2007 Conference on Gastrointestinal Function

Gleacher Center

Chicago IL, USA

April 16-18th, 2007

Final Program

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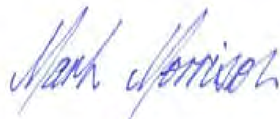
Chair's Welcome:

It is my pleasure to welcome you to the 2007 Conference on Gastrointestinal Function (CGIF)! The CGIF (formerly Rumen Function Conference) has been held on a biennial basis in Chicago since 1951. The Conference was the first venue for presenting the latest advances in our understanding of the biology underpinning the nutrition and health of ruminant animals under the intensive production practices. Because the interactions between the gut microbiome, nutrition and host physiology are now more widely recognized as being influential to the health and well being of humans as well as all food animals, the scope of the Conference expanded, and in 2003 we officially changed to the Conference on Gastrointestinal Function. The Organizing Committee is especially pleased that the expanded scope of our Conference has begun to attract many more scientists from a variety of disciplines, as evidenced by the podium and poster presentations this year. Indeed, we are especially pleased with the range of topics being presented, which includes some of the latest advances in our understanding of gastrointestinal function. We sincerely hope you enjoy these contributions.

On behalf of the CGIF 2007 organizing committee (Jane Leedle, JL Microbiology; Rod Mackie and Isaac Cann, University of Illinois; Jeff Firkins, The Ohio State University) I wish to acknowledge the generous financial support provided by our sponsors this year. Their support is truly critical to the success of Conference's presentation of emerging areas of research, the promotion of scholarly discourse, and the exchange of research methodologies and strategies. Thanks to all of you once again. I would also like to acknowledge the generous support of the Taylor and Francis Publishing group, for their willingness to publish the Conference abstracts in the journal *Microbial Ecology in Health and Disease.* We also appreciate the efforts of Nancy Henry and Chuck Kibler (University of Illinois) and Michael Chakerian (The Ohio State University) for providing assistance with the logistical details and website support of the Conference.

Last I thank all of you, the attendees, for selecting CGIF 2007 as one of the key venues for you to interact with colleagues and present your science this year. There are many scientific meetings that you can attend, and we sincerely hope that CGIF 2007 provides you with the professional and social interactions that warrant your continued participation and attendance at future CGIF Conferences. ENJOY!!!!



Mark Morrison
Chair CGIF 2007

Morning Session, Tuesday, April 17th:

09:00-09:40 New Insights Into The Prokaryotic Community Of The Mucosal Fraction Of Human Ileum: A Metagenomic Approach. **M. Leclerc[1]**, L. Sychova[2], C. Booijink[1,3] , P. Lepage[1], C. Caron[1], R. Nalin[4], M. Gelfand[2], Ph. Marteau[5] and J. Doré[1]; [1]INRA, Jouy en Josas, France. [2]NosGenetika, Moscow, Russia. [3]WCFS, Wageningen, The Netherlands. [4]Libragen, Toulouse, France, [5]HEGP, Paris, France.

09:40-10:00 Analysis Of Gastrointestinal Functions In Disease And Health: Molecular Microbial Ecology Of The Human Gut. **J.R. Marchesi**[1,2], P. Scanlan[2], F. Shanhan[1], E. Quigley[1,3], J.K. Collins[1,2,3], G. O'Sullivan[3], Y. Clone[1], E. Holmes[4], Y. Wang[4]; [1]Alimentary Pharmabiotic Centre; [2]University College Cork; [3]Cork University Hospital; [4]Imperial College London.

10:00-10:20 Functional Analysis Of The Ileum Microbiota. **E.G. Zoetendal[1,2]**, C.C.G. M. Booijink[1,2], H. Smidt[1,2], W.M. de Vos[1,2] , and M. Kleerebezem[1,3] [1]Wageningen Centre for Food Sciences, Wageningen, The Netherlands, [2]Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands, [3]Nizo food research, Ede, The Netherlands

10:20-10:40 COFFEE BREAK

10:40-11:00 Microbial Colonization And Subsequent Biofilm Formation By Ruminal Microorganisms On Fresh Perennial Ryegrass**. O.L. Mayorga**[1,2], S.A. Huws[1], E.J. Kim[1], A.H. Kingston-Smith[1], C. J. Newbold[2], and M.K. Theodorou[1]. [1]Institute of Grassland and Environmental Research, Aberystwyth, UK; [2]University of Wales, Aberystwyth, UK.

11:00-11:20 Comparative Metagenomics Of The Rumen Microbiome By Random Sampling Pyrosequencing. **J.M. Brulc[1]**, D.A. Antonopoulos[2], M.E. Berg[1], M.K. Wilson[1], R.E. Edwards[3,4,5], E.D. Frank[6], J. B. Emerson[7], K.E. Nelson[7, 8], and B.A. White[1], [1]University of Illinois, IL, USA; [2]Michigan State University, MI, USA; [3]Fellowship for Interpretation of Genomes, IL, USA; [4]San Diego State University, CA, USA; [5]The Burnham Institute, CA, USA; [6]Argonne National Laboratory, IL, USA; [7]The Institute for Genomic Research, MD, USA; and [8]Howard University, Washington, DC, USA.

11:20-11:40 Microbial Communities Using Real Time PCR And Fermentation Parameters *in vitro* As Affected By A Natural And A Synthetic Anti-Methanogenic Agent. **G. Goel,** H. P. S. Makkar and K. Becker University of Hohenheim, Stuttgart, Germany

Afternoon Session, Tuesday, April 17th:

13:00-13:40 Resistant Starch As A Vehicle For Delivering Health Benefits To The Human Large Bowel. **D.L. Topping[1,2]**, B.H. Bajka[1,3], A.R. Bird[2], J.M. Clarke[1], L. Cobiac[1,4], M.A. Conlon[2], M.K. Morell[2], S. Toden[2,3], [1]Preventative Health National Research Flagship, Australia, [2]Food Futures National Research Flagship, [3]Adelaide University, Adelaide, Australia, [4]Flinders University, Bedford Park, Australia.

13:40-14:00 RNA Stable Isotope Probing – Direct Identification Of Starch Fermenting Bacteria In The Human Colon. **P. Kovatcheva-Datchary[1,2]**, M. Egert[1,2], A.A. de Graaf[1,3], A. Maathuis[1,3], H. Smidt[1,2], W.M. de Vos[1,2] and K. Venema[1,3]; [1]Wageningen Center for Food Sciences, Wageningen, the Netherlands, [2]Wageningen University, Wageningen, the Netherlands, [3]TNO Quality of Life, Zeist, the Netherlands

14:00-14:20 *Prevotella bryantii* 25A Used As A Probiotic During The Transition Period In Dairy Cows: Effect On Ruminal Fermentation Characteristics And Milk Production. **J. Chiquette**[1], M.J. Allison[2] and M.A. Rasmussen[3]; [1]Dairy and Swine Research and Development Centre, Lennoxville, Canada; [2]Iowa State University, Ames, Iowa, USA; [3]Sar Tec Corp. Anoka, Minnesota, USA.

14:20-14:40 COFFEE BREAK

14:40-15:00 The Primary Starch Utilization Operon Of The Obligate Anaerobe *Bacteroides fragilis* Is Regulated By Carbon Source And Oxygen, And Expression Is Important For Survival During Oxidative Stress. **Cheryl Spence**[1] and C.J. Smith[2]; [1]Present address: USDA-ARS, National Center for Agriculture Research, Peoria, IL; [2]East Carolina University, Greenville, NC.

15:00-15:20 Changes Of Natural Food Antioxidants In The Intestinal Tract And Its Influence On Human Faecal Microflora M. Gumienna, J. Nowak**, K. Goderska** and Z. Czarnecki. The August Cieszkowski Agricultural University of Poznań, Poland.

15:20-16:00 Marvin P. Bryant Memorial Lecture. Syntrophy: The Lifestyle of the Thermodynamically Challenged. **Michael McInerney,** Department of Microbiology and Biology, Oklahoma University, Norman, OK.

16:00-18:00 MIXER AND POSTER SESSION ONE, TO INCLUDE POSTERS P-1.1 TO P-1.36.

Morning Session, Wednesday, April 18th:

09:00-09:40 Commensal-Derived Probiotics As Anti-Inflammatory Agents. C. Iyer[1,2], Y.P. Lin[1,2] and **J. Versalovic**[1,2] ; [1]Baylor College of Medicine, Texas, USA; [2]Texas Children's Hospital, Texas, USA.

09:40-10:00 Bacterial Imprinting Of The Neonatal Immune System: Lessons From Maternal Cells. P.F. Perez, J. Doré, **M. Leclerc,** F. Levenez, J. Benyacoub, P. Serrant, I. Segura-Roggero, E.J. Schiffrin and A. Donnet-Hughes. INRA, Jouy-en-Josas, France

10:00-10:20 Physiological And Molecular Indicators Of Change In The Intestinal Microflora Of Postmenopausal Women Consuming Soy And Fructooligosaccharides. **M. Nelson**[1], M. Geraghty[1], M. Failla[1], K. Walsh[1], A.M. Smith[1], S. Hertzler[1] Z. Yu[1], and M. Morrison[1,2]; [1]The Ohio State University, OH, USA; [2]CSIRO Livestock Industries, St Lucia, Australia.

10:20-10:40 Improved Criteria for Probiotic Strain Selection. M. Medina[1], E. Izquierdo[1,2], S. Ennahar[2] and **Y. Sanz**[1]. [1]Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Valencia, Spain. [2]Universite Louis Pasteur, Strasbourg, France.

10:40-11:00 Fate And Effects Of The Broad-Spectrum Bacteriocin Lacticin 3147 In The Mammalian Gut Following Oral Ingestion; **G.E. Gardiner**[1,2], B. O'Riordan[1], M. Rea[1,2], S. Morgan[1,2], P. O'Connor[1,2], P.G. Lawlor[3], P.B. Lynch[3], C. Hill[2], and R.P. Ross[1,2]; [1]Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland; [2]Alimentary Pharmabiotic Centre, University College, Cork, Ireland; [3]Pig Production Development Unit, Moorepark, Fermoy, Co. Cork, Ireland.

11:00-13:00 COFFEE AND POSTER SESSION TWO, TO INCLUDE POSTERS P-2.1 TO P-2.34.

Afternoon Session, Wednesday, April 18th:

14:00-14:40 Carbohydrate Metabolism In *Fibrobacter succinogenes*: What NMR Tells Us. **E. Forano**[1], A-M. Delort[2] and M. Matulova[3]; [1]INRA, Clermont-Ferrand-Theix, France; [2]CNRS-Université Blaise Pascal, Aubière, France; [3]Slovak Academy of Sciences, Bratislava, Slovak Republic.

14:40-15:00 Metabolomic Analyses Of The Gut Microbiota *in vitro* And *in vivo* Using ^{13}C-labeled Carbohydrates. **K. Venema**[1,2], A.A. de Graaf[1,2,3], A. Maathuis[1,2], N.E.P. Deutz[1,3], and W.M. de Vos[1,4]; [1]Wageningen Center for Food Sciences, Wageningen, the Netherlands, [2]TNO Quality of Life, Zeist, the

Netherlands; [3]Maastricht University, Maastricht, the Netherlands; [4]Wageningen University, Wageningen, the Netherlands.

15:00-15:20 Different Conjugated Linoleic Acids Are Formed By Different Mechanisms In Ruminal Digesta and Bacteria. **R. J. Wallace**[1], N. McKain[1], K. J. Shingfield[2] and E. Devillard[1]; [1]Rowett Research Institute, Aberdeen, UK; [2]MTT Agrifood Research Finland, Jokioinen, Finland

15:40-16:00 Hemicellulose Degradation By *Clostridium proteoclasticum* B316[T]. **E. Altermann[1]**, B. Kelly[1], J. Dunne[2], J. Bond[2], B. Jordan[3], and G.T. Attwood[1]. [1]AgResearch Limited, Palmerston North, New Zealand, [2]AgResearch Limited and VUW Proteomics laboratory, Victoria University of Wellington, Wellington, New Zealand and [3]Victoria University of Wellington, Wellington, New Zealand.

16:00-16:20 Cloning, Expression, And Characterization Of A Beta-Xylosidase From The Hemicellulose Degrading Rumen Bacterium *Prevotella ruminicola* 23. S.A. Kocherginskaya[1], **M.A. Spies[1]**, C. Abbas[2], K. Beery[2], S.K. Nair[1], I.K.O. Cann[1] and R.I. Mackie[1]. [1]University of Illinois at Urbana-Champaign, Urbana, IL, USA and [2]Archer Daniels Midland Co., Decatur, IL, USA.

16:20-16:30 CLOSING REMARKS AND INVITATION TO CGIF2009

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.1 Methane Production And Microbial Evaluation By q-PCR Of i*n vitro* Incubations Of Tannin-Rich Plants. **A.L. Abdalla**[1], C. Longo[1], I.C.S. Bueno[1], P.B. Goddoy[1,] V.A. Benedito[1,] D.M.S.S. Vitti[1], M.R.S.R. Peçanha[1], H. Louvandini[2], C.MacManus[2], E.J. Ambrosano[3]. [1]University of Sao Paulo, Piracicaba, Brazil; [2]University of Brasilia, Brasilia, Brazil; [3]APTA Centro Sul, Piracicaba, Brazil.

Keywords: methane mitigation, rumen microbial diversity

P-1.2 Methane Emission, Protozoa And Methanogens Counts In Sheep Fed Coconut Oil Or A Brazilian Tannin-Rich Plant (*Mimosa caesalpineaefolia*). **A.L. Abdalla[1]**, P.B. Godoy[1], C. Longo[1], J.C. Araujo Neto[1], M.R.S.R. Peçanha[1], I.C.S. Bueno[1], D.M.S.S.Vitti[1], S.M.A. Sallam[2], [1]University of Sao Paulo, Piracicaba, Brazil, [2]University of Alexandria, Egypt.

Keywords: tanniniferous plants, mitigation strategies, ruminant

P-1.3 Effect Of 2-Nitro-Methyl-Propionate On Ruminal Methane Production And Its Metabolism *in vitro*. **R.C. Anderson[1]**, D.J. Smith[2], J.K. Huwe[2], T.R. Callaway[1], T.S. Edrington[1], R.B. Harvey[1] and D.J. Nisbet[1]; [1]USDA/ARS, Southern Plains Agricultural Research Center, College Station, Texas, USA; [2]USDA/ARS, Biosciences Research Laboratory, North Dakota, USA.

Keywords: nitroethane, 2-nitro-methyl-propionate, methane-inhibitor

P-1.4 Profile Of Methane Emissions From Sheep Fed Oat Hay Basal Diet Supplemented With Ground Corn and Coconut Oil. X.Z. Ding[1], **R.J. Long[2]**, H.S. Shi[3] and X.D. Huang[3]. [1]Northwest Institute of Plateau Biology, Xining, China; [2]Lanzhou University, Lanzhou, China; [3]Gansu Agriculture University, Lanzhou, China.

Keywords: methane emissions, sheep, coconut oil, oat hay, corn grain

P-1.5 Rumen Methanogens in Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) and Norwegian Reindeer (*R. t. tarandus*), **M. A. Sundset[1]**, S. D. Mathiesen[2,3], M. Napal Fraile[1], R. Solana[1], T. Glad[1] and K. E. Præsteng[1]; [1]University of Tromsø, Norway; [2]The Norwegian School of Veterinary Science, Norway; [3] Saami University College, Norway.

Keywords: 16S rRNA gene, methanogens, rumen, reindeer, arctic

P-1.6 The Effect Of Encapsulated Fumaric Acid And Diet On Methane Formation From Ruminal Digesta. **T.A. Wood**, S. Mahmood and R.J. Wallace; Rowett Research Institute, Aberdeen, UK

Keywords: methane, encapsulated fumaric acid, pH, diet

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.7 A Meta-Analysis Of The Effects Of Malate On Ruminal Batch Cultures. **E.M. Ungerfeld** and R.A. Kohn; University of Maryland, Maryland, USA.

Keywords: rumen, methane, malate, electron acceptors, meta-analysis

P-1.8 Octadeca-Carbon Fatty Acids Affect Microbial Fermentation, Methanogenesis And Microbial Flora *in vitro*; C.M. Zhang[1], **J.X. Liu**[1], Y.Q. Guo[1], Z.P. Yuan[1], J.K. Wang[1] and W.Y. Zhu[2]; [1]Zhejiang University, MOE Key Laboratory of Molecular Animal Nutrition, Hangzhou, China; [2]Nanjing Agricultural University, Nanjing, China

Keywords: C_{18}-fatty acids, fermentation, methane, microbial flora, *in vitro*

P-1.9 Monitoring And Source Tracking Of Tetracycline Resistance Genes In Lagoons And Groundwater Underlying Swine Production Facilities. S. Koike[1], **A. Yannarell[1]**, I.G. Krapac[2], H.D. Oliver[1], J.C, Chee-Sanford[3], R.I. Aminov[4], and R.I. Mackie[1]; [1]University of Illinois at Urbana-Champaign, Illinois, USA; [2]Illinois State Geological Survey, Illinois, USA; [3]USDA Agricultural Research Service, Illinois, USA; [4]Rowett Research Institute, Aberdeen, UK.

Keywords: antibiotic resistance, quantitative PCR, livestock, groundwater

P-1.10 Bacterial Diversity And Antibiotic Resistance In The Colon Of The Hooded Seal. **T. Glad[1]**, M. A. Sundset[1], L. Nordgård[2], and K. M. Nielsen[1], [1]University of Tromsø, Norway; [2]Norwegian Institute of Gene Ecology, Norway.

Keywords: arctic seal, 16S rRNA, ampicillin resistance, tetracycline resistance

P-1.11 Evaluation Of Bio-Mos® Performance At Reducing The Levels Of Antibiotic Resistant Bacteria In Chicken Caecal Contents**. A. Corrigan** and K. Horgan Alltech Biotechnology Centre, Dunboyne, Ireland.

Keywords: antibiotic resistance, Bio-Mos® , real-time PCR, tetracycline.

P-1.12 Interventions To Reduce The Carriage Of Antimicrobial Resistance In Pigs. Determination Of The Inhibitory Spectrum Of Pre, Pro And Synbiotics Against *Salmonella typhimurium*. **A. Costabile[1]**, S. Martín-Peláez[2], S.M. Martín-Orúe[2], R. A. Rastall[1] and G.R. Gibson[1]. [1]The University of Reading, Reading, UK; [2]Universitat Autònoma de Barcelona.

Keywords: antibiotic resistance, probiotics, prebiotics, *S. typhimurium*, human health risk.

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.13 Development Of Antimicrobial Resistance Concurrent With Antimicrobial Uses In Cattle And Its Reduction During Composting. **J. Chen**[1], F. Fluharty[1], R. Sharma[2], M. Morrison[1,3] and Z. Yu[1]. [1]The Ohio State University, USA; [2]Lethbridge Research Centre, Alberta, Canada; [3]CSIRO Livestock Industries, St Lucia, Australia.

Keywords: manure, compost, *erm*, *tet*, antimicrobial resistance

P-1.14 Erythromycin Resistance Reservoirs In Different Swine Farms And At Different Locations Within Each Farm. J. Chen[1], **Z. Yu[1]**, R. Mackie[2], and M. Morrison[1,3]. [1]The Ohio State University, OH, USA; [2]University of Illinois at Urbana-Champaign, IL, USA; [3]CSIRO Livestock Industries, St Lucia, Australia.

Keywords: antimicrobial resistance, *erm*, *tet*, real-time PCR, swine farms

P-1.15 Changes In Normal Fecal Microbiota Of Dogs Related To Antibiotic Treatment. **A.M. Grønvold[1]**, T.M. L´Abée-Lund[1], H. Sørum[1], E. Skancke[1], and R.I. Mackie[2]. [1]Norwegian School of Veterinary Science, Oslo, Norway; [2]University of Illinois at Urbana-Champaign, Illinois, USA

Keywords: dog, fecal microbiota, antibiotic, PCR-DGGE

P-1.16 Isolation Of A Tylosin-Resistant *Clostridium hathewayi* From A Continuous Flow Culture Derived From A Feral Pig. **N. Ramlachan**, R.C. Anderson, K. Andrews and D.J. Nisbet; USDA/ARS, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, Texas, USA

Keywords: chemostat, antibiotic resistance, feral pig, *Clostridium hathewayi*

P-1.17 Diversity And Succession Of The Microbiota From The Small Intestine Of Broiler Chickens. **Lei Lei**, Rijun Zhang; China Agricultural University, Beijing, China.

Keywords: small intestine, microbiota, chlorotetracycline, *Lactobacillus plantarum*

P-1.18 Competitive Exclusion Of *Salmonella Enteriditis* By *Lactobacillus salivarius* And *Enterococcus faecium* In Poultry. A.J. Carter[1,2], R.M. La Ragione[1], M. Adams[2] and M.J. Woodward[1]; [1]Veterinary Laboratories Agency, Surrey, UK; [2]University of Surrey, Surrey, UK.

