount of antimicrobial resistance output from animal farms and thus reduce the dissemination of such antibiotic resistance to the environment.

#49 RUMEN DEFAUNATION USING ESSENTIAL OILS. S. Franklin, S. Carlson, M. Rasmussen, National Animal Disease Center, ARS, USDA, Ames, IA 50010.

Research in our laboratory has shown that engulfment and survival within rumen protozoa increases virulence of *Salmonella* strains carrying the DT104 gene cluster. These strains recovered from a mixed population of rumen protozoa following lysis demonstrated an 8-fold increase in virulence as measured by a tissue culture invasion assay (HEp-2 human carcinoma cells). When inoculated into calves, these bacterial strains caused a more rapid disease progression, including pyrexia (105°F), greater recovery of the bacterial pathogen from lymph nodes and spleen, and a less favorable prognosis resulting in premature euthanasia. These observations have implications for disease pathogenesis, fecal shedding of food borne pathogens from ruminants, and pathogen reservoir status of the rumen. Periodic defaunation of the rumen could reduce these implications. Research has shown that plant-derived oils can reduce protozoal numbers, but their effectiveness as defaunation agents in the rumen has not been fully examined. We screened 19 essential oils derived from common culinary spices for anti-protozoal activity using cell integrity and motility as indicators of viability. Of those screened showing substantial anti-protozoal activity, we selected rosemary oil for further study. Increasing concentrations of the essential oil from rosemary decreased protozoal viability and suppressed rumen fermentation as indicated by VFA (volatile fatty acids) production. Of the concentrations tested, 100 ppm had no effect upon VFAs or protozoa whereas 10,000 and 40,000 ppm greatly reduced protozoal viability (90%) and VFA production (70%). Rosemary oil at a concentration of 1,000 ppm was intermediate in effect, resulting in at least a 50% reduction of protozoal viability and a 19% decrease in VFA production.

#50 NOVEL CARBOHYDRATE-BINDING MODULES FROM THE *MANA* GENE OF *THERMOANAEROBACTERIUM POLYSACCHAROLYTICUM*. S. Ohene-Adjei, S. Kocherginskaya, R.I. Mackie, and I.K.O. Cann. Department of Animal Sciences, University of Illinois at Urbana-Champaign.

The mannanase gene *(manA)* from *Thermoanaerobacterium polysaccharolyticum* contains two putative carbohydrate binding modules (CBMs) C-terminal to the catalytic domain (CD). We hypothesized that the two putative CBMs contribute to binding, catalysis, thermostability, and pH tolerance. Six fragments from the *manA* gene (CD+CBM1+CBM2, CD+CBM1, CD, CBM1+CBM2, CBM1 and CBM2) were PCR-amplified, cloned, and expressed. Two fusion constructs (CD+CBM2 and CBM2+CBM1) were also investigated. Proteins were purified using a combination of affinity and anion chromatography and further tested for thermostability (70ºC; 10min), cellulose binding (pH 5.8-8.2), and mannan (locust bean gum, LBG) binding and hydrolysis. Each protein bound to Sigmacell and did not precipitate after heating, except for CBM1 and CBM2+CBM1. The optimum pH for the CBMs binding to cellulose was 5.8-6.2. Binding data suggested a model that saturates a proposed initial binding site followed by binding to secondary sites. There was no difference between the dissociation constants (K_d) for the tandem CBMs regardless of orientation and both had lower K_d compared to individual CBMs. Site directed mutagenesis of CBM2 suggested that, two mutated tryptophan residues were critical for binding. Single mutations resulted in over 60% reduction in binding although mutations at both positions did not further decrease binding. Circular dichroism scan of wild type and mutated CBM2 suggested that, the mutations decreased the level of exposure of aromatic amino acids. Scans for CBM1+CBM2 and CBM2+CBM1 suggested no difference in secondary structure. The CD without a CBM neither bound nor hydrolyzed LBG. However, CD+CBM1+CBM2, CD+CBM1, and CD+CBM2 hydrolyzed LBG. Catalytic activity decreased to a tenth in CD+CBM1+CBM2 and was lost in CD+CBM1 when pH was altered from 5.8 to 7.0. In conclusion, binding of the CBMs was necessary for catalysis. The tandem CBMs increased the affinity for substrate and the CD with either CBM1 or CBM2 was effective for enzyme catalysis.

#51 ANTIPROTOZOAL ACTIVITIES OF 'RUMEN-UP' PLANTS. R. Ningrat, I. Hussy, C. P. Walsh and R. J. Wallace. Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK.