Keywords: *Lactobacillus salivarius*, *Enterococcus faecium*, *Salmonella enteriditis*, competitive exclusion.

P-1.19 Effects Of Biological Pit Additives On Microbial Ecology Of Stored Pig Manure. C.J. Ziemer, **E. Coates,** B.J. Kerr, and S. Trabue. USDA National Soil Tilth Lab, Iowa, USA.

Keywords: manure, DGGE, additives, microbial diversity, pits

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.20 *Entodinium caudatum* Is The Most Abundant But Not The Most Bacteriolytic Ciliate In The Rumen Of Sheep. **S. Muetzel**[1], N.R. McEwan[2], Wallace, R.J[1]. [1]The Rowett Research Institute, Scotland, UK, [2]University of Wales, Wales, UK

Keywords: ciliate protozoa, *Entodinium caudatum*, bacteriolysis, rumen ecology

P-1.21 Culturing Ciliate Protozoa From The Kangaroo Forestomach. **Burk A. Dehority[1]** and Andre-Denis Wright[2]; [1]Ohio Agricultural Research and Development Center, The Ohio State University, Ohio, USA; [2]CSIRO, Queensland, Australia.

Keywords: kangaroos, in vitro culture, protozoa

P-1.22 Rumen Protozoa Can Alter Generation Time And 18S Ribosomal DNA Concentration To Adapt To Increased Transfer Rate, Starvation, And Monensin. **J.T. Sylvester**[1], S.K.R. Karnati[1], B.A. Dehority[1], M. Morrison[1,2], G.L. Smith[1], and J.L. Firkins[1]. [1]The Ohio State University, Columbus and Wooster, OH, USA; and [2]CSIRO, St Lucia, Australia.

Keywords: rumen protozoal culture, real-time PCR, monensin, rDNA copies

P-1.23 Development And Validation Of An Automated Ribosomal Intergenic Spacer Analysis For Anaerobic Fungi (*Neocallimastigales*). **J.E. Edwards**, H.R. Jimenez, S.A. Huws and M.K. Theodorou; Institute of Grassland and Environmental Research, Aberystwyth, UK.

Keywords: ARISA, rumen, *Neocallimastigales*, anaerobic fungi

P-1.24 Characterisation Of The Population Structure Of Anaerobic Fungi In The Ruminant Digestive Tract. **H.R. Jimenez[1,2]**, J.E. Edwards[1], N.R. McEwan[2] and M.K. Theodorou[1]; [1]Institute of Grassland and Environmental Research, Aberystwyth, UK; [2]University of Wales Aberystwyth, Wales, UK.

Keywords: ARISA, anaerobic fungi, *Neocallimastigales*, rumen, faeces

P-1.25 Phylogenetic Analysis Of Uncultured Butyrate Producing Bacteria From The Forestomach Content Of Wild Vicuña. **L.C. Arakaki[1],** F. Rigalt[2], M. Dunleavy[1], M.Cerón[1], S.Cravero[1], and A.Takenaka[3] [1]Instituto Nacional de Tecnología Agropecuaria, (INTA-CICVyA), Buenos Aires, Argentina; [2]EA- INTA Catamarca, Argentina; [3]National Institute of Livestock and Grassland Sciences, Tsukuba, Ibaraki, Japan.

Keywords: vicuñas, bacteria, Phylogenetic analysis, *Pseudobutyrivibrio*

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.26 Quantification By Real-Time PCR Of Cellulolytic Bacteria In The Rumen Of Sheep: Effect Of Supplementation Of A Forage Diet with Concentrate And Yeast. **P. Mosoni**[1], F. Chaucheyras-Durand[1,2], C. Béra-Maillet[1] and E. Forano[1]. [1]INRA France; [2]Lallemand Animal Nutrition France.
Keywords: rumen, cellulolytic bacteria, real-time PCR, diet, yeast

P-1.27 Real Time PCR Analysis Of Ruminal Microbial Populations In Sheep Fed Hay Or Corn:Hay Mix**.** **J. Stephens[1]**, J. Goopy[2], Mark Morrison[1,3], Z. Yu[1]. [1]The Ohio State University, OH, USA; [2]University of New England, Armidale, Australia; [3]CSIRO Livestock Industries, St Lucia, Australia.
Keywords: *Fibrobacter*, *Ruminococcus*, real-time PCR, rumen

P-1.28 Isolation And Partial Characterization Of An Uncultured Fiber-Associated Bacterium From Sheep Rumen. **S. Koike**, H. Goto, H. Yabuki, T. Shinkai and Y. Kobayashi; Hokkaido University, Japan.
Keywords: uncultured bacteria, rumen, isolation, LGCGP bacterial group

P-1.29 Development Of A Colorimetric Plate Assay For Detecting Defluorination By Microorganisms C.K. Davis[1,2], S.E. Denman[1], L. Sly[2] and **C.S. McSweeney[1].** [1]CSIRO, St.Lucia, Australia; and [2]University of Queensland, St. Lucia, Australia.
Keywords: dehalogenation, fluoroacetate, plate assay, rumen toxicity, enrichment culture

P-1.30 Screening Pure Culture Ruminal Bacteria For the Ability to Degrade 2,4,6-Trinitrotolulene. **M.J.M. De Lorme** and A. M. Craig. Oregon State University, Oregon, USA
Keywords: ruminants, bioremediation, rumen bacteria, 2,4,6-trinitrotolulene

P-1.31 Characterization Of Acetogenic Bacteria Associated With Skatole Production In Swine Lagoon Slurry. **K.L. Cook[1],** J.H. Loughrin[1], and M.R. Rothrock[1]. [1]USDA-ARS, Bowling Green, Kentucky, USA.
Keywords: skatole, acetogen, waste treatment, swine

P-1.32 An Efficient RNA Extraction Method For The Estimation Of Gut Microbial Diversity By PCR. S. Kang[1], S.E. Denman[1], M. Morrison[1,2], Z. Yu[2], and **C.S. McSweeney[1]**. [1]CSIRO, St. Lucia, Qld, Australia; [2]The Ohio State University, Columbus, OH, USA
Keywords: bacterial diversity, RNA extraction methods, RT-PCR

Poster Session One – Tuesday, April 17th, 16:00-18:00

P-1.33 Development And Evaluation Of Oligonucleotide Probes For Tracking Bacterial Strains Re-Introduced To The Reindeer Rumen. **K.E. Præsteng**[1], R.I. Mackie[2], I.K.O. Cann[2], S.D. Mathiesen[3,4] and M.A. Sundset[1]; [1]University of Tromsø, Norway; [2]University of Illinois at Urbana-Champaign, Illinois, USA, [3]The Norwegian School of Veterinary Science, Norway; [4]Saami University College, Norway.

Keywords: reindeer, intergenic spacer region, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*

P-1.34 Human Methanogens Are Inversely Related To Butyrate Concentration. **A.-D. Wright[1,2]**, B. MacHunter[2], A.F. Toovey[2], M. Conlon[3], D.L. Topping[3], A.R. Bird[3], I. Saunders[3], R. Miller[3], A.L. McOrist[3]. Preventative Health Flagship Program and [1]CSIRO, Queensland, Australia, [2]CSIRO, Western Australia, Australia, [3]CSIRO, South Australia, Australia.

Keywords: methanogens, butyrate, colorectal cancer, fibre, resistant starch

P-1.35 Bacterial Imbalance In The Duodenal Mucosa Of Children With Celiac Disease. **Y. Sanz[1]**, I. Nadal[1], M. C. Collado[1], E. Donant[2], C. Ribes- Koninckx[2] and M. Calabuig[3]. [1]Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Valencia, Spain. [2]Hospital Universitario La Fe, Valencia, Spain. [3]Hospital General Universitario, Valencia, Spain.

Keywords: intestinal microbiota, celiac disease, FISH, flow cytometry

P-1.36 Physiological And Molecular Indicators Of Change In The Intestinal Microflora Of Postmenopausal Women Consuming Soy And Fructooligosaccharides. **M. Nelson[1]**, M. Geraghty[1], M. Failla[1], K. Walsh[1], A.M. Smith[1], S. Hertzler[1] Z. Yu[1], and M. Morrison[1,2]; [1]The Ohio State University, OH, USA; [2]CSIRO Livestock Industries, St Lucia, Australia.

Keywords: FOS, soy, real-time PCR, isoflavone, *Bifidobacterium*

Poster Session Two – Wednesday, April 18th, 11:00-13:00

P-2.1 Application Of Quantitative PCR Assays To Detection Of Human *Bacteroides* Species In The Intestines Of Pigs. C. J. Ziemer and **M.J. Wimmer,** USDA National Soil Tilth Lab, Iowa, USA and Iowa State University, Iowa, USA.
Keywords: quantitative PCR, swine, *Bacteroides,* intestinal tract

P-2.2 Changes Of The Faecal Bacterial Communities Of Horses Under Transportation Stress And Effect Of An Active Dry Yeast. **C. Faubladier**[3], F. Chaucheyras-Durand[1,2], S.Masséglia[1,2], L. Da Veiga[3] and V.Julliand[3]. [1]Lallemand Animal Nutrition, Blagnac, France; [2]INRA Clermont-Ferrand-Theix, France; [3]ENESAD, Dijon, France.
Keywords: active dry yeast, equine microflora, transportation stress, TTGE

P-2.3 Effect Of Adding A Starter Culture Or Formic Acid To Fermented Liquid Feed On Gastrointestinal Ecology And Growth Performance Of Piglets. **N. Canibe[1]** , H. Miettinen[2] and B. B. Jensen[1]; [1]University of Aarhus, Tjele, Denmark; [2]Kemira Oyj, Helsinki, Finland.
Keywords: formic acid, fermented liquid feed, gastrointestinal ecology, piglets, starter culture

P-2.4 Characteristics Of *Bifidobacterium bifidum* Strains As Probiotics And Survivability Of Microencapsuled Cells *In Vitro*. **K. Goderska** and Z. Czarnecki. The August Cieszkowski Agricultural University of Poznan, Poland
Keywords: probiotic, *Bifidobacterium bifidum*, microencapsulation

P-2.5 Intestinal Morphology And Nutrient Ileal Digestibilities In Broiler Chickens Fed With Mannan Oligosaccharide Plus A Yeast Culture And/Or A Live Microorganism. **S. Gómez[1],** M.L. Angeles[1], E. Albaran[2], D. Avila[2] and M.C. Mojica[3]. [1]National Center of Disciplinary Research in Animal Physiology – INIFAP. Querétaro, México. [2]University of Guadalajara, Jalisco, México, [3]SynBios SA de CV
Keywords: broiler chickens, *Saccharomyces cerevisiae*, *Bacillus subtilis*

P-2.6 Effect Of Probiotic On Broiler Performance And Some Blood Factors. **M.R. Abdollahi**, A. Kamyab, A. Zareshahneh, and F. Zaefarian, The Tehran University, Tehran, Iran.
Keywords: probiotic, broiler, cholesterol, immune system

P-2.7 Novel Source For Probiotic Bacteria Capable Of Metabolizing Ergovaline. **R.M. Rattray**, S. Perumbakkam, A.M. Craig; Oregon State University, Oregon, USA.
Keywords: ergovaline, 16S rRNA, DGGE, earthworms, probiotic development

Poster Session Two – Wednesday, April 18th, 11:00-13:00

P-2.8 The Variability Of *Oxalobacter formigenes* And Oxalate In Stool Samples. S. Prokopovich, J Knight, D. G. Assimos and **R. P. Holmes**; Wake Forest University, NC, USA.
Keywords: oxalate, real-time PCR, urolithiasis

P-2.9 The Effect Of Distiller's Grain Products On The Prevalence And Growth Of *E. coli* O157. **M. E. Jacob**, J.T. Fox, A. Thornton, J.S. Drouillard, and T.G. Nagaraja. Kansas State University, Kansas, USA.
Keywords: *E. coli* O157, distiller's grains, cattle

P-2.10 Stimulation Of *E. coli* O157 In Batch Culture Fermentation By Constituents Of Intestinal Mucus. **J.T. Fox**, J.S. Drouillard, and T.G. Nagaraja; Kansas State University, Kansas, USA.
Keywords: *Escherichia coli* O157, *in vitro,* mucus, carbohydrates

P-2.11 Association Of *Providencia alcalifaciens* With Diarrhea In Dogs. **T.M. L'Abée-Lund[1]**, A.K. Fauske[1], M.N. Hirsch[1], and S.P. Hardy[1]. [1]Norwegian School of Veterinary Science, Oslo, Norway.
Keywords: *Providencia alcalifaciens,* dogs, diarrhea, PFGE

P-2.12 Alternatives To Antibiotics - Grasping Bacteria By Their Achilles Heels. **P. M. Becker[1]**, S. Galletti[1,2] and P. G. van Wikselaar[1]. [1]Animal Sciences Group of Wageningen UR, Lelystad, The Netherlands; [2]University of Milan, Milan, Italy
Keywords: gut health, pathogenic bacteria, *Salmonella*, adhesion test, food and feed

P-2.13 Improved RNA Extraction Procedures To Increase The Sensitivity Of Real Time Reverse Transcription-PCR Detection Of *Escherichia coli* O157:H7. S.C. de Wet[1,2], S.D. Denman[1], L. Sly[2], and **C. S. McSweeney[1]**. [1]CSIRO, St. Lucia, Australia; [2]University of Queensland, St. Lucia, Australia.
Keywords: RNA extraction, pathogen detection, environmental samples

P-2.14 The Digestion Of Newborn Lambs With Various Body Mass. **L.S. Kuchkarova[1]**, B.A., Sadikov[2], D.T. Sidikov[1], N.A. Ergashev[2] and G.A. Dustmatova[2]; [1]The National University of Uzbekistan, Thashkent, Uzbekistan; [2]Institute of physiology and biophysics, Tashkent, Uzbekistan.
Keywords: newborns lambs, digestion, colostrum, hydrolytic enzymes.

P-2.15 Changes In Serum Gastrin Level, Gastric Motility And Acidity After Roux-En-Y Biliary-Entereic Reconstruction In Patients With Obstructive Jaundice. E-S. Soliman, **H. Abdeldayem**, M. Osman, I. A-K. Salama, B. Soliman, R. Ghoneim, A.A. Moneim, T. El-Sefi and I. Marwan. Menoufeyia University, Egypt
Keywords: gastrin, motility, physiology, enteric reconstruction

Poster Session Two – Wednesday, April 18th, 11:00-13:00

P-2.16 Buffer Concentration Influences the Effects of Supplemental Yeast on pH in Rumen-Simulating Continuous Cultures. **J.M. Tricarico**, R. García-González, G.A. Harrison, and K.A. Dawson; Alltech Inc., Nicholasville KY, USA.

Keywords: yeast, buffer concentration, pH

P-2.17 Fe III Stimulates 4-Methyl Phenol and 3-Methyl Indole Production by *Clostridium scatologenes* ATCC 25775. **Kinchel C. Doerner** and Bryan P. Mason. Western Kentucky University, Bowling Green, Kentucky USA.

Keywords: 4-methyl phenol, p-cresol, 3-methyl indole, skatole, ferric iron

P-2.18 Phenolic Compounds Correlation With *in vitro* Gas Production And Degradability Of Selected Tibetan Shrub Species X.Z. Ding[1], **R.J. Long[2],** X.D. Huang[3] and S.H. Shi[3]. [1]Northwest Institute of Plateau Biology, Xining, China; [2]Lanzhou Universtiy, Lanzhou, China; [3]Gansu Agriculture University, Lanzhou, China.

Keywords: Tibetan shrubs, gas production; in vitro fermentation, tannins, phenolic compounds

P-2.19 Effects Of Rumen Undegradable Protein And *Yucca schidigera* Extract On Metabolism By Ruminal Microbes Maintained In Dual Flow Continuous Culture Fermenters. **M.R. Moreno** and M. Stern; University of Minnesota, Minnesota, USA.

Keywords: rumen, undegradable protein, *Yucca schidigera*

P-2.20 Effects Of Urea Or Optigen On Fermentation, Digestion, And N Flow In Rumen-Simulating Fermenters. **G.A. Harrison**, J.M. Tricarico, M.D. Meyer, and K.A. Dawson; Alltech Biotechnology, Inc., Kentucky, USA.

Keywords: non-protein nitrogen, urea, Optigen, ruminal metabolism

P-2.21 Effect Of Different Sources Of Nitrogen On Ruminal Microbial Growth And Fermentation of Pretreated Rice Straw *in vitro*; X.L. Chen, **J.K. Wang**, J.X. Liu and Y.M. Wu. Zhejiang University, Hangzhou, P.R. China

Keywords: ammonia bicarbonate, casein, ruminal fermentation, microbial growth, *in vitro*

P-2.22 Effect Of Protein Level In The Diet On Activity Of Tannins During Ruminal Breakdown Of Structural Polysaccharides. **Jorge Peña G,** Gaston Pichard D, and Antonio Hargreaves B. Pontificia Universidad Católica De Chile, Santiago De Chile.

Keywords: tannins, rumen, protein, gas production, digestibility, polysaccharides, cell wall.

Poster Session Two – Wednesday, April 18th, 11:00-13:00

P-2.23 Attachment, Colonization And Formation Of Ruminal Microbial Biofilms *In Vitro* On Inert Surfaces. **Jorge Peña G.**, Bruno Tesser O., Gaston Pichard D and Y Antonio Hargreaves B. Pontificia Universidad Católica De Chile, Santiago, Chile.
Keywords: attachment, biofilm, surface, flagella, ruminal microorganisms

P-2.24 Effects Of Forage To Concentrate Ratio On Intestinal Absorbable Amino Acid In Goats **X.L. Chen[1]**, G.Q. Zhao[2] and Y.H. Jia[2] [1]Zhejiang University, Hangzhou, P.R. China; [2]Yangzhou University, Yangzhou, P.R. China
Keywords: forage to concentrate ratio, absorbable amino acid, goats

P-2.25 An Investigation Of The Relationship Between Rumen And Fecal Bacterial Concentrations. **Laura M. Neumann** and Burk A. Dehority; Ohio Agricultural Research and Development Center, The Ohio State University, Ohio, USA.
Keywords: amylolytic, bacteria, cellulolytic, feces, rumen

P-2.26 Temporal Stability And Effect Of Preceding Diet On The Rumen Bacterial Population Structure In Sheep. **S. Sadet,** C. Martin, B. Meunier and D.P. Morgavi, INRA, Saint-Genès-Champanelle, France.
Keywords: PCR-DGGE, rumen bacteria, effect of preceding diet.

P-2.27 Composition Of The Rumen Microbial Populations Of Colombian Wild Herbivores: Capibara (*Hydrochaeris hydrochaeris*), Danta (*Tapirus terrestris*) And Aguti (*Agouti taczanowskii*). E. Martin, C. Laverde, and **F. Rodriguez.** CORPOICA, Colombia
Keywords: quantitative PCR, wild herbivores, Colombia, gut bacteria, methanogens

P-2.28 Comparative Metagenomics Of The Chicken Cecal Microbiome Using 16S rDNA Terminal-Fragment Length Polymorphism And Random Sampling Pyrosequencing. **M.K. Wilson[1]**, B.F. Law[2], L.A. Joens[2], M.E. Konkel[3], R.E. Edwards[4,5,6], and B.A. White[1], [1]University of Illinois, IL, USA; [2]University of Arizona, AZ, USA; [3]Washington State University, WA, USA, [4]Fellowship for Interpretation of Genomes, IL, USA; [5]San Diego State University, CA, USA; and [6]The Burnham Institute, CA, USA.
Keywords: pyrosequencing, metagenome, chicken, cecum

P-2.29 Differential Regulation By Nitrogen Source Of Gene Expression, Enzyme Activity And Growth In *Ruminococcus albus* 8. **J.N. Kim,** I.K.O. Cann, and R.I. Mackie; University of Illinois at Urbana-Champaign, Illinois, USA.
Keywords: rumen, *Ruminococcus albus,* nitrogen metabolism, ammonia assimilation, regulation of gene expression

P-2.30 Differential Translocation Of Green Fluorescent Protein Fused To Signal Sequences Of *Ruminococcus albus* Cellulases By The Tat And Sec Pathways Of *Escherichia coli.* J. Esbelin, C. Martin, E. Forano and **P. Mosoni**; INRA, Clermont-Ferrand, France.
Keywords: secretion, *Ruminococcus albus*, cellulases, SS-GFP fusions, rumen

P-2.31 Xylanolytic System Of Two New Xylan-Degrading Species From The Human Colon: *Bacteroides* sp. And *Roseburia intestinalis.* C. Mirande[1]**,** C. Chassard[1], A. Bernalier-Donadille[1], M. Leclerc[2], **E. Forano[1]** and C. Béra-Maillet[1]. [1]INRA, Clermont-Ferrand-Theix, France and [2]INRA, Jouy-en-Josas, France.
Keywords: human colon, *Bacteroides*, *Roseburia*, phylogeny, xylanase.

P-2.32 A Novel Clamp Loader Complex, A Molecular Switch In DNA Replication, From Methanogenic Archaea. **Yi-Hsing Chen[1]**, Mary-Cathleen McKinney[1], Aya Yoshinaga[2], Liu Cheng[1], Yuyen Lin[1], Roderick I. Mackie[1], Yoshizumi Ishino[2], Taekjip Ha[1], and Isaac K. O. Cann[1]. From the [1]University of Illinois at Urbana-Champaign, IL 61801, [2]USA and Kyushu University, Fukuoka, Japan.
Keywords: methanogens, DNA replication, clamp, clamp loader, evolution

P-2.33 DNA Replication Proteins Required For Origin Recognition In The Methanogenic Archaeon *Methanosarcina acetivorans* C2A. **Y. Lin,** Y-H. Chen, S. Kocherginskaya, R.I. Mackie, and I.K.O. Cann. University of Illinois at Urbana-Champaign, IL USA.
Keywords: *Methanosarcina acetivorans*, origin recognition complex (ORC), cell division control 6 protein (Cdc6), minichromosome maintenance protein (MCM), AAA+ ATPase

P-2.34 Proteomic Analysis Of Proteins Expressed By *Bifidobacterium longum* S3 When Grown On Galactooligosaccharides Or Glucose. **M-S. Kim[1]**, T. Premaraj[1], Z. Yu[1], M. Morrison[1,2]. [1]The Ohio State University, OH, USA; [2]CSIRO Livestock Industries, St Lucia, Australia.
Keywords: *Bifidobacterium longum*, GOS, proteomics, prebiotics

P-1.30 Screening Pure Culture Ruminal Bacteria for the Ability to Degrade 2,4,6-Trinitrotolulene

M.J.M. De Lorme and A. M. Craig. Oregon State University, Oregon, USA

The clean up and degradation of munitions wastes is a growing concern as environmental restrictions continue to become more stringent. It has been estimated that over 700,000 cubic yards of soil and 10 billion gallons of groundwater require treatment for munition waste at tremendous costs to the Department of Defense. The removal of unexploded ordnances on military ranges has the potential to be the largest environmental clean-up program ever implemented in the United States, estimated to cost $35 billion. One of the major munition wastes in the United States is 2,4,6-trinitrotolulene (TNT). Anaerobic microbes found in the rumen are well equipped with reductive enzymes that are potentially responsible for the rapid reduction of TNT in bioremediation systems. Previous work has shown whole bovine rumen fluid is capable of rapidly degrading TNT *in vitro*. We feel it may be possible to use sheep as bioreactors for the bioremediation of munitions wastes. To this end, ruminal bacteria were screened for their ability to degrade TNT. Thirteen species of pure culture ruminal bacteria were grown in monocultures, then incubated with 100 mg/L TNT for 24 hours. Eight species displayed a greater than 90% degradation of TNT, 3 species were able to degrade 80-90% of the TNT, while one species was able to degrade 50-60% of the TNT. One species was not able to transform TNT. The concentration of metabolites observed after 24 hours varied by species, with the majority of the metabolites being an unknown polar compound. The mono-amine reduction products (2-amino,4,6-dinitrotolulene and 4-amino,2,6-dinitrotolulene) were more common (ranged from 0 to 41% depending on species) than the di-amino products (2,4-diamino,6-nitrotolulene and 2,4-diamino,6-nitrotolulene; ranged from 0 to 10% depending on species). However, some species of bacteria, such as *Selenomonas ruminatium* HD4, had > 98% of the initial TNT converted into an unknown metabolite. This work shows that the ability to degrade TNT is common amongst ruminal microbes, lending support to the idea of using sheep as bioreactors to detoxify TNT contaminated sites.

Key Words: ruminants, bioremediation, rumen bacteria, 2,4,6-trinitrotolulene

P-2.17 Fe III Stimulates 4-Methyl Phenol and 3-Methyl Indole Production by *Clostridium scatologenes* ATCC 25775.
Kinchel C. Doerner and Bryan P. Mason. Western Kentucky University, Bowling Green, Kentucky USA.

Introduction: 4-Methyl phenol (4-MP, p-cresol) and 3-methyl indole (3-MI, skatole) are generated by microbial processes in ruminal, fecal, and animal waste environments. 4-MP is a strong and a predominant odorant emanating from both cattle and swine facilities. 3-MI is generated in the colon of swine, absorbed by the host, and largely responsible a displeasing pungent odor of boar taint. 3-MI is also generated in the rumen and can cause emphysema. 4-MP and 3-MI are derived from the side chain oxidation of tyrosine and tryptophan, respectively. Here we test the effect of inorganic electron acceptors on *C. scatologenes* ATCC 25775 4-MP and 3-MI culture supernatant levels. **Materials and Methods:** *C. scatologenes* ATCC 25775 was cultured anaerobically at 37ºC in a semi-defined medium supplemented with 1% (w/v) casamino acids or brain-heart infusion (BHI) medium for 48 hr. Na_2SO_4, KNO_3, MnO_2, and $FeCl_3$ were prepared as anoxic 100 mM aqueous solutions. To produce Fe III, $FeCl_3$ was adjusted to pH 7.0. Each compound was added to 10 mM final concentration. Analytes were quantified using an HPLC equipped with a C-18 reverse phase column and a fluorescence detector. **Results:** Neither 4-MP or 3-MI levels differed from control culture levels when *C. scatologenes* ATCC 25775 was grown in BHI medium supplemented with Na_2SO_4, KNO_3, or MnO_2**.** However, at 48 hr of growth in BHI, 4-MP levels were 18.3±0.8 and 38.9±1.7 mg ml^{-1}, for control and Fe III cultures, respectively. Also, at 48 hr of growth in BHI, 3-MI levels were 119.5±4.8 and 171.6±3.2 mg ml^{-1}, for control and Fe III cultures, respectively. When semi-defined medium +1% casamino acids was used, 4-MP levels at 24 hr of culture were 0.025±0.002 and 0.124±0.007 for control and Fe III cultures, respectively. Similarly, 3-MI levels at 24 hr of culture were 0.038±0.004 and 0.120±0.008 for control and Fe III cultures, respectively. **Conclusions:** *C. scatologenes* ATCC 25775 did not exhibit increased culture supernatant levels of 4-MP or 3-MI in response to Na_2SO_4, KNO_3, or MnO_2. However, addition of Fe III increased both 4-MP and 3-MI levels in BHI medium and in semi-defined +1% casamino acids medium.

Keywords: 4-methyl phenol, p-cresol, 3-methyl indole, skatole, ferric iron

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O-1.1

New insights into the prokaryotic community of the mucosal fraction of human ileum: A metagenomic approach

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In order to investigate the functions of uncultured microorganisms associated with the human ileum mucosa, a 20,000 clone-fosmid library was constructed from an ileum segment obtained from an individual. The high molecular weight DNA obtained was used for both constructing a fosmid library and performing PCR-based 16S rDNA archaeal and bacterial molecular inventories. A total of 76 clones from the metagenomic library were randomly selected, and full inserts were sequenced. Diversity of the fosmid inserts, based on protein identification, compared, at a phylum level, with its counterpart from the 16S rDNA molecular inventory performed on the same DNA: the dominant members of the ileum mucosa are part of the *Bacteroides* and *Firmicutes* phylum. Annotation of 76 full 40 kb fosmid inserts provided preliminary data of the putative functions of the microorganisms. Among the set of 1172 putative proteins, we will highlight the genes indicating the occurrence of an anaerobic trophic chain in the ileal mucosa, such as genes involved in sugar and carbohydrate catabolism. Sequence comparisons with the published TIGR metagenomic dataset were performed. Among the 43 fosmids with no hits with the published TIGR metagenomic dataset, 37 corresponded taxonomically to the Bacteroidetes phylum. Conversely, strong matches were observed for 12 fosmid inserts, described taxonomically as Firmicutes and *Enterococcus*. Two examples of biological validations of annotations will be given. Firstly, the expression of several proteins of interest could be obtained in the host strain background. We will show the xylanase activity assay and substrate utilization by fosmid clones. For the latter, the whole ileum fosmid library was screened for its capacity to grow on sugars and polymers including cellulose and cellobiose, on mucin and its components (fucose, N-acetyl glucosamine, N-acetyl galactosamine) and on the prebiotics GOS and FOS. Secondly, the prevalence of bacteriophages in our sequence data set (4/76 inserts consisted entirely of phage genes), prompted us to investigate the abundance and diversity of Virus-Like-Particles in human GIT. VLP numbers averaged 3.5×10^8/g in fecal samples and strikingly 1.2×10^9/biopsy in the mucosal samples, indicating that the mucosal environment of the intestine may represent an ecological niche for dense communities of bacteriophages.

Keywords: metagenome, carbohydrate, bacteriophages, functional screening, diversity

O-1.2

Analysis of gastrointestinal functions in disease and health: Molecular microbial ecology of the human gut

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Many bacterial functions which reside in the human gut can be either beneficial or detrimental to the host. Since many bacteria are still uncultured in this ecosystem we undertook to investigate the ecology of the bacterial residents and their functions in the gut and relate them to diseases commonly associated with this organ. We used molecular based approaches to look at the bacterial diversity (16S rRNA gene DGGE and RISA) in several disease situations: Crohn's disease (CD), Polyposis (PP) and Colorectal cancer (CRC). We investigated the temporal stability and diversity of the dominant bacteria, the *Bacteroides*, Clostridia (*C. leptum* and *C. coccoides*), Bifidobacteria, and Lactic acid Bacteria in CD, CRC and PP. Investigation of the temporal stability of bacteria involved taking faecal samples from all patients over several time points, n = 11; CD in remission; n = 5, CD relapsed (a total of 49 samples were processed for CD), n = 22 for CRC and n = 20 for PP (a total of 154 samples were processed for CRC and PP). In addition, metabonomics analysis of the faecal water was undertaken for a subset of samples from each of the diseases in order to determine metabolite profiles. This analysis was performed on CD (n = 10), CRC (n = 10) and PP (n = 10) using high resolution ^{1}H NMR spectroscopy and multivariate pattern recognition techniques. In all cases the data was compared to a healthy cohort of volunteers. The analysis of the CD subjects presented an image of an ecosystem in flux. The stability of the dominant bacteria was much less when compared to the control group. In addition, a significant proportion of the group showed a loss of Clostridia and *Bacteroides* species and this was reflected in a change in the host's metabonome and in particular the short chain fatty acid profile. The CRC groups showed a similar stable bacterial community to healthy subjects, however, the diversity of the Clostridia was significantly increased, but there was no significant alteration of the SCFA profiles.

Keywords: bacterial functions, gut diseases, molecular approaches, bacterial diversity

O-1.3

Functional analysis of the ileum microbiota

E.G. Zoetendal[1,2], C.C.G. M. Booijink[1,2], H. Smidt[1,2], W.M. de Vos[1,2], and M. Kleerebezem[1,3]
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The microbial biomass in our gastrointestinal (GI) tract consists of a myriad of microbes that are pivotal to health and disease. In recent years, 16S ribosomal RNA-based approaches provided a phylogenetic framework of more than 1,200 microbial species inhabiting our GI tract and gave insight into the temporal, spatial and inter-individual microbial diversity. Nevertheless, most studies have focused on the colon, while insight into structure, dynamics and functionality of the small intestinal microbiota remains limited. To study the microbial ecology of the small

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intestine we combined a metagenomics and metatranscriptomics approach. Effluent samples from an ileostomy patient were used as model for ileum samples to realize multiple sampling without the use of invasive procedures. Ileostomy patients have their colon removed. As a consequence the small intestinal content leaves their body at the end of the ileum via a stoma. High molecular weight DNA was isolated from the stoma effluent and was used to construct a metagenomic library consisting of 25,344 fosmids (~700 Mb). Partial end-sequence analysis provided a framework of 13 Mb of microbial sequences from which more than 75% did not show a significant hit when compared to 50 non-redundant microbial genomes and a fecal metagenome. For metatranscriptomic analyses RNA was extracted from the same effluent and profiled by cDNA- Amplified Fragment Length Polymorphism (cDNA-AFLP) after mRNA enrichment. Sequence analysis of 48 cDNA-AFLP amplicons revealed that 31 sequences encoded bacterial transcripts. The remaining sequences encoded bacterial rRNA (6), human RNA (2) and sequences without a significant database hit (9). Using PCR and blotting approaches, 7 of 14 selected *in situ* expressed genes could be detected in the metagenomic library. These included genes encoding proteins involved in carbohydrate metabolism (4) and hypothetical proteins (3). Currently, the full inserts of those fosmids that were identified by this screening method are sequenced to gain insight into the gene context and potential pathways in which these genes are involved. Overall, our data indicated that the combination of metatranscriptomics and metagenomics provides insight into the *in situ* gene expression of uncultured microbes in the ileum.

Keywords: metagenomics, metatranscriptomics, human ileum microbiota

O-1.4

Microbial Colonization And Subsequent Biofilm Formation By Ruminal Microorganisms On Fresh Perennial Ryegrass

O.L. Mayorga[1,2], S.A. Huws[1], E.J. Kim[1], A.H. Kingston-Smith[1], C.J. Newbold[2], and M.K. Theodorou[1]
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It is widely recognised that ruminal microorganisms attach to forages as a strategy to optimally access available nutrients. In nature attached microbes commonly exist as biofilms, yet there is very little mention of this architecture and it's implications within the rumen ecosystem. The purpose of this work was to investigate the occurrence of biofilm communities on fresh perennial ryegrass and its components (leaf and stem) as well as investigate the temporal colonization of fresh plant material. *In vitro* incubations were set up in media with 10% strained rumen fluid and 5% fresh cut (approximately to 5 mm) 6-week old whole perennial ryegrass (WP), leaf (L), or stem (S) material, and attached communities were harvested at regular time intervals. Scanning electron microscopy was used to follow the biofilm formation and the quantity and composition of the EPS (extracellular polymeric substances) in terms of protein, carbohydrate, and DNA were also assessed following chemical extraction (3% glutaraldehyde in phosphate buffered saline, pH 7.4). Temporal microbial colonization was assessed using denaturation gradient gel electrophoresis (DGGE) and total eubacterial quantitative PCR (qPCR). Forage degradation was measured by the *in vitro* dry matter digestibility (IVDMD) technique. Scanning electron microscopy and EPS extraction data revealed that biofilm formation was greatest at 2 h for WP, 1 h for L, and 4 h for S after incubation. Forage degradation after 24 h of *in vitro* incubation for WP, L and S was 76%, 69% and 59%, respectively. At maximum EPS production (average 42.7microgram for WP, 28.6microgram for L, and 29.73 microgram for S) carbohydrate, protein and DNA composition averaged at 62.7%, 11%, and 5.6% for WP, 37.5%, 27.1%, and 0.9% for L and 56.9%, 28.9%, and 1.1% for S respectively. DGGE revealed that time dependent sub-clusters were apparent for attached (for WP, L, and S) communities, with earlier (0 to approximately 4 h) and later time points (8 and 24 h particularly) forming reasonably distinct clusters. It was also evident that attached communities on leaf and stem material clustered distinctly from each other, but following 24 h of incubation distinct clustering was no longer apparent. qPCR data revealed that maximum attachment for WP, S, and L was at 8 h (approximately 4.5 Log_{10} 16S rRNA copy number bacteria (ng/g remaining dry matter), and the kinetics of attachment were similar for all of subtracts. In conclusion, we have demonstrated the occurrence of dynamic biofilms on plant material under conditions mimicking the rumen. We are now investigating the consequences of this process on nutrient use efficiency.

Keywords: rumen, biofilm, *in vitro*, DGGE, and qPCR

O-1.5

Comparative Metagenomics Of The Rumen Microbiome By Random Sampling Pyrosequencing

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The complex microbiome of the rumen functions as a uniquely effective system for the conversion of plant cell wall biomass to microbial protein, short chain fatty acids, and methane. In order to gain a greater understanding of the ecology of this microbiome, we used comparative metagenomics to examine 838,900 reads (79,913,722 bases) of random sampled 454 pyrosequence data from the fiber-associated microbiomes of three different bovine rumens. We also compared these sequences with 251,716 reads (24,016,021 bases) from a single pooled microbiome from the planktonic microbiomes from these same three animals. These sequence reads were compared using either the BLASTX algorithm (E value $<1\times10^{-5}$) for coding sequences or the BLASTN algorithm for 16S rDNA genes (E value $<1\times10^{-5}$, length hit >50 bases). The distribution of identified phylotypes was dominated by *Bacteroidetes*, *Bacilli*, *Clostridia* and *Gammaproteobacteria*, and was strikingly different for the fiber-associated microbiome from the rumen of animal #710. Sixty-six percent of the identified 16S rDNA sequences from this animal were *Gammaproteobacteria*, whereas in the other fiber-associated samples were only 4.3 to 8%. Moreover, there was a shift between the distribution of phylotypes between the *Bacteriodetes* and *Bacilli*, when compared to the other fiber-associated samples. We also used the subsystems-based annotations available in the SEED database to gain a better understanding of the metabolic potential of these microbiomes. The distribution of coding sequences is remarkably congruent with the distribution of 16S rDNA phylotypes. The fiber-associated microbiome from the rumen of animal #710 contains significantly more coding sequence hits to metabolisms that are consistent with a community that has shifted from a carbohydrate-based metabolism to a protein and amino acid based metabolism. We also compared the glycoside hydrolase and cellulosome (dockerins and cohesins) functional gene content in

the different microbiomes. These genes are in lower abundance in the fiber-associated microbiome from the rumen of animal #710, congruent with the apparent shift in metabolism. This analysis showed that even though the three animals were fed the same diet, the community structure and metabolic potentials were markedly different from each other.

Keywords: pyrosequencing, metagenome, rumen

O-1.6

Microbial Communities Using Real Time PCR And Fermentation Parameters *in vitro* As Affected By A Natural And A Synthetic Anti-Methanogenic Agent

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A crude protein preparation and a purified protein from a tropical plant and bromochloromethane were evaluated for methane reduction activity, effects on fermentation parameters, and microbial population when incubated (2.5 and 5 mg per ml of the protein and 10 µM bromochloromethane) with roughage based substrate (hay: 380 mg, 40 ml incubation buffer) using the Hohenheim gas test for 24 h. The crude protein preparation decreased methane production by 7.5 and 18% on volume basis, and by 8 and 21% when expressed as 'ml methane produced/ 100 mg truly degraded substrate (M/TDS)'. These two levels of the protein increased the Partition Factor, PF (an index of efficiency of microbial protein synthesis – the higher the PF, higher is the efficiency) from 3.39 to 3.42 and 3.48, and decreased molar proportion of acetate to propionate from 3.11 to 2.7 and 1.8. When compared to the control, 73%, 71%, and 42% reductions in methanogens, *Ruminococcus flavefaciens,* and ruminal fungal population, respectively, and 21% increase in population of *Fibrobacter succinogenes* relative to total bacterial population were observed when the crude protein preparation was used at 5 mg level. A protein purified from the tropical plant increased the PF from 3.39 to 3.52 and 4.30 at 1.25 and 2.5 mg per ml. This protein reduced methane production by 15.7 and 35.7% on volume basis, and by 35.7 and 49% when expressed as M/TDS. Decreases in molar proportion of acetate to propionate, from 3.11 to 2.65 and 1.73 at these two levels of the pure protein were observed. Reductions of 83%, 71% and 42% in methanogens, *R. flavefaciens* and ruminal fungi population and a 4% increase in population of *F. succinogenes* were observed on using the pure protein at 2.5 mg level. A wide variation was observed within different treatments at different days using bromochloromethane, probably due to its volatile nature. At a concentration of 10 µM, 94% reduction in methane on both the volume basis and on M/ TDS basis was observed. The PF increased from 3.02 to 3.57 while acetate: propionate proportion decreased from 3.06 to 2.84. A complete inhibition of methanogens, 48% decrease in *R. flavefaciens*, and 68% increase in *F. succinogens* and 30% increase in rumen fungi were observed when quantified relative to total bacterial population. In summary, the protein and bromochloromethane have potential to reduce methane production and to increase efficiency of rumen fermentation.