The Rumen-Up project (Ruminal Metabolism Enhanced Naturally Using Plants) is a shared cost research programme funded by the European Commission (FP5 project QLK5-CT-2001-00992). The aim of the project is to explore the potential of new plant species or plant extracts as feed additives for ruminants in order to overcome long-standing problems of animal welfare and environmental damage. A collection of 500 plants and their extracts from species not used previously in animal feeding supplied from industrial and academic sources in Europe was tested for their general effects on rumen fermentation and digestion processes. Among other properties, samples in the collection were screened for their antiprotozoal activities using an *in vitro* bacteriolysis assay. The aim was to suppress protozoal activity, which would enhance microbial protein flow to the intestine. More than twenty samples were identified to have potential for development as natural antiprotozoal agents without having detrimental effects on overall fermentation. The most promising included *Bellis perennis*, *Gentiana asclepiadea, Lonicera japonica, Eugenia caryophyllata, Olea europaea, Aster frikartii, Arbutus unedo, Athyrium filix-femina, Primula florindae, Macleaya cordata, Symphytum officinale* and *Clematis vitalba.* Further detailed experiments were carried out for the most promising antiprotozoal plants i.e. *B. perennis* and *G. asclepiadea* on persistence, dose response, acceptability and toxicity. Differential solvent extraction indicated that saponins were responsible for the effect in *B. perennis.* The active component of the other plants has yet to be established.

#52 RE-EVALUATION OF THE FROTHY BLOAT COMPLEX IN CATTLE GRAZING WINTER WHEAT IN THE SOUTHERN PLAINS: EVOLUTION OF A NEW INTEGRATED RESEARCH PARADIGM. W. E. Pinchak[1], B. R. Min[1], D. P. Malinowski[1], J. W. Sij[1], R. J. Gill[1], R. Puchala[2] and R. A. Anderson[3]. [1]Texas Agricultural Experiment Station, Vernon, TX. [2]Langston University, Langston OK. [3]USDA-ARS, College Station, TX.

An integrated, multi-disciplinary systems approach has been developed to elucidate to the temporal and spatial manifestation, etiology, and impacts of frothy bloat in stocker cattle grazing winter wheat. Frothy bloat is caused by the formation of a low gas permeable polysaccharide bio-film that results from rumen microbial digestion of wheat soluble protein. The underlying questions are: Why doesn't bloat occur throughout the grazing season on wheat? Why is it not severe every year? Why is there so much spatial variability in occurrence and severity within years? Why are intervention strategies often minimally effective in prevention? Our research paradigm is based on understanding the mechanisms and causal factors in the episodic occurrence of wheat pasture bloat. The focus of the research is on the interactions of management actions (nitrogen fertilization, grazing management, and intervention strategy), environmental conditions (solar radiation, ambient temperature dynamics and dew), plant physiologic state (growth phase, growth rate, soluble protein dynamics, and in situ phenolic compound changes) and animal factors (forage intake dynamics, rumen microbial ecology, in vitro and in situ rumen fluid, ruminal gas production and foam characteristics, and grazing behavior). A complete summary of recent research findings and an integrated model system of frothy bloat in cattle grazing winter wheat will be presented.

#53 PHAGE INCIDENCE IN FEEDLOT CATTLE FECES, AND THE USE OF PHAGE TO REDUCE *E. COLI* O157:H7 *IN VIVO*. **T. R. Callaway[1], T. S. Edrington[1], K. J. Genovese[1], J. E. Keen[2], R. C. Anderson[1], A. D. Brabban[3], E. Kutter[3], T. L. Poole[1], and D. J. Nisbet[1], [1]USDA-ARS, Food and Feed Safety Research Unit, 2881 F&B Road, College Station, TX 77845; [2]USDA-ARS, Meat Animal Research Center, Clay Center, NE; [3]Evergreen State College, Olympia, WA.**

Escherichia coli O157:H7 can be specifically killed by phage (bacterial viruses), which are commonly found in the gastrointestinal tract of cattle. However, no examination of the incidence of anti-*E. coli* O157:H7 phage in cattle has been performed. Therefore, the current study was designed to determine 1) the incidence of anti-*E. coli* O157:H7 phage in the feces of commercial feedlot steers in the United States, and 2) to determine the efficacy of anti-*E. coli* O157:H7 phage as a pre-harvest intervention strategy to reduce *E. coli* O157:H7 in cattle. Fecal samples (n = 60) were collected from four feedlots in two Southern Great Plains states (total n = 240 fecal samples). *Salmonella* and *E. coli* O157:H7 were found in 3.8% and 11.7% of the fecal samples, respectively. Anti-*E. coli* O157:H7 phage were found in 15% of all fecal samples, in 55% of all cattle pens, and were present in all four feedlots. Sheep (n=20) were inoculated with 10^{10} CFU *E. coli* O157:H7 at -72 h, and feces were monitored daily for inoculated *E. coli* O157:H7 fecal shedding. Anti-*E. coli* O157:H7 phage (n=24 isolates from feedlots) were grown in the laboratory and were inoculated into the treated sheep via oral gavage (1×10^7 PFU/phage/sheep) at t = 0, 24, and 48 h. Sheep were humanely euthanized at 72 h, and intestinal populations of inoculated *E. coli* O157:H7 were reduced by phage treatment in the rumen, cecum ($P < 0.05$) and rectum ($P < 0.12$). Our results indicate that anti-*E. coli* O157:H7 phage are very widespread in feedlot cattle, indicating that further research into the ecological role of bacteriophage in the gastrointestinal tract is needed before bacteriophage can be considered as a pre-harvest intervention strategy to reduce *E. coli* O157:H7 in cattle.