Keywords: natural anti-methanogenic agent, bromochloromethane, methane, microbial population

O-1.7

Resistant Starch As A Vehicle For Delivering Health Benefits To The Human Large Bowel

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Non-starch polysaccharides (NSP; major components of dietary fibre) are effective faecal bulking agents and laxatives but have proved rather disappointing in the prevention and management of large bowel inflammatory diseases (IBD) or colo-rectal cancer (CRC). Resistant starch (RS) is that starch fraction which escapes small intestinal digestion and enters the large bowel. RS contributes to total dietary fibre and may be as important as NSP in promoting large bowel health. Unlike NSP, RS is a poor bulking agent and acts largely (but not exclusively) through its bacterial fermentation products. In adults, these are the short chain fatty acids (SCFA) principally acetate, propionate and butyrate. Collectively, SCFA have several non-specific positive actions on large bowel physiology including lowering of luminal pH. However, butyrate has attracted the most attention. It is a major metabolic fuel for colonocytes and it seems to promote a normal phenotype in these cells. Recent data from our laboratory support the latter suggestion. We have shown that, in rats, higher dietary protein (as casein, red meat or soy) increases colonocyte genetic damage and thinning of the colonic mucus barrier. However, feeding of RS as a high amylose maize starch ameliorated both of these changes in proportion to increased colonic butyrate. These data accord with prospective population data showing lower CRC risk with consumption of total dietary fibre. RS intakes appear to be low in most affluent industrialized countries so increasing its consumption by modifying consumer foods is one strategy to improve public health. CSIRO and its partners are developing new high amylose cereal cultivars for this purpose. Colonic delivery of specific SCFA could also be useful clinically and we have shown that acetylated, propionylated and butyrylated starches resist small intestinal amylolysis. In the large bowel these starches are metabolized by the microflora releasing SCFA and raising their digesta levels with the greatest increase being in the esterified acid. Feeding studies with butyrylated starch in rats have confirmed the opposition of diet-induced genetic damage, supporting a role for this SCFA in promoting colonic health. Further human and animal interventions are planned to determine the health potential of these new types of RS.

Keywords: starches, large bowel, short chain fatty acids, humans, bacterial fermentation

O-1.8

RNA Stable Isotope Probing – Direct Identification Of Starch Fermenting Bacteria In The Human Colon

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The human colonic microbiota comprises a complex microbial ecosystem vital for human gut health. An essential function of this community is the fermentation of dietary substrates, which escape digestion in the upper digestive tract. Such substrates, in particular dietary fibers, prebiotics and resistant starch (RS), are known to be important promoters of intestinal health, mainly through their interactions with the human colonic microbiota. However, these interactions are far from being fully understood. To provide a link between distinct groups of microorganisms and the fermentation of dietary relevant carbohydrates in the human colon we used RNA-stable isotope probing (SIP). In a pilot study, the microbial community of an *in-vitro* model of the human colon (TIM-2) was supplemented with 1 g of [U-^{13}C]-starch (~98% ^{13}C; isolated from potatoes grown in presence of $^{13}CO_2$). Within

8 h starch was fermented yielding acetate, butyrate and propionate. After 4 and 8 h of incubation with [U-^{13}C]-starch, isotopically labeled RNA could be isolated. 16S rRNA-based phylogenetic analyses revealed a microbial community consisting of four major groups: *Bifidobacterium, Bacteroidetes, Clostridium* cluster IV *(Clostridium leptum* group), as well as *Clostridium* cluster XIVa (*Eubacterium*, *Dorea*, *Roseburia* and *Ruminococcus*). Terminal-restriction fragment length polymorphism analyses indicated that species closely related to *Ruminococcus bromii*, *Bacteroides-Prevotella* group and *Eubacterium rectale* were strongly involved in starch metabolism *invitro*. Integrating molecular and metabolite data, it can be hypothesized that there is metabolic cross-feeding in the system, where species closely related to *Ruminococcus bromii* and the *Bacteroides-Prevotella* group ferment starch to acetate, which is subsequently utilized by *Eubacterium rectale* for the formation of butyrate. Further studies on this metabolic cross-feeding are underway to confirm our assumption. In conclusion, RNA-SIP combined with metabolic analyses using ^{13}C-labeled substrates proved instrumental for the identification of bacteria actually involved in colonic fermentations and link them to metabolic products in the course of *invitro* studies, opening promising avenues for unraveling functionality of the intestinal microbiota *in vivo*.

Keywords: carbohydrates, stable isotope probing, colon, digestion

O-1.9

Prevotella bryantii 25A Used As A Probiotic During The Transition Period In Dairy Cows: Effect On Ruminal Fermentation Characteristics And Milk Production

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Rumen sub-acute acidosis is considered to be one of the most important nutritional disorders in the feedlot and dairy industries today. The economic impact is estimated to be in the billions of dollars. It is occasioned by the ingestion of high levels of rapidly fermented carbohydrates which exceed the buffering capacity of the rumen and causes a rumen pH drop. Ruminal acidic conditions result in animal discomfort, anorexia, depression, decreased digestibility and decreased milk production. Probiotics represent an alternative to the use of antibiotics to alleviate those symptoms. In the present study we have introduced daily a strain of *Prevotella bryantii* (25A) which was shown to ferment carbohydrates without producing lactate but rather succinate which is further metabolized to propionate. A total of 12 rumen-fistulated cows were used. Three weeks before calving, all cows received an increasing amount of an energy dense diet containing barley grains and were distributed evenly between the control group and the group receiving *P. bryantii*. The cows were fed once a day. Six cows were given daily a dose of *P. bryantii* (2×10^{11} cells/dose) from three wks pre-partum up to 7 wks post-partum. Rumen fluid was sampled prior to feeding as well as 2 h and 3 h post-feeding on the third week pre-partum and on weeks 1, 2, 3, 4, 6 and 7 post-partum. Feed intake was recorded daily as well as milk yield and composition up to wk 7 of lactation. Rumen fluid samples were analyzed for pH, VFA, NH_3 and lactate. Feed intake was similar between control and treated cows. *P. bryantii* did not change milk production but milk fat tended to be greater ($P < 0.06$) in treated cows compared with control cows (3.9 vs 3.5%). Rumen pH was similar between the two groups. Rumen lactate concentration was similar before feeding between control and treated cows, however, 2 to 3 hours following feeding, lactate concentration was lower ($P < 0.05$) in cows receiving *P. bryantii* compared with control cows (5.8 vs 11.6 mg/dl). Ammonia concentration was greater ($P < 0.03$) in treated cows than in control cows (175 vs 141 µg/ml). Acetate (65.5 vs 57.8 mM), butyrate (12.7 vs 10.5 mM) and isobutyrate (0.90 vs 0.75 mM) concentrations were greater ($P < 0.01$) in post feeding samples of treated cows. Supplementing early lactating cows with *P. bryantii* 25A modified ruminal fermentation characteristics.

Keywords: *Prevotella bryantii* 25A, transition dairy cows, probiotics

O-1.10

The Primary Starch Utilization Operon Of The Obligate Anaerobe _Bacteroides fragilis_ Is Regulated By Carbon Source And Oxygen, And Expression Is Important For Survival During Oxidative Stress

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The obligate anaerobe *Bacteroides fragilis* is a commensal organism of the human large intestine where it utilizes both dietary and host-derived polysaccharides as a source of carbon and energy. This study describes the characterization of a novel operon, designated oxygen-induced starch utilization (*osu*), which encodes four genes, *osuABCD* whose translational products are involved in starch utilization. The first three genes of this operon, *osuABC*, are predicted to encode outer membrane/starch binding proteins, whereas the fourth gene, *osuD* has been shown to encode the primary α-amylase involved in starch hydrolysis in this organism. We have identified a gene, *osuR*, which encodes a transcriptional activator of this operon, responsive to maltose and higher glucosides. This obligate anaerobe is also an opportunistic pathogen and has evolved a complex oxidative stress response (OSR) to allow it to combat the toxic effects of oxygen exposure outside of the anaerobic confines of the GI tract, where it is the most frequent isolate from anaerobic infections such as intra-abdominal abscesses. The results of this study demonstrated that expression of the *osu* operon was oxygen-responsive, with expression of *osuA* induced approximately 20-fold upon exposure to air. Differential expression of *osu* results from oxygen stress or anaerobic starch utilization. The transcriptional activator OsuR, although involved in anaerobic regulation of this operon, did not play a role in activation during oxygen exposure. Consistent with this observation a shift in the transcriptional start site was observed during oxygen stress, compared to anaerobic growth on starch and this suggests the involvement of a different regulator under these conditions. Building on the hypothesis that expression of this operon was important for survival during oxidative stress, results from *osu* mutational studies demonstrated that expression of the *osu* operon was important for survival of *B. fragilis* during oxygen exposure. The mechanism of *osu* protection has not been fully determined but may involve providing a utilizable energy source to the cell during pathogenesis to maintain the OSR during oxygen exposure, thereby enhancing the pathogenic potential of *B. fragilis*.

Keywords: *Bacteroides*, anaerobe, starch, oxygen

O-1.11

Changes Of Natural Food Antioxidants In The Intestinal Tract And Its Influence On Human Faecal Microflora

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Food is considered as a major source of exposure to ingested contaminants. Intestinal microflora is the main component of the

digestive tract and plays an important role in digestive functions. The role of colonic microflora remains generally unclear regarding both polyphenol degradation and formation of phenolic compounds and other food components, as well as microorganisms responsible for that action and mechanisms involved. The aim of this research was to analyse changes in microbial composition and metabolic function of colonic microflora as well as interaction of intestinal microflora with biologically active compounds. Extruded products from Red Kidney bean (chips) and chokeberry juice (*Aronia melanocarpa*) were selected for this project. The digestive tract model, which comprises 3 parts: stomach, small intestine and large intestine, was used for controlling and regulating the environment in which digestion processes occurred. In each parts of the model the total amount of phenolic compounds (mg gallic acids), antoxidant $ABTS^{\cdot +}$ (mg Trolox) activity as well as polyphenols (quantitative and qualitative) and endproducts of microflora metabolism (short-chain fatty acids) were determined by HPLC. Changes in microbial composition were controlled during the digestion process. Faecal flora was isolated from feces of 3 human volunteers. The intestinal model was inoculated with bacterial flora at concentration of 10^6 cfu/ml. Growth of *Lactobacillus, Bifidobacterium, Entercoccus* and *Enterobacteriacea* was enumerated on selective media. The numbers of bacteria after digestion in the large intestine reached 10^6 cfu/ml for *Enteroccocus* and 10^8 cfu/ml for other groups of microorganisms. The product from Red Kidney beans stimulated bacterial growth in the model intestinal tract while chokeberry juice inhibited *Enteroccocus* growth. The main microbial metabolic endproducts observed in the digested material were short-chain fatty acids. Lactic acid was the main product detected in the digested Red Kidney bean product. The highest antioxidant activity, as well as amount of phenolic compounds after the digestion process, was determined in red bean chips, while a decrease in antioxidant activity and total amount of phenolic compounds was observed for aronia juice. Quantitative and qualitative analysis of compounds in chokeberry juice showed decreases in phenols, especially anthocyanins

Keywords: polyphenol, antioxidant activity, faecal human microbiota

O-2.1

Commensal-Derived Probiotics As Anti-Inflammatory Agents

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Indigenous bacteria in the mammalian gastrointestinal tract comprise a rich ecosystem of microorganisms and secreted factors with direct effects on intestinal immunity. Commensal constituents of the gastrointestinal microbiota include lactic acid bacteria such as *Lactobacillus* species. The genus *Lactobacillus* includes a restricted set of species that is derived from an amalgamation of greater than 100 species. Scientific challenges include the definition of indigenous species and the isolation of immunomodulatory bacterial clones from a vast array of intestinal microbes. Our studies have resulted in the identification of *Lactobacillus* strains from mice and humans with potent anti-inflammatory effects. Mouse-derived *Lactobacillus paracasei* and *Lactobacillus reuteri* strains were isolated from healthy mice lacking evidence of intestinal inflammation. Less than 10% of commensal *Lactobacillus* strains had potent TNF-α-inhibitory activity. These anti-inflammatory strains suppressed the production of murine TNF-α by macrophage cell lines and abrogated inflammation in murine colitis models. Human-derived *L. reuteri* strains have been identified with potent human TNF-α-inhibitory effects when combined with LPS-activated human myeloid cell lines and primary monocyte-derived macrophages from children with Crohn's disease. Human commensal-derived probiotics suppress transcription of the human TNF-α gene by inhibition of MAP kinase signaling via AP-1. Alternatively, commensal-derived human *L. reuteri* strains suppress NF-κB signaling in TNF-α-stimulated human myeloid cells. Suppression of NF-κB signaling in TNF-stimulated myeloid cells promotes apoptosis of activated immune cells and provides a novel mechanism for anti-inflammatory action in the mammalian intestine. In summary, commensal-derived immunoprobiotics provide opportunities for understanding mechanisms of microbial-host interactions in the gastrointestinal tract. By understanding mechanisms of probiosis, novel therapeutic strategies may be developed for prevention and treatment of inflammatory bowel disease and other intestinal disorders

Keywords: commensal, immunomodulation, inflammation, *Lactobacillus*, probiotics

O-2.2

Bacterial Imprinting Of The Neonatal Immune System: Lessons From Maternal Cells

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Breast milk is suspected to convey live bacteria. We have examined the presence of a natural bacterial inoculum in breast milk and its intracellular transport from the maternal intestine to the breast via the circulation. The presence of viable bacteria in breast milk and peripheral blood, collected aseptically from healthy donors at various times postpartum, was determined by plating. Temporal Temperature Gradient-gel Electrophoresis (TTGE) was used to examine bacterial rDNA patterns in maternal feces, peripheral blood mononuclear cells (PBMC) and milk cells, and in corresponding infant feces. Bacterial translocation to extra-intestinal tissues was also evaluated in virgin, pregnant and lactating mice. Breast milk contained a low, total concentration of $<10^3$ cfu/mL microbes. TTGE revealed a high diversity of bacterial signatures in maternal blood and milk cells. Some bacterial signatures were common to infant faeces and to samples of maternal origin. Bacterial translocation from the gut to mesenteric lymph nodes and mammary gland occurred during late pregnancy and lactation in mice. Hence, intestinally derived bacterial components are transported to the lactating breast within mononuclear cells. This phenomenon could contribute to program the neonatal immune system to recognize specific molecular patterns of intestinal commensal bacteria of maternal origin

Keywords: neonate, bacteria, breast milk, transmission

O-2.3

Development Of A Novel Synbiotic Targeted Towards Dogs

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Dogs and cats are now considered as family members. Pet owners seek for nutritional diets that assure the health of their pets and therefore improve their quality of life. The Pet Food Industry has recently considered the use of diets enriched with functional foods such as probiotics and prebiotics in order to promote a better balanced diet that helps the capacity to resist disease and thereby enhance health. The overall aim of this

project is the development of a synbiotic that supports and enhances the beneficial effect of the probiotic bacteria present in the canine GI tract and reduces the numbers of pathogens that cause frequent GI diseases. For the selection of a potential probiotic strain *Bifidobacterium longum* 05, *Bifidobacterium bifidum* 02450B, *Lactobacillus acidophilus* 14150B and *Lactobacillus plantarum* 115400B were studied for their ability to utilise and grow on various carbohydrate substrates, in order to find out on which oligosaccharides further investigation should be focused. *B. bifidum* 02450B and *L. acidophilus* 14150B showed the highest growth rates with all carbohydrates. Inulin, fructo-oligosaccharides (FOS), rice bran, citrus pectin, galacto-oligo-saccharides (GOS) and banana flakes were selected to be further evaluated in a mixed bacterial environment for their prebiotic potential. The prebiotic properties of the oligosaccharides were studied using stirred, pH controlled batch cultures using faecal samples of three Golden Retriever dogs as inoculum. Changes in bacterial population numbers were determined by fluorescence *in situ* hybridization (FISH). All substrates showed an increase of 2 log in lactobacilli and bifidobacteria at 24 h of fermentation, however GOS demonstrated the best prebiotic properties over the 24 h of fermentation. Bifidobacteria and lactobacilli population numbers increased during the first 24 h while clostridia numbers showed a 10 fold decrease. GOS also showed no changes in bacteroides numbers over the fermentation time. This study suggests the potential prebiotic properties of GOS that could be enhanced by the addition of a suitable probiotic strain so as to develop a synbiotic mixture targeted towards dogs. Antimicrobial activity data against *Campylobacter jejuni*, *Clostridium perfringens* and *Salmonella typhimurium* is currently being measured

Keywords: probiotic, prebiotic, synbiotic, companion animals

O-2.4

Improved Criteria for Probiotic Strain Selection

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The objective of this study was to assess whether a comparative *in vitro* evaluation of the biological properties of different *Bifidobacterium longum* strains could lead to a better definition of the probiotic concept linked to the *in vivo* functionality of the so-called probiotic strains. Different *B. longum* strains including currently commercialized probiotics, human commensals, and reference strains were studied. The ability of these stains to interact with the host was comparatively evaluated by assaying their adhesion to mucin and their immunomodulatory properties on peripheral blood mononuclear cells (PBMC). The studied *B. longum* strains showed significantly different adhesion ability to mucin. The percentage of adhesion of these strains ranged from 2 to 11%. The studied probiotic strains were not those showing the highest adhesion ability, although this has been considered to be a positive trait of probiotics to ensure their residence and interaction with the human host. The mechanisms of adhesion of these strains were studied, and the results point for a role of proteins associated to the cell-envelope of bifidobacteria. The studied *B. longum* strains also showed differences in their ability to stimulate cytokine production by PBMC. This leaded to differential increases in inflammatory (TNF-α) and regulatory (IL-10) cytokines that could have opposite effects on the host in healthy and disease situations. This could be particularly important for the administration of probiotics to population groups immunocompromised or prone to inflammatory biased immune responses. Overall, *Bifidobacterium* strains belonging to the same species showed remarkable differences in the biological properties related to their interaction with the host epithelial surface and immune system. The properties of particular probiotic strains were not always those desired according to the criteria generally accepted for their selection (FAO/WHO; 2001). The establishment of soundest links between the *in vitro* and *in vivo* evaluation tests of probiotic strains as well as the identification of biological markers of their functional traits will be of pivotal importance for their rational selection and use, particularly for risk population groups

Keywords: *Bifidobacterium*, probiotics, adhesion, immunomodulation

O-2.5

Fate And Effects Of The Broad-Spectrum Bacteriocin Lacticin 3147 In The Mammalian Gut Following Oral Ingestion

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Lacticin 3147, a two-component bacteriocin, composed of the peptides LtnA1 and LtnA2, has a broad spectrum of antimicrobial activity. The aim of this study was to investigate the fate and effects of lacticin 3147 in the mammalian gut following oral ingestion, using pigs as a model for humans. Initial *in vitro* experiments showed that lacticin 3147 is unaffected by either pepsin or trypsin, but is inactivated by the small intestinal enzyme α-chymotrypsin. *Ex-vivo* experiments confirmed this, as lacticin 3147 activity was retained during 2 h of incubation in porcine gastric digesta but was reduced by 50% following 5 h of incubation in ileum digesta. Lacticin ingestion was then evaluated in pig-feeding trials. Firstly, one group of weaned pigs ($n = 10$) was fed reconstituted lacticin 3147 skim milk powder for seven days (1 million AU lacticin per day) while a control group ($n = 10$) was fed reconstituted skim milk. No differences in faecal counts of *Lactobacillus*, *Enterococcus*, coliform or total anaerobes were observed between lacticin-fed and control pigs ($P > 0.05$) and no lacticin 3147 activity was detected in the faeces. In a separate experiment four pigs were fed either reconstituted lacticin 3147 skim milk powder (1 million AU lacticin) or reconstituted skim milk and sacrificed 2 h later. There were no differences in *Lactobacillus*, *Enterococcus*, coliform or total anaerobe counts either in the stomach or jejunum digesta between lacticin-fed and control pigs ($P > 0.05$). In addition, neither LtnA1 nor LtnA2 were detected by MALDI-TOF mass spectrometry in the jejunum or ileum contents of the pigs fed lacticin. Overall, these data suggest that lacticin 3147 is degraded in the small intestine, thereby explaining its lack of effects on the gut microbiota following oral ingestion. In contrast, a previous study showed that the addition of lacticin 3147 directly to a faecal environment *in vitro* reduced Gram-positive populations, confirming that the lack of effects *in vivo* is due to lacticin degradation in the upper gastrointestinal tract. The implication of these findings is that ingestion of antimicrobial peptides such as lacticin is not likely to perturb the gut microbiota but that strategies that ensure targeted delivery to the colon (for example, encapsulation) would be necessary for therapeutic applications

Keywords: Lacticin 3147, antimicrobial, gut microflora, pig

O-2.6

Carbohydrate Metabolism In *Fibrobacter succinogenes*: What NMR Tells Us

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Fibrobacter succinogenes is a major rumen fibrolytic bacterium found in high numbers when ruminants are fed cellulose-rich diets. It produces a very efficient fibrolytic system comprising numerous enzymes, but the organisation and catalytic features of this system remain unclear. We have been using NMR to study carbohydrate metabolism by *Fibrobacter* spp., well before the term "metabolomics" was coined. We first analysed in detail the NMR spectra arising from resting cells of *F. succinogenes* provided glucose, cellobiose or cellulose as a substrate. We were able to show carbohydrate cycling and reversibility of some steps. The use of 1D and 2D ^{1}H and ^{13}C NMR showed the synthesis by the cells of unexpected oligosaccharides: maltodextrins, maltodextrin-1-phosphate, and other glucose derivatives. We also showed that maltodextrins and maltodextrins-1-phosphate were synthesised and excreted out of the cell then degraded, and could originate from exogenous sugars or endogenous glycogen. According to substrate and physiological state of the bacterium (resting or growing cells), metabolic deviations toward the synthesis of these oligosaccharides were observed. We have also used liquid and solid-state NMR to monitor wheat straw degradation by *F. succinogenes*. The originality of this NMR approach was to investigate the activity of an entire fibrolytic system on an intact complex substrate (wheat straw). No preferential degradation of amorphous versus crystalline cellulose was observed, nor of cellulose versus hemicelluloses. We showed for the first time a sequential activity of some enzymes of *F. succinogenes* S85 on wheat straw. The degradation of wheat straw by *Ruminococcus albus*, another major rumen cellulolytic bacterium, was also studied and it showed that the two bacteria act differently in the degradation process. In conclusion, our studies illustrate the utility of various NMR approaches to study and better understand sugar and polysaccharide metabolism in rumen bacteria

Keywords: carbohydrate metabolism, *Fibrobacter succinogenes*, NMR

O-2.7

Metabolomic Analyses Of The Gut Microbiota *in vitro* And *in vivo* Using ^{13}C-labeled Carbohydrates

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The aim of the overall program is to study the role of the colonic microbiota in gut health and disease. This is done by integrating state-of-the-art technologies with the development of novel tools. These include use of stable isotope techniques, development of novel gastroenterological methods and nano-technology. In this presentation we will focus on metabolomics using stable isotope (^{13}C)-labeled substrates. ^{13}C-labeled carbohydrates (glucose, lactose, inulin and starch) were used *in vitro*, in pigs and in human volunteers to study which metabolites are produced by the microbiota, by tracing the ^{13}C-label to the metabolites using NMR and LC-MS. This allows determination of microbial pathways that are active on these substrates as well as measurement of fluxes through these pathways. From a constructed database of NMR signals from gut microbial metabolites and from ^{13}C-isotopomer distributions in these metabolites (from NMR and LC-MS), a first-generation *in silico* model of the metabolic network of the microbiota has been generated with the model substrate glucose. On this substrate production of ^{13}C-labeled lactate, formate, acetate, butyrate and small amounts of succinate, aspartate, glycerol and ethanol were observed. The isotopomer patterns of acetate and butyrate indicated the presence of the Wood-Ljungdahl pathway of acetate formation from carbon dioxide and evidence of cross-feeding between different micro-organisms. This first-generation model was used to establish the microbial activity on the more relevant carbohydrates lactose, inulin and starch in an *in vitro* colon model. Clearly different activities were observed when studying the isotopomer distribution of the SCFA. For instance, the M+1 isotopomer of butyrate was only observed upon lactose fermentation, whereas the M+3 isotopomer was only evident with starch. Also, differences in time of peak enrichment and maximum enrichment were observed. For instance, the tracer-tracee ratios (TTR) for the M+2 isotopomer of butyrate were 9%, 16% and 22% for lactose, inulin and starch, respectively. By integrating these research disciplines with microbiological methods such as stable isotope probing (SIP) and analyses that focus on gut health parameters we start to unravel the processes that occur in the colon and their relevance to gut health

Keywords: gut microbiota, gut health, metabolomics, nanotechnology

O-2.8

Different Conjugated Linoleic Acids Are Formed By Different Mechanisms In Ruminal Digesta and Bacteria

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Samples of digesta from the ovine rumen and pure cultures of ruminal bacteria were incubated with linoleic acid (*cis*-9, *cis*-12-18:2; LA) in buffer containing 43% deuterium oxide in order to investigate the mechanisms of formation of different isomers of conjugated linoleic acids (CLA). In ruminal digesta, rumenic acid (*cis*-9, *trans*-11-18:2) and *trans*-9, *trans*-11-18:2 were the major CLA products, with smaller amounts of *trans*-10, *cis*-12-18:2 also formed. Mass spectrometric analysis indicated that the (M+1) isotopomers of rumenic acid and *trans*-9, *trans*-11-18:2 were 56–62% labelled, while *trans*-10, *cis*-12-18:2 was unlabelled. The deuterium atom in both labelled CLA was located at C-13. In pure culture, *Butyrivibrio fibrisolvens* and *Clostridium proteoclasticum* produced predominantly rumenic acid, which was labelled at C-13. Traces of *trans*-10, *cis*-12-18:2 produced by these bacteria were labelled in the same way. In contrast, the type strain and a ruminal isolate of *Propionibacterium acnes*, which produces *trans*-10, *cis*-12-18:2, formed an unlabelled product. Despite the labelling pattern indicating that ricinoleic acid (12-OH, *cis*-9-18:1) might be an intermediate, the rate of metabolism of ricinoleic acid in ruminal digesta was low. Ricinoleic acid was extremely toxic to pure cultures of ruminal bacteria. The *B. fibrisolvens* linoleate isomerase producing rumenic acid showed anomalous kinetics and appeared to have a cofactor requirement. It was concluded that the mechanism of formation of *trans*-10, *cis*-12-18:2 differs from other CLA isomers, particularly rumenic acid, in the rumen and that formation of the latter appears not be a simple isomerization reaction

Keywords: biohydrogenation, linoleate isomerase, rumenic acid

O-2.9

Hemicellulose Degradation By *Clostridium proteoclasticum* B316[T]

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Microbial degradation of forage material in the reticulo-rumen is the main source of energy for the ruminant animal. Complex plant hemicelluloses constitute a significant proportion of the energy in forage and degradation to their component sugar moieties requires specialised sets of genes. *Clostridium proteoclasticum*, a low G+C Gram positive bacterium within the *Butyrivibrio-Pseudobutyrivibrio* assemblage, is able to break down plant hemicelluloses and *in vitro* is an efficient degrader of xylan. The draft phase genome sequence of *C. proteoclasticum* has revealed the presence of at least 17 glycoside hydrolases, mostly classified as xylanases, xylosidases and arabinosidases. *In silico* analyses of *C. proteoclasticum* ORFs has also confirmed the presence of biochemical pathways utilizing arabinans and pentosans. Protein expression profiling of the secreted, membrane and intracellular fractions has demonstrated differential expression of hemicellulases and other carbohydrate metabolism-related proteins when *C. proteoclasticum* was grown on xylan or xylose. Growth phase-dependent variations were observed during the later stages of growth on xylan in the secretome and intracellular fractions. Most prominently, two secreted endoxylanases were dramatically upregulated in the presence of xylan, while the expression of endocellulases was found to be growth phase-dependent. Furthermore, tandem mass spectrometry identified a number of xylanolytic enzymes in the intracellular fraction, several of which appear to be involved in the removal of groups attached to the xylan backbone

Keywords: xylan degradation, proteomics, genome sequence, *Clostridium proteoclasticum*

O-2.10

Cloning, Expression, And Characterization Of A Beta-Xylosidase From The Hemicellulose Degrading Rumen Bacterium *Prevotella ruminicola* 23

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Ruminal microorganisms are capable of efficient hydrolysis of plant cell walls in the rumen. Therefore, they are a rich source of genes encoding enzymes essential for degradation of cellulose and hemicellulose, currently targets for biofuel production. Corn fiber, mainly composed of arabinoxylan, is a byproduct of the wet milling process. It accumulates in large amounts at processing plants, and its bioconversion to value-added products is of major interest. To hydrolyze arabinoxylan using a "green chemistry" approach, it is necessary to use a set of enzymes acting synergistically, and beta-xylosidase, one of the enzymes, releases xylose from xylooligosaccharides. From the closed genome sequence of *Prevotella ruminicola* 23, we identified a gene predicted to code for a beta-xylosidase (glycosyl hydrolase family 3). The gene was amplified, cloned, and expressed in *E. coli* cells. Since the recombinant enzyme had a six-Histidine-tag sequence at the N-terminus, we purified it using affinity chromatography and biochemically characterized the highly purified product. We tested six different *p*-nitrophenyl (pNP) substrates, and only pNP-beta-D-xylopyranoside was efficiently hydrolyzed. Using this model substrate, we determined pH and temperature optima (pH 5.0 and 45 C, respectively) and kinetic parameters of the hydrolysis reaction. Using a method that measures continuously the release of product (*p*-nitrophenol) at 45 C, the K_m was 0.374 mM, V_{max} was 5.7 micromol/min/microgram protein, and k_{cat} was $570\ min^{-1}$ or $9.5\ s^{-1}$. As expected, beta-xylosidase did not efficiently hydrolyze oat spelt xylan, but released xylose from xylooligosaccharides (X2-X6). Mutational analysis based on a computer generated three-dimensional structure of the beta-xylosidase is currently being undertaken

Keywords: *Prevotella ruminicola*, arabinoxylan, beta-xylosidase, enzyme kinetics

P-1.1

Methane Production And Microbial Evaluation By q-PCR Of *in vitro* Incubations Of Tannin-Rich Plants

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In vitro assays have been used for searching ways of inhibiting ruminal methane production. The aim of this study was to measure total gas and methane (CH4) production during *in vitro* incubation of shrubs to be used as potential agents for CH4 mitigation. Five plant species were used as substrates: *Leucaena leucocephala* (LEU), *Mucuna cinereum* (MCZ), *Mucuna aterrima* (MPR), *Mimosa caesalpiniaefolia* (SAN) and Tifton-85 (*Cynodon* sp) hay (TIF). The *in vitro* gas production (GP) assay was carried out in bottles incubated (39 C, 24 h) with 0.5 g of substrate, 50 mL of buffer and 25 mL of inoculum from Santa Ines sheep. Methane production (at 24 h) was estimated from the volume of gas and the gas composition data. Incubation was stopped and bottle contents were collected for evaluation of microbial numbers and the organic matter apparently digested (OMAD). DNA was extracted; purified, adjusted to 10 ng μL-1 and used as templates for qPCR. The small subunit rRNA genes were amplified using primer pairs for rumen bacteria (BACT), rumen fungi (FUNG), rumen methanogens (METH), *Ruminococcus flavefaciens* (RUMI) and *Fibrobacter succinogenes* (FIBRO) in a real-time thermal cycler. The comparative Delta-Delta Ct method for relative template quantification was used to quantify the percentage of the target population relative to a normalizer population (BACT). GP and CH4 production were significantly different among substrates. LEU, MCZ and MPR had significantly ($P<0.05$) higher GP values (134, 120 and 119 (SE 12.4) mL kg DM-1) than SAN and TIF (63 and 90 (SE 12.4) mL kg DM-1). Methane production was significantly ($P<0.05$) lower for SAN (22 mL Kg OMAD-1) than the other substrates (38, 42, 39 and 53 (SE 3.3) mL Kg OMAD-1 for LEU, MCZ, MPR and TIF). The relative abundance of FUNG and RUMI expressed as proportion of BACT was different among substrates, while FIBRO (1.4% (SE 0.566)) and METH (0.12% (SE 0.026)) were not different ($P>0.05$) among the substrates. FUNG expression was 0.16, 0.79, 0.70, 0.35 and 0.65% (SE 0.151) respectively for LEU, MCZ, MPR, SAN and TIF ($P=0.039$). TIF showed significantly ($P<0.01$) higher RUMI expression than in all other substrate (0.006, 0.015, 0.013, 0.007 and 0.084% (SE 0.0051) respectively for LEU, MCZ, MPR, SAN and TIF). The findings suggested that the studied shrubs may be suitable for CH4 mitigation strategies

Keywords: methane mitigation, rumen microbial diversity

P-1.2

Methane Emission, Protozoa And Methanogens Counts In Sheep Fed Coconut Oil Or A Brazilian Tannin-Rich Plant (*Mimosa caesalpineaefolia*)

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The aim of this work was to measure methane (CH_4) released by sheep fed coconut oil or a tannin-rich plant (*M. caesalpineaefolia*). Twelve Santa Ines sheep were allocated into 3 groups according to liveweight (LW $=47 \pm 11.9$ Kg). For 30 days, all animals were fed a basal diet (Tifton-85 hay (*Cynodon* sp), corn grain, soybean meal, cottonseed meal and mineral mixture). The control group (CNTRL) received only the basal diet and the tested groups received on top of that, 22 g $Kg^{-1}d^{-1}$ of coconut oil (CNOIL group) in the concentrate (2% in total diet) or 127 g $Kg^{-1}d^{-1}$ of *M. caesalpineaefolia* (SNSAO group) replacing the forage (13% in total diet). For CH_4 measurements, the animals were individually kept in four chambers (1.9 m^3) with an outflow rate of 168 L min^{-1} and the outgoing air sampled into bags by peristaltic pump at 100 mL min^{-1} rate. The gas collections were done on two occasions for each sheep, and CH_4 production was determined by GC. Rumen samples were collected at day 0 and day 28 and kept in a freezer. The extractable tannin content of *M. caesalpineaefolia* was 99, 59 and 72 g Kg^{-1} respectively for total phenol, total tannin (tannic acid equivalent) and condensed tannin (leucocyanidin equivalent) and tannin bioactivity was 234% (as increase in gas production in the presence of PEG). The forage to concentrate ratio of diets was 69:31; 43:57, and 66:34, for CNTRL, CNOIL, and SNSAO, respectively. Dry matter intake did not differ ($P > 0.05$) among treatments (0.9 (SE 0.21) Kg d^{-1} or 2.1 (SE 0.25% LW). LW changes were significantly different ($P < 0.044$) for CNOIL in relation to the other groups (-40, 101 and 125 (SE 38.1) g d^{-1} for CNOIL, CNTRL and SNSAO, respectively) and CNTRL released significantly ($P < 0.023$) more CH_4 (25 L d^{-1}) than CNOIL (12 L d^{-1}) or SNSAO (18 L d^{-1}) (SE 3.4). Methane release in relation to animal's metabolic weight ($Kg^{0.75}$) was significantly ($P < 0.05$) different among treatments (1.4, 0.7 and 1.0 (SE 0.12) L d^{-1} for CNTRL, CNOIL and SNSAO). Protozoa numbers showed a tendency ($P = 0.07$) to be lower in CNOIL and SNSAO than CNTRL (7.5, 8.5 and $10.7 \pm 1.05 \times 10^5$ cell mL^{-1}, respectively). It is concluded that coconut oil can reduce CH_4 release but it compromise animal performance, and there was evidence that supplementation with *M. caesalpineaefolia* has potential to mitigate CH_4 production in ruminants

Keywords: tanniniferous plants, mitigation strategies, ruminant

P-1.3

Effect Of 2-Nitro-Methyl-Propionate On Ruminal Methane Production And Its Metabolism *in vitro*

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The production of methane (CH_4) within the rumen results in the loss of 2–12% of the gross energy consumed by the host and contributes nearly 20% of the total CH_4 produced in the United States. A series of *in vitro* experiments were conducted to compare the CH_4-inhibiting ability of the known CH_4 inhibitor, nitroethane, and a newly synthesized nitrocompound, 2-nitro-methyl-propionate. In our first experiment, freshly collected ruminal fluid obtained from a pasture fed cannulated cow was incubated ($n = 3$/treatment) at 39°C under H_2:CO_2 (1:1) in 18 × 150 mm tubes (10 ml/tube) preloaded with 0.2 g alfalfa and with or without added nitrocompound. Consistent with earlier findings, CH_4 production after 24 h by incubations containing 2.5, 5.0 or 10.0 mM nitroethane was reduced ($P < 0.05$) 34, 92 and 96%, respectively, from that produced by controls containing no added nitrocompound (20.8 ± 1.9 μMol CH_4 ml^{-1} ruminal fluid). By comparison, CH_4 production was more effectively reduced ($P < 0.05$) from that produced by controls by incubations with 2.5 but not 5.0 and 10.0 mM 2-nitro-methyl-propionate (94, 94 and 97%, respectively). To test our hypothesis that 2-nitro-methyl-propionate would primarily be reduced by ruminal microbes to alanine, washed cell suspensions (in anaerobic buffer containing 60 mM formate) of nitroethane-enriched or none-nriched ruminal microbes (via 3 consecutive 24 h batch incubations with or without 12 mM nitroethane) were incubated under H_2:CO_2 (1:1) with 10 mM added 2-nitro-methyl-propionate. Analysis of fluids collected at 0, 6 and 24 h of incubation revealed that more than 23 and 63% of the 2-nitro-methyl-propionate had disappeared by 6 and 24 h of incubation, respectively, but rates of disappearance did not differ ($P = 0.22$) between nitroethane-enriched and nonenriched cells (0.34 ± 0.02 vs 0.26 ± 0.09 μMol 2-nitro-methyl-propionate ml^{-1} h^{-1}). In both cases, accumulations of alanine averaged less than 10% the amount of 2-nitro-methyl-propionate metabolized and accumulations of ammonia average < 1.0 μMol ml^{-1} thus indicating that 2-nitro-methyl-propionate is either incompletely reduced or catabolized to products other than alanine. Results confirm that 2-nitro-methyl-propionate exhibits considerable CH_4-inhibiting activity but the fate of its carbon and nitrogen has yet to be determined

Keywords: nitroethane, 2-nitro-methyl-propionate, methane-inhibitor

P-1.4

Profile Of Methane Emissions From Sheep Fed Oat Hay Basal Diet Supplemented With Ground Corn and Coconut Oil

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Five wether crossbred sheep (11 mouths old, with an average weight of 25 kg), were alternatively assigned to receive one of the following diet treatments (1) oat hay alone; (2) oat hay+ground corn; (3) oat hay+ground corn+coconut oil and (4) oat hay+coconut oil. When fed together, the oat hay and ground corn were fed at a ratio of 70:30, while the coconut oil was added at a level of 12 g/d. The diets were allocated according to calculated requirements of metabolizable protein and energy. The sheep were adapted to each treatment for 2 weeks prior to methane measurement. A tunnel approach was employed to monitor methane (CH_4) emissions, which enables the donor animals to be enclosed under as near a natural condition as possible. Continuous measurement of the CH_4 emission was made over a period of two days for each diet. The result showed that there was a significant depression of CH_4 release ($P < 0.001$) when coconut oil was added compared to either the pure oat hay or oat hay+ground corn diet. The emission of methane from sheep fed the 4 different diets of oat hay, oat hay+ground corn, oat hay+ground corn+coconut oil and oat hay+coconut oil was 16.4, 12.8, 5.7 and 4.1 g day^{-1} per animal, respectively. Moreover, the CH_4 released from the sheep, when expressed as $w^{0.75}$ kg^{-1}, were decreased by 75.4%, 65.5% and 21.9% for oat hay+coconut oil,

oat hay+ground corn+coconut oil and oat hay+ground corn, respectively, when compared to the diet of oat hay only ($P < 0.001$). However, there were no significant difference between the treatments of pure oat hay and oat hay+ground corn ($P > 0.05$). We conclude that the methane suppression seems to arise, at least partially, by adding coconut oil to ruminant diets

Keywords: methane emissions, sheep, coconut oil, oat hay, corn grain

P-1.5

Rumen Methanogens in Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) and Norwegian Reindeer (*R. t. tarandus*)

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Svalbard reindeer survive austere nutritional conditions on the high-arctic archipelago of Svalbard, where energy intake optimization is essential to cope with food scarcity and starvation during 8 months of winter when vegetation is covered by snow and ice. In domestic ruminants, 2–12% of the energy intake is lost as methane produced by rumen methanogens using H_2 to reduce CO_2. Rumen methanogens have been isolated at levels of 10^4 (summer) -10^7 (winter) cells/ml from Svalbard reindeer, but to this date no methane has been detected in their expired air. Methane production has been reported to be low also in semi-domesticated Norwegian reindeer. In the current study, populations of rumen methanogens in reindeer (Svalbard late summer (n = 5) and winter (n = 5), and Norway late (n = 5) and mid summer (n = 3)) were screened by PCR amplification of the 16S rRNA gene using 9 different primers targeting the domain Archaea, the group Methanogens, and down to the genus *Methanobrevibacter*. DNA extracts (Power Soil™ DNA isolation kit, MO BIO Laboratories) from the 18 animals averaged 28.2ng/µl (SD = 10.3), and were diluted to 10ng/µl prior to the PCR reactions (30 cycles). Five of the nine primer combinations examined gave PCR products of expected size and with bands visible enough on a gel after electrophoreses to allow quantification by the use of a standard. The methanogen specific primers (Met1346R and Met85F, size fragment 1259 bp) gave the largest amount of PCR product averaging 556–1034 ng in the targeted bands (5 µl samples loaded on the agarose gel). Quantification of the PCR products indicated that methanogens appear less abundant in Svalbard reindeer compared to Norwegian reindeer, and in winter compared to summer in Svalbard reindeer. We are currently generating 16S rRNA gene clone libraries to determine the molecular diversity of rumen methanogens in Svalbard reindeer and Norwegian reindeer on late summer pastures

Keywords: 16S rRNA gene, methanogens, rumen, reindeer, arctic

P-1.6

The Effect Of Encapsulated Fumaric Acid And Diet On Methane Formation From Ruminal Digesta