#54 NOVEL GLYCOSYL HYDROLASE ARCHITECTURE FROM THE *RUMINOCOCCUS FLAVEFACIENS* FD-1 GENOME. **M. E. Berg[1], D. A. Antonopoulos[1, 4], M. Morrison[2, 4], K. E Nelson[3, 4], and B. A. White[1, 4]. [1]Department of Animal Sciences, University of Illinois, Urbana, IL 61801; [2]Department of Animal Science, The Ohio State University, Columbus, OH 43210; [3]The Institute for Genomic Research, Rockville, MD 20850; and [4]The North American Consortium for Genomics of Fibrolytic Ruminal Bacteria.**

The *Ruminococcus flavefaciens* FD-1 sequencing project at the University of Illinois at Urbana-Champaign is currently at 3.36x coverage of the predicted 4.4 Mb genome. The total length of the contigs is approximately 4.04 Mb with an average contig length of approximately 3.1 kb. Analysis of the assembly revealed a contig (9.28 kb) containing genes encoding for the putative scaffoldin proteins, ScaA and ScaB. ScaA consists of an N-terminus signal peptide, an X domain of unknown function, two nearly identical cohesins, and a dockerin. ScaB is downstream of ScaA and contains an N-terminus signal peptide, nine cohesins, and an X domain. Part of the ScaC protein, composed of a partial cohesin linked to a dockerin, was also found at a location upstream of ScaA. Threonine-rich linkers separate each domain in all of the scaffoldin proteins. Compared to *R. flavefaciens* 17 the architecture of these proteins is very similar, except for the number of cohesins in ScaA and ScaB. ScaA has one less cohesin, and ScaB has two more cohesins than *R. flavefaciens* 17. Additionally, ScaB in *R. flavefaciens* FD-1 has short P-T linkers separating cohesins five, six, and seven, similar to those seen in ScaB and ScaC of *Acetivibrio cellulolyticus*. A different contig (4.67 kb) appears to encode a novel cellulase protein, which has glutamine-asparagine rich linkers rather than T-rich linkers similar to the bifunctional xylanase from *R. flavefaciens* 17. This protein is comprised of two family 11 glycosyl hydrolases, a family 10 glycosyl hydrolase, a carbohydrate binding domain, a dockerin, and a polysaccharide deacetylase. Little addition to sequence information was detected, however, with respect to the two ORFs with repeated X domains. These approaches will facilitate the identification of relevant ORFs for further study using microarray and proteomic-assisted technologies.

#55 PCR-DGGE ANALYSIS OF PROKARYOTE DIVERSITY AND COMMUNITY STRUCTURE IN DIFFERENT FRACTIONS OF RUMEN DIGESTA. **R. Garcia-Gonzalez[1], Z. Yu[2], R. C. Larue[3] and M. Morrison[2]. Current address: [1]Dept. of Animal Production, University of Leon, 24071 Leon, Spain; [2]Dept. of Animal Sciences, The Ohio State University, Columbus, OH 43210; [3]Dept. of Microbiology, The Ohio State University, Columbus, OH 43120.**

Four canulated sheep were fed grass hay or a combination of corn and grass hay (70:30) in a cross-over design. After four weeks of adaptation to each diet, rumen contents were sampled and ruminal microbes were separated into three factions: free-living in the liquid phase, firmly attached to plant particles, and loosely associated to those particles. The bacterial community profiles were examined using PCR-DGGE targeting the V3 region with universal primers, while the methanogenic community profiles were also examined using PCR-DGGE employing methanogen-specific primers. The resulting DGGE banding patterns were compared by clustering and multidimensional scaling analyses using BioNumerics and XLSTAT programs. The profiles for both prokaryote groupings tended to be first clustered with respect to animal, but the number of resolvable bands in the bacterial DGGE gels suggested that a more diverse subcommunity exists within the biofilm adherent to plant biomass, and that hay-based diets promote greater bacterial species diversity than hay:corn. We interpret these findings as showing that host-derived factors as well as diet are relevant influences on rumen prokaryote diversity. The DNA amplicons from the methanogen-specific DGGE gels were excised, reamplified, and then sequenced. Most of the 16S rRNA sequences were most similar to *Methanobrevibacter* spp., although there were also some amplicons most similar to *Methanosphaera* spp., *Methanomicrobium* spp., and an uncultured rumen *Archaeon*.

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