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A decrease in methane could mean lower energy losses from ruminant animals and an increase in gluconeogenesis leading to increased production efficiency. This would benefit the agricultural industry but also the environment, by decreasing a greenhouse gas that has 21 times the global warming potential of carbon dioxide. Encapsulated fumaric acid (**EFA**), a novel ingredient in tortilla flour, was found to decrease methane formation from growing lambs fed a concentrate diet (**W**) by 76%. However, *in vitro* incubations with ruminal digesta from cows receiving a grass silage/concentrate diet indicated that the encapsulated fumaric acid particles were resistant to degradation in bovine digesta, with no effect on methane formation compared to a 15% decrease ($P = 0.078$) with free fumaric acid. Further *in vitro* incubations were carried out with 8 mM, 16 mM, 32 mM and 48 mM EFA added to buffered ruminal fluid from three sheep fed one of four diets (a grass silage based diet, **GS**; a 50:50 grass hay:concentrate diet, **GHC**; a grass pellet diet, **GP**; the concentrate diet used in previous successful lamb trials, W) methane and pH were recorded after 24 h. The greatest effect on pH and methane was observed in ruminal fluid from all diets when incubated with 48 mM EFA. This effect was greatest with digesta from sheep fed a GHC diet (16% decrease in methane, $P < 0.001$; 10% decrease in pH, $P = 0.001$). This study shows that the ability of EFA to decrease methane emissions from ruminants is influenced by a combination of diet and rumen pH

Keywords: methane, encapsulated fumaric acid, pH, diet

P-1.7

A Meta-Analysis Of The Effects Of Malate On Ruminal Batch Cultures

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The objective of this analysis was to understand the effects of malate addition on ruminal fermentation and methane production. Malate is an intermediate of propionate formation, and its conversion to the latter requires the incorporation of one pair of reducing equivalents. Therefore, the addition of malate to ruminal cultures has been studied as a means of decreasing methanogenesis through competition for electrons. A meta-analysis of the effects of added malate on production of methane and volatile fatty acids (VFA) by ruminal batch cultures was carried out using 76 treatment means from 6 different studies. Treatment means were weighted by the reciprocal of their variance. All regressions included a random study effect. There was no effect of malate addition or initial concentration on methane production or concentration. Only approximately half of added malate seemed to be converted to propionate as hypothesized. However, about 20% of added malate seemed instead to be converted to acetate. There was no effect of malate on butyrate production. Total VFA production was increased by 0.79 mol/mol of added malate. Malate addition increased the final pH, although the response was of no biological importance (2.27×10^{-4} pH units/mol of added malate). There were no effects of malate on ammonium-N concentration. The conversion of malate to acetate through known biochemical pathways results in the release of two moles of reducing equivalents per mole of malate. This would almost entirely offset the uptake of reducing equivalents that occurred in the conversion of added malate to propionate. The conversion of an important fraction of added malate to acetate occurred because it was thermodynamically feasible. The partition of malate into acetate and propionate was similar to that of fumarate, as shown by a previous meta-analysis on fumarate addition to ruminal batch cultures. Therefore, the dehydration of malate to fumarate would appear to be close to equilibrium in the rumen, as occurs in aerobic organisms. This analysis shows that thermodynamic considerations should be taken into account when designing strategies for methane abatement through the addition of external electron acceptors

Keywords: rumen, methane, malate, electron acceptors, meta-analysis

P-1.8

Octadeca-Carbon Fatty Acids Affect Microbial Fermentation, Methanogenesis And Microbial Flora *in vitro*

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The effects of type and level of C_{18}-fatty acids on rumen fermentation, methane emission and microbial community structure were examined using an *in vitro* gas production technique (Reading Pressure Technique). Three levels (0, 3.5 and 7.0% of substrate DM) were evaluated for four types of C_{18}-fatty acids: stearic acid, oleic acid, linoleic acid and linolenic acid. The 24-h gas production and methane emission were significantly decreased by type, level and their interactions ($P<0.001$). Methane suppressing effect of unsaturated C_{18}-fatty acids was more profound than on gas production. Compared to the control, addition of C_{18}-fatty acids had little effect on pH, concentration of ammonia-N and total volatile fatty acids. However, the fermentation patterns were significantly changed ($P<0.001$), with lower proportion of acetate and higher propionate with increasing levels and unsaturation of C_{18}-fatty acids. Methanogens and protozoa population relative to total bacteria were decreased by linoleic and linolenic acids, with the linolenic acid being more effective. However, addition of these unsaturated C_{18}-fatty acids also inhibited growth of fibrolytic microbes including fungi, *F. succinogenes* and *R. flavefaciens.* From the present study, it is demonstrated that there is a significant effect of unsaturated C_{18}-fatty acids on suppressing methanogenesis, mediated probably by a direct action against the rumen microbes involved in methane formation and by a decreased hydrogen supply

Keywords: C_{18}-fatty acids, fermentation, methane, microbial flora, *in vitro*

P-1.9

Monitoring And Source Tracking Of Tetracycline Resistance Genes In Lagoons And Groundwater Underlying Swine Production Facilities

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The frequent use of antibiotics in the livestock industry can select for antibiotic resistance within the intestinal microflora of animals, and antibiotic resistant bacteria or their genes can then spread into the environment. To investigate the long-term environmental consequences of antibiotic usage in confinement animal feeding operations, the occurrence of tetracycline resistance genes (Tc^r) in waste treatment lagoons and on-site groundwater wells was determined. Samples were collected from groundwater wells ($n=124$) and waste treatment lagoons ($n=12$) from two different hog confinement feeding facilities from 2000 to 2003. PCR assays were conducted for seven different Tc^r genes [*tet*(M), *tet*(O), *tet*(Q), *tet*(W), *tet*(C), *tet*(H) and *tet*(Z)] previously shown to be present at these sites in hog feces, waste lagoons, and groundwater wells. Quantitative PCR protocols were developed to determine the concentration of these genes in lagoons and selected wells. All seven genes were consistently found in waste lagoons, and elevated concentrations of Tc^r genes relative to background control wells were detected in wells down-gradient of lagoons. For more rigorous source-tracking of genes, clone libraries of a 1152 bp segment of the *tet*(W) gene were constructed from lagoons and selected groundwater wells. Phylogenetic analysis of *tet*(W) genes revealed that genes from impacted wells down-gradient of lagoons were nearly identical (99.8% sequence identity) to those found in waste lagoons, while *tet*(W) genes from up-gradient control wells formed their own distinct sub-cluster. Thus, while native populations of groundwater organisms can harbor their own Tc^r genes, these results strongly suggest that selection for antibiotic resistant organisms within the intestinal tracts of livestock can significantly influence the movement of antibiotic resistance into the environment

Keywords: antibiotic resistance, quantitative PCR, livestock, groundwater

P-1.10

Bacterial Diversity And Antibiotic Resistance In The Colon Of The Hooded Seal

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At present there is little information on the phylogenetic diversity of the gut microflora in animals inhabiting the pristine Arctic. Furthermore, environmental populations of bacteria may be a reservoir of clinically important resistance genes. Hooded seals (*Cystophora cristata*) are abundant in the arctic/subarctic regions of the North Atlantic Ocean, feeding on a variety of pelagic fish, squid and to some extent crustaceans. The purpose of this project was to improve our understanding of the colon bacterial diversity in these animals based on sequence heterogeneity of the 16S rRNA genes, and to investigate tetracycline and ampicillin resistance determinants. Colon contents were collected from six female hooded seals harvested during a three weeks expedition to the Greenland Sea March-April 2004, and processed within 15 minutes of sampling. Numbers of anaerobic bacteria ranged between $8,1(\pm 1,1)\times 10^8 - 5,2(\pm 1,2)\times 10^9$ cfu/ml, and numbers of aerobic bacteria between $1.2(\pm 0,3)\times 10^8 - 3.1(\pm 1,1)\times 10^9$cfu/ml. No anaerobic tet^r and amp^r isolates were detected after 72 hrs growth (detection limit > 20 cfu/ml). Aerobic tet^r isolates were found in all samples, the numbers ranging from $4.8(\pm 3,7)\times 10^2$ to $1.8(\pm 1,5)\times 10^3$ cfu/ml. Aerobic amp^r isolates were found in three of the samples, the numbers ranging between $1.4(\pm 1,7)\times 10^1 - 1.6(\pm 0,3)\times 10^4$ cfu/ml. A selection of phenotypical amp^r isolates ($n=208$) was investigated for presence of ampicillin resistance gene bla_{TEM} by PCR, and no genes were detected. Community DNA isolated directly from the colon contents were bla_{TEM} PCR negative as well, but two samples showed a weak positive result when PCR products were hybridized with bla_{TEM} probes. Sequencing of 16S rRNA genes showed that the hooded seal colon includes bacterial species considered to be a part of the normal flora in humans, pigs, cats, sheep and cows, while novel bacterial species not yet isolated or characterized were also identified

Keywords: arctic seal, 16S rRNA, ampicillin resistance, tetracycline resistance

P-1.11

Evaluation Of Bio-Mos® Performance At Reducing The Levels Of Antibiotic Resistant Bacteria In Chicken Caecal Contents

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Bio-Mos® represents a new era in biological gut micro flora modifiers. It is derived from yeast cell wall mannan oligosaccharides which have been processed with the animal feed industry's requirements in mind. A floor pen study was conducted to investigate whether dietary supplementation with Bio-Mos® had

an effect on antibiotic resistance gene levels in the microbial population of chicken caecal contents. The study consisted of two treatment groups, control and Bio-Mos® (1 kg T^{-1}). Each group consisted of 32 birds, 4 of which were allocated at random to 8 pens. Birds were sampled on two days, day 16 and 21, 2 birds from each pen were euthanized and their caecal contents extracted and lyophilised. DNA was then extracted from the caecal contents using a modified CTAB extraction protocol. The levels of four different tetracycline resistance genes Tet A, B, L and M, were analysed by real-time quantitative PCR. These results were subsequently analysed by one way ANOVA and evaluated to assess whether there was a statistically significant decrease ($p \leq 0.05$) in the resistance gene levels, following treatment with Bio-Mos®. No significant difference was noted in birds sacrificed on day 16, however a significant decrease was observed in the mean log copy number of Tet A gene in the Bio-Mos® treated group, log 5.9045, when compared to the control, log 7.8770, on day 21. This value was statistically different with a p value of $p < 0.001$. This result indicates that Bio-Mos® did have a significant effect at reducing the load of a specific sub-group of tetracycline resistant bacteria in the caecal contents of chickens at a level of 1 kg T^{-1} of feed

Keywords: antibiotic resistance, Bio-Mos®, real-time PCR, tetracycline

P-1.12

Interventions To Reduce The Carriage Of Antimicrobial Resistance In Pigs. Determination Of The Inhibitory Spectrum Of Pre, Pro And Synbiotics Against *Salmonella typhimurium*

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Salmonellosis is considered as one of the most important foodborne illnesses, traditionally associated with the consumption of eggs, poultry and pork meat. The aim of this study was to evaluate the influence of different prebiotics and probiotics on the growth and survival of multi-resistant *Salmonella enterica serovar typhimurium* (ST1344). Preliminarily, a cohort of probiotics ($n \sim 12$) was selected with different carbohydrate sources to study powerful anti-salmonellae capacity and then tested their survival in mixed culture experiments. In separate experiments, we also determined the prebiotic capacity of these carbohydrates suitable for inclusion into the pig diet. Prebiotics identified as being preferentially utilized by the probiotics tested were xylooligosaccharide, gentioligosaccharides, raftilose P95, FOS, synergy 1, lactulose and actilight. Strains of *B. longum*, *L. acidophilus*, *L. plantarum* and *L. rhamnosus* showed the highest growth rates in presence of lactulose. A significant decrease in colony forming units (CFU) of *ST1344* was observed in presence of these synbiotics, the best combinations were *B. longum* and lactulose and *L. rhamnosus* and lactulose. In vitro experimental infections were carried out using a batch model of the pig colon to simulate microbial activities of lower gut bacteria. Bacterial populations (and their metabolic products) were studied under the effects of various fermentable substrates. Bacterial groups of interest were evaluated by fluorescence in situ hybridization (FISH) and also short-chain fatty acid profiles (SCFA) produced during the fermentations. The CFU/ml of *ST1344* was determined by plate count on a selective smedium. Lactulose had a stimulatory effect upon colonic lactobacilli at concentrations of 1% (w/v). This prebiotic showed an increase of 2 log in lactobacilli between 10 and 24 h of fermentation, while clostridial cluster XIV and IV numbers showed a significant decrease. The SCFA production was stimulated, particularly the levels of butyrate (beneficial for host colonocytes). Lactulose also significantly reduced *ST1344* counts from 10^7 to 10^5 CFU/ml. This study suggests the potential beneficial properties of this prebiotic to reduce the incidence of gastrointestinal infection. Further studies are underway to evaluate the efficacy of lactulose in an in vivo pig trial

Keywords: antibiotic resistance, probiotics, prebiotics, *Salmonella typhimurium*, human health risk

P-1.13

Development Of Antimicrobial Resistance Concurrent With Antimicrobial Uses In Cattle And Its Reduction During Composting

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Erythromycin, tylosin, and tetracyclines are widely used in food animals. Being the same superfamily, erythromycin and tylosin cross select for resistance to all drugs belonging to macrolide-lincosamides–streptogramin B (MLS_B) superfamily, including several important drugs used to treat human infections (e.g., clarithromycin, clindamycin and azithromycin). As such, the use of erythromycin or tylosin in food animals can pose risk to human health. In this study, we examined the development of resistance to MLS_B and tetracyclines in a feeding trial and the dynamics of these two types of resistance during manure composting. The feeding trial involved three groups of beef cattle housed together: tylosin (11 mg/kg diet dry matter) + monensin (29.9 mg/kg), Amaferm (2 g/head/day), and control (no additions). Real-time PCR was used to quantify *tet* and *erm* genes in cattle manures and compost samples. The abundance of both *tet* and *erm* genes increased concurrent with the use of tylosin. However, different classes of *tet* and *erm* genes exhibited different increases, with ribosomal protection protein genes having the biggest, while *tet*(G) the smallest increase. The control and the Amaferm groups also had similar abundance of *tet* and *erm* genes two weeks after the the feeding trial began, probably because of clonal dissemination of resistant bacteria from the tylan + monensin group. These results corroborate a positive correlation between antibiotic use and development of antimicrobial resistance (AR) in food-animals. The abundances of *tet* and *erm* genes were also examined during composting of cattle manures from three groups of beef cattle fed chlortetracycline and sulfamethazine, tylosin or no antibiotic. Low abundances of either *erm* or *tet* genes ($< 10^6$ copies/g) were found in all the compost samples, including those collected at the beginning of the composting (1 - 4 days). No significant difference in *erm* or *tet* gene abundance was observed among the manures of the three groups of cattle. Over 11-week composting, different *erm* and *tet* genes displayed different dynamics: with most of the *erm* and *tet* gene classes decreasing, while two classes increasing. These results suggest that composting can be an effective means to reduce AR in food-animal manures, minimizing AR dissemination to soil and other environments

Keywords: manure, compost, *erm*, *tet*, antimicrobial resistance

P-1.14

Erythromycin Resistance Reservoirs In Different Swine Farms And At Different Locations Within Each Farm

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Antibiotics of the macrolide–lincosamides–streptogramin B (MLS_B) superfamily, such as clarithromycin, clindamycin and

azithromycin, are important drugs in treating human infections owing to their safety and tolerance. Erythromycin and tylosin are macrolides and can select for resistance to all MLS_B drugs. As such, the use of erythromycin and tylosin in food animal production might contribute to an increase in MLS_B resistance, posing a potential risk to human health. In this study we quantified the reservoirs of *erm* genes, which are the most common genes encoding MLS_B resistance, in various locations of four conventional swine farms and one organic swine farm. We used class-specific real-time PCR assays to quantify five classes (*erm*A, B, C, T, and X genes) present in samples collected from feed, swine manures, lagoons, soils amended with the swine manures, and one on-farm well. All the analyzed feeds, including the feed from the organic farm, contained at least two classes of *erm* genes at low level ($10^2 - 10^5$ copies/g). All the swine manures and the waste lagoons had high but similar levels of *erm* genes, which were at least five logs greater than those found in the feeds. Among the five *erm* classes, *erm*(B) was the most abundant, while *erm*(C) the scarcest in all the farms. All the manure-amended soils had the targeted *erm* genes, though at several logs lower abundance than the manures. The organic farm appeared to have significantly less *erm* genes than the conventional farms. Collectively, these results suggest that (i) animal feeds can be a source of MLS_B resistance genes; (ii) large reservoirs of MLS_B resistance genes are present in manures; (iii) swine manure management through solids separation and lagoon storage does not result in appreciable reduction of MLS_B resistance; (iv) MLS_B resistance genes are present in manure-amended soils and; (v) *erm* genes are also present within an organic farm environment but perhaps at lower levels, although more research is required with more organic farm environments

Keywords: antimicrobial resistance, *erm*, *tet*, real-time PCR, swine farms

P-1.15

Changes In Normal Fecal Microbiota Of Dogs Related To Antibiotic Treatment

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The gastrointestinal tract contains a complex microbial ecosystem, which plays a vital role in several functions of the host animal. In veterinary medicine, little is known concerning the effect antibiotic treatments have on the patients' normal microbiota. The main objective of this study was to monitor changes in fecal bacterial populations of dogs as influenced by antibiotic treatment. Seven healthy dogs with no bacterial infection and no history of antibiotic treatment the past six months were included in the study. The dogs were of random age and sex, three different breeds, kept in three different households and on three different diets. Each dog was given 10 mg amoxicillin per kg body weight orally twice daily for seven days. A total of 13 fecal samples were collected from each dog over a period of 30 days. Four samples were collected before antibiotic treatment to monitor fluctuations in normal bacterial populations. Samples were taken every day during treatment, in addition to one and two weeks after end of treatment. All samples were kept at −20 C until further processing. Genomic DNA was extracted from the frozen fecal samples using DNA Stool Mini Kit (QIAGEN). Denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S ribosomal DNA fragments was performed. To identify phylotypes within the gels, representative bands were excised from DGGE gels for reamplification, cloning and DNA sequence analysis. Analysis of PCR-DGGE banding patterns showed a tendency for each individual dog to have its own characteristic banding pattern, demonstrated by clustering of individual dog profiles together. Dogs from the same household clustered closer together than dogs from different households. However, the outstanding conclusion obtained from these fecal bacterial profiles was that within individual dogs, samples taken before antibiotic treatment were significantly different from samples taken during and after treatment. These changes observed in the normally stable and characteristic individual banding patterns indicate that the normal bacterial populations of the canine gastrointestinal tract are affected by antibiotic treatment. The effect was persistent since patterns had not returned to pretreatment levels 14 days after antibiotic withdrawal

Keywords: dog, fecal microbiota, antibiotic, PCR-DGGE

P-1.16

Isolation Of A Tylosin-Resistant *Clostridium hathewayi* From A Continuous Flow Culture Derived From A Feral Pig

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The chemostat model has been an important tool in studying intestinal microflora of several domesticated animal species. Several competitive exclusion products have been developed from such studies for use as prophylactic treatments against pathogenic bacteria. Presently, we report on the establishment and characterization of a continuous flow culture with gut contents from a wild boar native to East Texas. The culture contained at least the following species: *Enterococcus hirae*, *Bacteriodes uniformis*, *Streptococcus bovis*, *Proteus mirabilis*, *Staphylococcus epidermis*, *Alcaligenes denitrificans*, *Escherichia coli*, *Clostridium clostridiforme* and *Clostridium hathewayi*. When tested for antibiotic susceptibility, none of these species showed tylosin resistance except the *Clostridium hathewayi*, which was resistant to 512 μg tylosin/ml. Because *C. hathewayi* has been documented to be pathogenic to both humans and animals, the *C. hathewayi* isolate was subjected to further characterization. When E tests^{TM} were used, *C. hathewayi* also showed resistance to erythromycin (>256 μg/ml), cefoxitin (>4 μg/ml), chloramphenicol (>64 μg/ml), ciprofloxacin (>32 μg/ul), azithromycin (>256 μg/ml), ceftriaxone (>32 μg/ul), clindamycin (256 μg/ml) and imipenmem (>32 μg/ml). PCR analysis also indicated the presence of a *cmlA*-like resistance element encoding resistance to chloramphenicol. No products were recovered when PCR was performed to investigate presence of the *Flo$_{st}$*, *spvC*, *invA*, *int*, sipB/C, *erm A*, *erm B* or *erm C* genes. When the PSE-1 primer set was used in a PCR amplification, a product of approximately 250 bp was produced but this was considerably larger than the expected product size of 132 bp. Attempts to isolate a plasmid from *C. hathewayi* were not successful. Feral pigs may be carriers of a potentially emerging zoonotic pathogen possessing multiple antimicrobial resistance traits and may provide a potential source for transfer to domestic animals and the environment

Keywords: chemostat, antibiotic resistance, feral pig, *Clostridium hathewayi*

P-1.17

Diversity And Succession Of The Microbiota From The Small Intestine Of Broiler Chickens

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Cultivation-based approaches and denaturing gradient gel electrophoresis (DGGE) technique were used to study the bacterial flora in the small intestine of chickens and the effects of chlorotetracy-

cline and *Lactobacillus plantarum* on chicken small intestine microbiota. Three hundred and sixty 1-day-old healthy Avian broiler chickens were randomly divided into three groups and were fed for 6 weeks. Group A was fed corn-soy basal diet, groups B and C were fed additives chlorotetracycline (100 microgram/kg) and 0.5% *Lactobacillus plantarum* fermentation liquid, respectively. At 1, 3, 7, 14, 21, 28 and 42 days of age, the small intestine contents were collected aseptically and then were used to cultivate nine types of bacteria (*Escherichia coli, Enterococcus, Staphylococcus, Actinomycetes, Bacteroides, Lactobacillus, Bifidobacterium, Peptococcus* and *Clostridium*), with cultivation-based techniques. The results indicated that the predominant bacteria of the duodenum was *Lactobacillus* (3×10^7), while in the jejunum it was *Lactobacillus* and *Bifidobacterium* (8×10^6, 1.5×10^6), but in the ileum the majority of the bacteria were *Lactobacillus, Bifidobacterium, Escherichia coli* and *Enterococcus* (5×10^6, 5×10^6, 6×10^6, and 2.9×10^6, respectively). Before day 7, the levels of aerobes were higher than anaerobes, but after day 14, anaerobes predominated in intestine. The small intestine had a stable bacterial community structure between 21 to 28 days of age, but there was a unique community structure at 3 and 7 days of age, especially at day 3, suggesting that the structure at 3 and 7days of age was transient. Results showed that major bacterial species were decreased ($p < 0.05$) by chlorotetracycline and *L. plantarum* treatments, but the effect of chlorotetracycline was mainly at 1–21 days of age. Conversely, the effect of *L. plantarum* increased with age and also increased the quantities of *Lactobacillus* and *Bifidobacterium*. The duodenal and ileal contents were collected for DNA isolation at 14 and 42 days of age. The V6-V8 region of 16S rDNA was amplified by PCR and analyzed using DGGE. The results indicated that the bacterial community of the duodenum at the d14 and d42 differed significantly. Total band numbers decreased in chlorotetracycline-treated chickens, and the effect was more evident at 14 days of age than at 42 days of age, whereas *L plantarum* increased band numbers compared with controls

Keywords: small intestine, microbiota, chlorotetracycline, *Lactobacillus plantarum*

P-1.18

Competitive Exclusion Of *Salmonella Enteriditis* By *Lactobacillus salivarius* And *Enterococcus faecium* In Poultry

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Probiotic bacteria are thought to act as competitive exclusion agents against food borne pathogens such as *Salmonella enteriditis*. However, the exact mechanisms of action are still to be elucidated. Preliminary *in vitro* studies were conducted to elucidate possible mechanisms of inhibition of *S. enteriditis* by *Lactobacillus salivarius* and *Enterococcus faecium*. The inhibition of growth of *S. enteriditis* by *L. salivarius* and *E. faecium* in Brain Heart Infusion Broth (BHIB) was evaluated. Conditioned medium was prepared by growing the probiotic strains in the medium for 16 hrs which was subsequently filtered and adjusted to pH 7.2. Conditioned and control media was inoculated with 10^4 cfu/ml of *S. enteriditis*. The growth rate of *S. enteriditis* was 0.35 in the control medium, 0.05 in *E. faecium* conditioned medium and 0.13 in *L. salivarius* conditioned medium. To test the competitive exclusion properties of the probiotic organisms *in vivo*, day old White Leghorn SPF chicks were inoculated by oral gavage with either 10^9 cfu of *L. salivarius* and *E. faecium* as mono or mixed preparations. On day 2 the birds were challenged with 10^5 cfu of *S. enteriditis* by oral gavage. *S. enteriditis* colonisation of the caeca, ileum and colon was evaluated by direct bacterial counts. In the group treated with the dual probiotic culture, colonisation of the evaluated tissues by *S. enteriditis* was reduced by approximately two logs on day 43, compared to the control group. The ability of the combined probiotic inoculum to inhibit *S. enteriditis* was evaluated in a seeder bird model. White Leghorn chicks were inoculated with the combined probiotic preparation on different days. *Salmonella* challenged seeder birds were introduced to the control and treatment groups on day 11 of the study. The greatest inhibition was observed when the birds were dosed on day 12 and 20; ~1 log reduction was observed in the three evaluated tissues on day 43 of the study. The demonstration of pH independent inhibition of *S. enteriditis* in BHIB indicates the possible presence of metabolite(s) which inhibit *S. enteriditis*. We have demonstrated that inoculation with *L. salivarius* and *E. faecium* mixed cultures can significantly reduce *S. enteriditis* infection in adult birds. As probiotics are relatively cheap and easy to produce a preparation of these cultures could be used in industry to reduce the risk of *S. enteriditis* contamination of poultry meat at the time of slaughter

Keywords: Lactobacillus salivarius, Enterococcus faecium, Salmonella enteriditis, competitive exclusion

P-1.19

Effects Of Biological Pit Additives On Microbial Ecology Of Stored Pig Manure

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The effects of biological pit additives on microbial ecology in stored pig manure were investigated using a dynamic manure storage system, which allowed for continual addition of swine feces and urine. After 13 weeks of manure collection and storage, 4 treatments were added to tanks (900 L capacity with 400 L): control (no treatment), Indigo (1.1 ml/L), Pit Remedy 1X (1 ml/L) and Pit Remedy 4X (4 ml/L) with three tanks per treatment. At predetermined intervals (0, 1, 3 and 5 weeks after treatment); 100 ml of sample was removed for analysis. Anaerobic serial dilutions were prepared within 2 h of sampling and the remainder of the sample was stored at −20°C until DNA extraction. Total anaerobes and aerobes were determined by spiral platting dilutions onto reduced Wilkens-Chalgren agar and Nutrient agar, respectively. To estimate Gram-positive and negative bacterial counts, 0.03 mg/ml of nalidixic acid or vancomycin were included in agars. All plates were incubated at 37 C and colonies enumerated automatically. Data were transformed into log counts for statistical analysis. Total DNA was extracted from samples and the 16S rRNA gene V3-region was amplified prior to DGGE analysis. Biological pit additives had no effect on total aerobic and anaerobic bacteria. Pit additives did significantly affect both nalidixic acid and vancomycin resistant anaerobes, but had no effect on resistant aerobic bacteria. Treatment of manure did not result in differences in DGGE banding patterns. While manure pit additives did affect numbers of nalidixic acid and vancomycin resistant anaerobes, these changes were not reflected in the microbial community based on DGGE analysis.

Keywords: manure, DGGE, additives, microbial diversity, pits

P-1.20

***Entodinium caudatum* Is The Most Abundant But Not The Most Bacteriolytic Ciliate In The Rumen Of Sheep**

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Entodinium caudatum is one of the most abundant protozoal species in the rumen and it is thought to be the most highly

predatory organism since it takes up bacteria very quickly and in an unspecific manner. Three cannulated sheep monofaunated with *E. caudatum* were refaunated by the introduction of 5 ml ruminal fluid from a conventionally faunated donor sheep. Samples were taken twice a week for 3 weeks before and 4 weeks after the refaunation. Rumen protozoa were counted microscopically. The bacteriolytic activity was determined by the release of radioactivity from ^{14}C labelled *Selenomonas ruminantium*. Quantitative PCR was performed using primer specific for eubacteria, *Fibrobacter* species and *Ruminococcus flavefaciens*. After refaunation at the beginning of week 4 the non-*E. caudatum* population established itself within 14 days to a plateau of 1.8×10^5 cells per ml rumen fluid. The *E. caudatum* population decreased significantly from 2.6×10^5 to 1.2×10^5. During refaunation the concentration ammonia of was decreased by 75% and the concentration of bacterial DNA in the rumen was increased by 45%. But while the proportion of *Fibrobacter* DNA was more than doubled after refaunation the proportion of *R. flavefaciens* was decreased by 62%. The bacteriolytic activity of the rumen fluid was increased 5fold upon refaunation. Our data suggest that *E. caudatum* is not the most bacteriolytic protozoal species in the rumen of sheep. The fact that lysis of *Selenomonas ruminantium* was increased 5-fold by the refaunation, while the total bacterial population was decreased only by 45% suggests that protozoal predation is not a random and unspecific process but that certain types of bacteria are more likely engulfed and digested than others. This hypothesis is supported by differences in DNA abundance of *R. flavefaciens* and *Fibrobacter.* A more detailed examination of the effect of refaunation on the bacterial community is currently underway

Keywords: ciliate protozoa, *Entodinium caudatum*, bacteriolysis, rumen ecology

P-1.21

Culturing Ciliate Protozoa From The Kangaroo Forestomach

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In earlier studies, attempts to culture kangaroo forestomach ciliates in vitro or establish them in the rumen of defaunated sheep, were unsuccessful. The rumen and forestomach environments are similar; however, there are some basic differences. This study was undertaken to determine if incorporating these differences in our culture procedure would allow growth of the kangaroo ciliates in vitro. From literature reports, forestomach pH, % acetic acid, % H_2 in the gas phase and % DM of forestomach contents are all slightly higher than in the rumen. In addition, body temperature of marsupials is 2–5 degrees lower than in ruminants. A total of seven kangaroos were used to inoculate in vitro cultures, three on 2/3/06 and four more on 2/19/06. Increasing % H_2 in the gas phase, raising medium pH to 6.8–6.9, increasing the daily substrate concentration and feeding only forage did not increase culture longevity; however, cultures incubated at 34–36C remained viable for periods ranging from 8 to 28 days. Although concentrations were low, at least four species of protozoa were observed to survive in vitro: three species of *Bitricha* and one of *Macropodinium.* In general, three of four kangaroos used to inoculate cultures on 2/19 became monocultures of *Macropodinium yalanbense* (concentrations ranged from 400 to 2000 cells/ml). In addition, the fourth also contained *Bitricha.* One culture started from the first three kangaroos (2/3) still contained two species of *Bitricha* in low numbers at 28 days, around 250 cells/ml. One of the species appeared different from described species, and 18S rRNA analysis confirmed it as a new species. In addition, bacterial concentrations in the forestomach fluid of five of the kangaroos was determined with an MPN assay. Total bacteria concentrations ranged from $4.3-93 \times 10^8$/ml and cellulolytic bacterial concentrations from $9.3-2400 \times 10^6$/ml. Incubation of cultures at 33–35C is the likely starting point for future studies on in vitro cultivation of kangaroo forestomach ciliate protozoa

Keywords: kangaroos, in vitro culture, protozoa

P-1.22

Rumen Protozoa Can Alter Generation Time And 18S Ribosomal DNA Concentration To Adapt To Increased Transfer Rate, Starvation, And Monensin

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Previously, we developed a real-time PCR assay targeting the DNA encoding synthesis of 18S rRNA (rDNA) to quantify protozoal biomass in ruminants. The macronucleus of rumen protozoa has high (thousands) and variable copies of rDNA, but factors influencing the copies per cell are not clear. Monensin is commonly used in beef and dairy rations, but its effects on protozoa are equivocal. In experiment 1, our objectives were to determine how abruptly changing transfer rate affects the growth rates of *Entodinium caudatum* (Ento), mixed *Entodinium* species (Mix), *Epidinium caudatum* (Epi), or *Ophryoscolex caudatus* (Oph) cultures. The cultures were fed a combination of starch and ground alfalfa (1.5% and 1.0% w/v, respectively) every 24 h, with or without monensin. When the transfer time was decreased from 72 h, protozoal generation time recovered to approach but not go below respective transfer times of 48 or 24 h, except for the slower-growing Oph. Monensin inhibited cell growth in all cultures when it exceeded 0.5 µmol/L. In experiment 2, our objectives were to determine diurnal changes in rDNA, N and total RNA concentrations in protozoa quantitatively recovered and washed with respect to feeding and monensin (0.5 µmol/L). Cultures were transferred every 72 h but fed every 24 h. Then 24 h after the last transfer, the cultures were further subdivided into unfed, fed, or fed with monensin. Although total cell count per 400 ml differed ($P < 0.05$) among species, total protozoal N was similar among species, decreasing at 16 h. The log10 copies of rDNA per culture decreased ($P < 0.08$) at 16 h for Ento and Mix and tended ($P = 0.12$) to decrease for Epi. In contrast, log copies increased ($P < 0.05$) at 6 and 16 h for Oph, even when expressed per cell, per mg N, or per mg RNA. When not fed, log rDNA copies decreased ($P < 0.05$) at 16 h; however, the log copies tended to increase at 6 h after feeding without ($P = 0.12$) or with ($P = 0.06$) monensin, but copies decreased ($P = 0.06$) again at 16 h only with monensin. Results were similar when expressed per mg RNA. Experiment 1 indicates that transfer rate seems to regulate the growth rate on a cell basis, whereas with constant transfer rate (Experiment 2), substrate supply seems to regulate synthesis of protozoal growth on a biomass basis independent of cell size or number. Monensin apparently disrupts substrate availability and therefore the replication of rDNA (or other genes) needed for cell growth

Key words: rumen protozoal culture, real-time PCR, monensin, rDNA copies

P-1.23

Development And Validation Of An Automated Ribosomal Intergenic Spacer Analysis For Anaerobic Fungi (*Neocallimastigales*)

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Anaerobic fungi (*Neocallimastigales*) are inhabitants of the gastrointestinal tract of herbivores, where they play an important role in the degradation of complex lignocellulosic substrates. Six genera have been classified to date although recent application of molecular techniques has demonstrated that other novel groups exist, and that anaerobic fungi appear to be more widely distributed in the environment than previously thought. Due to the high similarity of 18S rRNA between genera, sequence differences in the internal transcribed spacer 1 (ITS1) region have been used to determine relationships between the genera. In addition, as the ITS1 region is polymorphic in size, the development and application of an ITS1 size polymorphism analysis has enabled the direct profiling of *Neocallimastigales* within environmental samples. Here we describe the further development of this approach into a semi-quantitative Automated Ribosomal Intergenic Spacer Analysis (ARISA) method. A new primer pair spanning the complete ITS1 region was designed, and the specificity for *Neocallimastigales* was confirmed *in silico*. The primers were then confirmed to match all available *Neocallimastigales* sequences, for the six genera as well as the recently identified novel groups. PCR cycling conditions were optimised using DNA from pure cultures of anaerobic fungi, and then separated on a capillary sequencer. Analysis of the spectra demonstrated that within isolates the ITS1 region was polymorphic in size, with variable numbers and relative intensities of the ITS1 region fragments observed. Interestingly, identical profiles occurred for isolates with distinct geographical origins (China v UK). ARISA spectra of a rumen digesta sample, from a dry Holstein-Fresian dairy cow fed a barley straw diet, contained twelve ITSI amplicon fragment sizes. Direct cloning and sequencing of the ARISA PCR products confirmed the specificity of the primers, and sequence lengths matched the size determined by the capillary sequencer. Unique sequences belonging to the *Caecomyces* genera were found to represent the dominant peaks. Application of this new ARISA methodology will enable the diversity of *Neocallimastigales* to be effectively characterised within environmental samples, overcoming limitations associated with previous approaches

Keywords: ARISA, rumen, *Neocallimastigales*, anaerobic fungi

P-1.24

Characterisation Of The Population Structure Of Anaerobic Fungi In The Ruminant Digestive Tract

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Anaerobic fungi can be isolated from the entire digestive tract of herbivores and freshly voided faeces. A range of fungal species have been isolated from both fresh and air-dried digesta and faecal samples but the inability to isolate them from air-dried rumen digesta led to the suggestion that the rumen and hindgut populations of anaerobic fungi differ. Comparisons of anaerobic fungi present in representative samples of rumen and faeces were made to determine if this was the case. Rumen and faecal samples from a dairy cow fed barley straw were collected and a most-probable number (MPN) technique used to culture and isolate anaerobic fungi present in fresh and air-dried samples. A semi-quantitative Automated Ribosomal Intergenic Spacer Analysis (ARISA) method was used to profile those populations present. Anaerobic fungi were cultivated from all fresh samples but with air-dried samples isolates were only obtained from faeces. In terms of population dominance, a bulbous and a rhizoidal isolate appeared to dominate in fresh and dried samples respectively. Axenic cultures generated distinctly different, simple ARISA profiles. ARISA profiles of fresh rumen and faecal samples on the other hand were all similar (>92%), with profiles showing multiple distinct peaks with a range of relative intensities. Peaks of the same position as the axenic isolates were present in all the rumen and faecal samples. The bulbous isolate correlated to the dominant peak (356 bp) within the profiles (23–30% of total peak height), whereas the monocentric isolate represented a minor member of the population (1–2% of total peak height). Despite this finding, no bulbous isolates were recovered from air-dried samples from either the rumen or faeces. Similar populations of anaerobic fungi in the rumen and faeces occurred, yet only a minor subset of the fungi in the faeces remained culturable after samples were air-dried. On this basis, it is concluded that fungi in the rumen and hindgut are similar but that some fungi can produce post- ruminal 'survival structures', thus enabling them to survive an air-drying process. It is also concluded that the medium used to cultivate fungi from digesta and faecal samples has a major (biasing) enrichment influence on the dominant population that develop in MPN based cultivation methodology

Keywords: ARISA, anaerobic fungi, *Neocallimastigales*, rumen, faeces

P-1.25

Phylogenetic Analysis Of Uncultured Butyrate Producing Bacteria From The Forestomach Content Of Wild Vicuña

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The South American Camelids, which include species of vicuña, guanaco, alpaca and llama, harbor a large and complex microbial population in their forestomachs. The microbial population plays a key role in the bioconversion of lignocellulosic feeds into energy and protein nutrients for maintenance and milk, meat or muscle production. Although the microbial ecosystem of ruminants is well studied, information on the microbial composition in the gastrointestinal tract of camelids like vicuñas is scarce. We report the PCR amplification of 16 rDNA and phylogenetic studies of uncultured butyrate producing bacteria from two camelids. Samples were collected from the forestomach content of two vicuñas kept under wild conditions in Laguna Blanca, (3260 m altitude), Catamarca Province, Argentina. Total genomic DNA was extracted from the forestomach contents and 16S rDNA amplified using specific primers derived from some butyrate-producing bacteria. By comparative analysis with known 16S rRNA genes, the PCR products were identified as signatures of *Pseudobutyrivibrio* and *Butyrivibrio fibrisolvens*. For further analysis, PCR products from *Pseudobutyrivibrio* were cloned into a TA-cloning vector (pGEMT vector, Promega) and sequenced. Phylogenetic analysis of the sequence data at NILGS (Tsukuba-Japan) suggested that our amplified 16S rRNA genes from the

forestomach of the wild vicuñas derived from novel strains of the genus *Pseudobutyrivibrio*

Keywords: vicuñas, bacteria, Phylogenetic analysis, *Pseudobutyrivibrio*

P-1.26

Quantification By Real-Time PCR Of Cellulolytic Bacteria In The Rumen Of Sheep: Effect Of Supplementation Of A Forage Diet with Concentrate And Yeast

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In ruminant nutrition, rumen cellulolysis is known to be negatively affected by the addition of readily fermentable carbohydrates in the diet while additives such as *Saccharomyces cerevisiae* have been used for their beneficial effect on ruminal fermentation. The impact of such supplementations on growth of cellulolytic bacteria *in vivo* remains to be unraveled. In order to examine the effect of concentrate and yeast on the number of cellulolytic bacteria in the rumen of sheep, the three major cellulolytic bacterial species, *Fibrobacter succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* were quantified using real-time PCR in parallel to cellulolytic flora enumeration with microbial techniques. Whatever the conditions tested, *R. flavefaciens* was slightly more abundant than *F. succinogenes*, with both species outnumbering *R. albus*. Surprisingly, before feeding, the shift from hay to hay plus concentrate diet had no negative effect on the number of the three species; this result is perhaps explained by a rumen pH close to neutrality on both diets. On the contrary, just after feeding, the concentrate-supplemented diet induced a decrease (−1 Log) of the number of the three cellulolytic species concomitant with rumen acidification. Whatever the diet and sampling time, the presence of the yeast additive resulted in a significant increase (2- to 4-fold) of *Ruminococcus albus* and *Ruminococus flavefaciens*. In conclusion, the use of real-time PCR allowed us to show slight changes in the population dynamics of cellulolytic bacterial species *in vivo* in response to diet shift and additives that could not be as easily evidenced by classical microbial methods

Keywords: rumen, cellulolytic bacteria, real-time PCR, diet, yeast

P-1.27

Real Time PCR Analysis Of Ruminal Microbial Populations In Sheep Fed Hay Or Corn: Hay Mix

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Extensive research has been conducted to determine how changes in the diet affect the microbial communities in the rumen. However, very little is known regarding the partitioning of different bacterial populations in the rumen (adhering vs. free-living) and the mechanisms underpinning such partitioning. In this study we analyzed, using specific real-time PCR assays, a number of bacterial populations of both known species and uncultured species that have been revealed by DNA cloning and sequencing from the adhering and the liquid fractions of rumen digesta. Rumen contents were taken from four sheep, two of which were fed hay while the remaining two fed a mix of corn and hay (70:30). Community DNA was extracted from the adhering and the liquid fractions and subjected to real time PCR assays. The total bacteria abundance (*rrs* copies/μg DNA) was similar between the adhering and liquid fractions and among all the four sheep fed either diet. Among the three major recognized cellulolytic bacterial species, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* had similar abundance (1.1×10^4 to 1.7×10^6 *rrs* copies/μg DNA for *F. succinogenes*, 1.2×10^3 to 1.2×10^6 *rrs* copies/μg DNA for *R. flavefaciens*), which was much more (up to 3 logs) abundant than that of *R. albus*. The hay-fed sheep always had more *F. succinogenes* and *R. flavefaciens* than the two corn:hay-fed sheep. The abundance of these two cellulolytic species was much greater (1–2 log) in the adhering fraction than the liquid fractions. However, *R. albus* appeared to be more abundant in the corn:hay-fed than the hay-fed sheep. The abundance of several amylolytic bacteria and uncultured bacteria are being quantified. These preliminary data suggest that *F. succinogenes* and *R. flavefaciens* may play a bigger role than *R. albus* in the cellulose degradation in the rumen of these sheep. Given the small number of animals tested, further studies involving more animals are required to test these conclusions. Coupled with the genome sequencing data, ecological knowledge of cellulolytic bacteria in the rumen will help us better understand the degradation of diet and thus nutrition in the rumen

Keywords: Fibrobacter, Ruminococcus, real-time PCR, rumen

P-1.28

Isolation And Partial Characterization Of An Uncultured Fiber-Associated Bacterium From Sheep Rumen

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Ruminant animals harbor a diverse and dense bacterial population in the rumen. In recent years, the genetic diversity of the rumen bacterial community has been revealed by sequence analysis of the 16S ribosomal RNA gene (16S rDNA). Through the process of analyzing this community, the presence of uncultured bacteria has been recognized. However, the roles of these uncultured bacteria in rumen fermentation have not been described. In the present study, we attempted isolation of uncultured rumen bacteria to retrieve their physiological information. Among previously reported uncultured bacteria, the fiber-associated group U2 belonging to Low GC Gram positive bacterial group was targeted. Rice straw fragments were incubated in the rumen of sheep for 6 h and used as a source for isolation. The straw fragment was inoculated into a medium containing 40% rumen fluid and ball-milled rice straw. In order to inhibit the growth of Gram negative bacteria, phenylethyl alcohol was added to the medium (0.5% v/v final concentration). The medium was incubated for up to 5 d to enrich U2. During the incubation, population sizes of total bacteria and U2 in the medium were monitored by real-time PCR assays specific to the respective target. Isolation was made by the roll tube method using the above medium. The isolates obtained were screened by PCR with U2-specific primers followed by 16S rDNA sequencing. On the basis of 16S rDNA copy number analysis, proportions of U2 as a % of total bacteria were 0.4 and 1.1% for sheep rumen content and ruminally incubated rice straw, respectively. After 5 d incubation, the proportion of U2 increased up to 2.9%. These results demonstrate enrichment through the ruminal incubation of straw fragment followed by *in vitro* incubation with milled straw and phenylethyl alcohol. Among 109 isolates obtained in the present study, one isolate was grouped into U2, based on similarity of 16S rDNA sequence. The isolate was a Gram positive coccus and/or short rod, and was clearly visualized by U2-specific FISH

Keywords: uncultured bacteria, rumen, isolation, LGCGP bacterial group

P-1.29

Development Of A Colorimetric Plate Assay For Detecting Defluorination By Microorganisms

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To the best of our knowledge, there is no published research about attempts to investigate whether fluoroacetate-degrading microorganisms exist naturally in the digestive systems of herbivores, which can protect the host animal from this highly toxic substance present in important forage plants in Australia and Africa. The aim of the present study is to conduct an environmental survey of microorganisms that can degrade fluoroacetate and to assess the possibility of inducing a specific population, or introducing them into the digestive system of livestock, to reduce the incidence of fatal poisonings. There are currently several known species of bacteria and fungi that can degrade fluoroacetate however they are unlikely candidates for colonization into digestive ecosystems as they originate from soil. To facilitate the isolation of new microorganisms able to degrade fluoroacetate under anaerobic conditions, an agar growth plate assay has been developed. The assay is based on a colorimetric reaction between a xylenol orange-zirconium complex, and fluoride ions released from fluoroacetate, and provides a sensitive, reliable and rapid method for detection of defluorinating microorganisms. An enrichment consortium originating from the rumen of a cow *(Bos taurus)* was obtained in a nutrient-limited media containing rumen fluid, casamino acids and 20 mM fluoroacetate using transfer and serial dilution methods over a period of 4 weeks. Using this agar plate assay under anaerobic conditions, the enrichment consortium was screened, and a bacterium with defluorination activity was isolated. The bacterium was able to release fluoride ions at a rate of 5.92 mM day^{-1}from media containing fluoroacetate. This is over four times greater than the rate of 1.38 mM day^{-1} that was reported for a soil Pseudomonad. The new rumen isolate is currently being characterised, and has been classified as a *Synergistes* sp., by 16S rDNA sequencing

Keywords: dehalogenation, fluoroacetate, plate assay, rumen toxicity, enrichment culture

P-1.30

Characterization Of Acetogenic Bacteria Associated With Skatole Production In Swine Lagoon Slurry

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Skatole is a potent odorant produced by anaerobic degradation of stored animal waste materials. Little is known of the phylogeny of skatole-producing microorganisms or the conditions that favor their growth. These deficiencies hamper attempts to reduce skatole production. Previous studies suggest that acetogens may be important in the conversion of indole-3-acetic acid (IAA) to skatole. The goal of this study was to enrich for homoacetogenic microorganisms present in swine lagoon slurry (SLS) which are also capable of skatole production. To this end, SLS (100 µl) was added to anaerobically incubated acetogen media containing no added carbon source except 100 µM IAA. GC-MS was used to measure skatole production in the acetogen enrichments after 0, 7, 21, 28 and 42 days incubation. The enrichments were tested for an increase in total cell concentration using quantitative, real-time PCR of the 16S rDNA gene and the presence of acetogens was evaluated by PCR amplification using primers specific to the formyltetrahydrofolate synthetase (FTHFS) gene (a highly conserved gene important in a key step in the formation of acetate). Results show that skatole production increased from 0 ng mL^{-1} on day 0 to a high of 4.7 ng mL^{-1} on day 28 before decreasing to below detectable levels on day 42. Skatole concentrations in sterile and non-sterile controls with no IAA added never increased above the limit of detection. Total cell concentrations increased from $1.30 \pm 0.50 \times 10^5$ cells mL^{-1} following inoculation on day 0 to $1.41 \pm 0.25 \times 10^7$ cells mL^{-1} by day 42. FTHFS analysis of day 42 samples show that acetogens were present in IAA supplemented samples, but were absent in sterile and non-sterile control samples. PCR products (16S rDNA and FTHFS) from these enrichment samples are currently being sequenced to provide phylogenetic data about the identity of organisms in these samples. These data suggest that acetogens may be associated with skatole production

Keywords: skatole, acetogen, waste treatment, swine

P-1.31

An Efficient RNA Extraction Method For The Estimation Of Gut Microbial Diversity By PCR

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PCR-based analysis of the gut microbial community using ribosomal RNA as template is a suitable method to describe the predominant members of a population. The amount of rRNA produced by microbial cells is directly correlated with the growth activity of the organism, and some recent studies have shown it to be a preferred template for PCR-enabled microarray-based studies. However, few studies have been undertaken using this approach because RNA isolation from gut digesta is laborious, the RNA is more prone to enzyme degradation than DNA, and inhibitors associated with the extracted RNA often impair amplification by PCR. RNA isolation from rumen or mouse faecal samples was optimized, and metabolically active groups from these samples analysed. Five extraction methods were tested on different amounts of sample and compared for efficiency and purity of RNA using the NanoDrop® ND-1.00 Spectrophotometer. Trizol (Invitrogen) extraction after bead beating produced a better quantity and quality of RNA than a similar method using phenol/chloroform. Optimal extraction of RNA was achieved using 200 µl (rumen liquid) and 50 mg (rat faeces) of sample, which yielded 5 µg and 8 µg RNA, respectively. Dissociation buffered saline (DBS) produced a 1.5 to 2-fold increase in RNA when compared with phosphate buffered saline (PBS) during the dissociation of bacteria from rumen or faecal particles. Reverse transcriptase PCR (RT-PCR) using the different RNA extracts showed we could detect bacteria which are present in lower abundances, such as *Ruminococcus flavefaciens* and *Streptococcus bovis.* Bacterial diversity in the samples was also analysed by sequencing 96 amplicons produced using Bac-specific 16S rDNA primers, with cDNA synthesised from the extracted RNA as the template. Amplicons representing the major phyla encountered in the rumen (Bacteroidetes, Firmicutes and Proteobacteria) were recovered, showing that the techniques developed provide a good representation of both numerically dominant and minor bacterial groupings in these microbiomes

Keywords: bacterial diversity, RNA extraction methods, RT-PCR

P-1.32

Development And Evaluation Of Oligonucleotide Probes For Tracking Bacterial Strains Re-Introduced To The Reindeer Rumen

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Reindeer (*Rangifer tarandus tarandus*) in the northern part of Norway are semi-domesticated animals that experience large seasonal variations in the abundance and quality of feed. During the winter periods, thawing and freezing of snow occasionally blocks the pastures, and animals are exposed to starvation, which results in a reduced number of rumen bacteria. To avoid reduced digestion of feed following starvation, we aimed at studying the effect of treating reindeer with probiotic bacteria during re-feeding. The probiotic bacterial strains (strain 8/94-32 with 98% identity to *Ruminococcus flavefaciens*, and 8/9293-9 with 98% identity to *Butyrivibrio fibrisolvens* based on 16S rDNA sequence) tested were fibrolytic strains isolated from the rumen of healthy reindeer. Probes for tracking the probiotic bacterial strains were developed based on sequences generated from the intergenic spacer region (ISR) of 16S-23S rRNA genes. The ISR sequences of 10 *Butyrivibrio fibrisolvens*- isolates (98–99% identity based on 16S rDNA sequencing) from reindeer were used in alignments to develop a *Butyrivibrio*-probe. For the development of a *Ruminococcus*- probe, since no other *Ruminococcus*-isolates than strain 8/94-32 were retrieved from reindeer, its ISR together with those of *Ruminococcus albus* Ra-8 and *Ruminococcus flavefaciens* FD-1, both from a cultural collection, were used in the alignments. The ISR sequences were between 498 and 626 nucleotides long, and there were greater similarities between the *Butyrivibrio* sequences than between the *Ruminococci* sequences. Hybridisation experiments were carried out to determine the specificity of the probes and also their efficacies in evaluating how well probiotic bacterial strains (8/94–32 and 8/9293-9) establish in reindeer rumen. Our preliminary results showed that the *Butyrivibrio*-probe can detect 4 of 8 *B. fibrisolvens* strains tested, whereas the initial probe for the *Ruminococci* has proven non-specific for strain 8/94-32. More probes for the Ruminococci will be designed and evaluated

Keywords: reindeer, intergenic spacer region, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*

P-1.33

Human Methanogens Are Inversely Related To Butyrate Concentration

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The presence, abundance, and diversity of methanogens in human faeces were elucidated using cultivation-independent methods and their relationship with short chain fatty acids (SCFA), especially butyrate, was examined using a cross-over design. Forty-six healthy individuals were divided into 2 equal groups. Both groups maintained their usual diet for an initial 4 weeks (wks 1–4). In the subsequent 4 week period, one group consumed a high fibre diet (D1) while the other group consumed the high fibre diet plus resistant starch (RS) from a variety of foods (D2, wks 5–8). At week 9, both groups returned to their usual diet. At week 11 the volunteers returned to the experimental diets, but crossed-over so that the group that received D1 was assigned D2 and vice-versa (wks 11–14). Six faecal samples (wks 1, 6, 8, 10, 12, 14) were collected from each individual and frozen. DNA was extracted and the methanogens amplified by PCR using methanogen-specific 16S rRNA gene primers. Almost 40% of the individuals tested positive for methanogens. There were 3 distinct methanogen phylotypes found, but as many as 14 phylotypes were present in some individuals. DNA sequencing of denaturing gradient gel electrophoresis (DGGE) bands and 16S rRNA clones indicated that all phylotypes were >99.1 similar to *Methanobrevibacter smithii*. Real-time PCR analyses revealed that faecal methanogen numbers varied significantly between individuals and over time ranging from 3.5×10^3 to 7.7×10^9 cells/ gram wet weight (gww). SCFA concentrations were significantly higher during D2 than D1, consistent with the greater intake of RS. There was a significant negative relationship between the presence or absence of methanogens $\geq 10^7$ and butyrate concentration ($p = 0.0497$), and significantly higher methanogen numbers for the combined diets than for the usual diet ($p = 0.0301$). There was also a significant negative relationship between the methanogen counts and faecal output ($p = 0.0485$), but not pH, acetate, propionate, total SCFA, subject age, height, weekly weight, mass index, diet, gender (male, female), or diet order (i.e. whether the individual consumed D1 or D2 first). Butyrate is believed to be an important factor in promoting colonic health so the inverse relationship with methanogens reported here suggests that methanogens may be useful biomarkers of bowel health

Keywords: methanogens, butyrate, colorectal cancer, fibre, resistant starch

P-1.34

Bacterial Imbalance In The Duodenal Mucosa Of Children With Celiac Disease

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Celiac disease (CD) is the most common immune-mediated enteropathy, characterized by chronic inflammation of the small intestinal mucosa. The ingestion of gluten is responsible for the symptoms of CD, but other environmental factors associated with its presentation are unknown. In this study, the composition of the intestinal microbiota of celiac children and controls was investigated in order to progress on knowledge of the possible role of specific intestinal bacteria in CD. Three groups of children were included in this study: CD patients with active disease, symptom-free CD patients on a gluten-free diet, and control children without known food intolerance. Bacteriological analysis of duodenal biopsy specimens were carried out by fluorescent *in situ* hybridization (FISH) coupled with flow cytometry. The composition of the duodenal microbiota differed between control and celiac children, particularly in the case of those showing active CD. Most of the quantitatively important gut bacteria reached intermediate numbers in samples of celiac infants with inactive disease, when compared with those of controls and patients with active disease. *Bactericides*, *Clostridium*, and sulphate reducing bacteria were the groups showing the most remarkable differences among the studied population groups. The proportions of these bacteria were significantly higher in duodenal mucosal samples of celiac infants with active disease than in those of controls. Therefore, the presentation of CD in children is associated with

an imbalance in the composition of the duodenal mucosal microbiota, which is not completely normalized in patients with inactive disease. The bacterial groups that were overrepresented in CD patients have been previously associated with other immune-related disorders and could contribute to perpetuate an intestinal inflammatory milieu in celiac children

Keywords: intestinal microbiota, celiac disease, FISH, flow cytometry

P-1.35

Physiological And Molecular Indicators Of Change In The Intestinal Microflora Of Postmenopausal Women Consuming Soy And Fructooligosaccharides

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Dietary isoflavones are predominantly glycoside conjugates, which require enzymatic cleavage of the glucose moiety prior to absorption from the gut. Fructooligosaccharides (FOS) are preferentially metabolized by beneficial bacteria (e.g., bifidobacteria and lactobacilli) that produce glucosidases. It has been reported that feeding of FOS enhanced the absorption and metabolism of isoflavones in Sprague-Dawley rats. Here we report the results of a prospective, randomized controlled trial conducted with 24 postmenopausal women randomized to soy+FOS or soy+placebo treatment. Isoflavone absorption was moderately enhanced by FOS ingestion as determined by HPLC (daidzein: 5–17%; genistein: 17–34%, and glycitein: 12–20%). Fecal β-galactosidase activity increased significantly in the FOS group ($P=0.02$), suggesting an induction of β-galactosidase and/or increase in β-galactosidase-producing bacteria in response to FOS supplementation. Bacterial diversity also increased in women consuming soy and FOS, as monitored by PCR-DGGE analysis. A moderate correlation ($r^2=0.74$) also existed between β-galactosidase activity and bacterial diversity index. Although real-time PCR analyses showed no significant increase in total bacteria, *Bifidobacterium*, *Lactobacillus*, or the *Clostridium leptum* group for either group of the postmenopausal women, pronounced individual changes were observed. These results suggest a trend for the modest enhancement of urine isoflavone absorption in the FOS group. An increase in diversity of the total intestinal bacteria, β-galactosidase activity, and the adjusted breath hydrogen underscore the magnitude of the physiological effects of FOS and soy on the intestinal microflora of postmenopausal women. This study provides a hypothetical model that can be used to design further studies to address the effects of soy and or prebiotics on the human microbiota

Keywords: FOS, soy, real-time PCR, isoflavone, *Bifidobacterium*

P-1.36

The Human Biota Associated Rat As A Model For The Human Intestinal Microbiota

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Human Biota Associated (HBA) animals are created by association of germ-free animals with microbiota derived from human fecal samples. HBA animals are used in studies where gut models are needed to mimic effects of various interventions on the human gut microbiota and associated parameters. There is no doubt that the composition of the fecal microbiota in HBA animals differs from the composition of the microbiota in the human sample with which they were inoculated, since a certain selection takes place in the animal gut. However, no studies have systematically characterized this difference. In the present study, Denaturing Gradient Gel Electrophoresis (DGGE) was applied to separate PCR-amplified 16S rRNA genes originating from HBA rat feces as well as from the human fecal sample used for inoculation of the animals. Subsequently, a total of 15 dominant bands were excised from the DGGE gels, cloned and sequenced. Comparison of the sequences to sequences in the Ribosomal Database revealed that species of *Bacteroides/Prevotella* and *Faecalibacterium* gave rise to most of the dominant bands in the human sample as well as in the HBA rats. There was a tendency that *Prevotella* bands were mostly represented in the human sample, while *Bacteroides* bands were mostly represented in the rats. In the HBA rats, two very dominant bands, which were not present in the human DGGE profile, originated from species of *Ruminococcus*. With the exception of the *Ruminococcus* sequences, sequences originating from both rats and human samples were represented in all major branches of a maximum parsimony tree, indicating that the rat gut does not select for specific taxonomic units of the human microbiota. Bands representing *Prevotella* and *Faecalibacterium*, which were found in identical positions of the DGGE gels originating from human and HBA rat fecal samples, respectively, originated from completely identical sequences, indicating that the same strains of these species were dominating in the human and the HBA rat gut. In conclusion, we find that in spite of major differences in the DGGE profiles, the microbes of the HBA rat gut provide an applicable model for the human gut microbiota

Keywords: human biota associated rats, animal model, DGGE

P-2.1

Application Of Quantitative PCR Assays To Detection Of Human *Bacteroides* Species In The Intestines Of Pigs

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When evaluating the efficacy of probiotic bacteria, it is beneficial to know whether a fed bacterium reaches the appropriate location in the digestive tract. Use of quantitative PCR could detect specific bacteria in a sample with a complex microbial community. In order to determine whether 3 human *Bacteroides* isolates fed to pigs reach the cecum and feces, we investigated the application of *Bacteroides* targeted quantitative PCR primers/ probes developed by Layton et al. (2006). These PCR assays targeted human (HuBac) and all *Bacteroides* (AllBac) species. One concern for application of HuBac was the close relation among 16S rRNA gene sequences *Bacteroides* isolates and clones from pigs and humans. Concentrations of 24 h growth for *Bacteroides* isolates were determined by anaerobic growth on Wilkens-Chalgren agar (approximately 2×10^9 CFU/ml for all three isolates). Feces were obtained from 2 pigs, fed either standard diet or high fiber diet. Dilutions (0 through 10^{-7}) were added to both fecal samples (1 ml dilution with 1 g feces) and mixed well (spiked feces). Using a MJ Research Opticon thermocycler, DNA extracted from pure cultures and spiked feces was used to determine detection limits and optimize standard curves for both AllBac and HuBac. DNA from pig fecal and cecal samples was use to determine whether HuBac amplified native *Bacteroides* present in the pig gut. The AllBac was more sensitive, detecting 10^2 CFU/ml compared to 10^4-10^5 CFU/ml using the HuBac with all 3 isolates. No amplification occurred when HuBac was applied to any pig samples. Both AllBac and HuBac detected the addition of human Bacteroides isolates to fecal samples; AllBac detected the increased concentration of *Bacteroides* while the HuBac detected isolate additions of at least 10^4 CFU/ml. This pair of *Bacteroides*

quantitative PCR primers/probes can detect intestinal survival of human *Bacteroides* isolates in pigs

Keywords: quantitative PCR, swine, *Bacteroides*, intestinal tract

P-2.2

Changes Of The Faecal Bacterial Communities Of Horses Under Transportation Stress And Effect Of An Active Dry Yeast

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In ruminants, active dry yeasts (ADY) exert a positive role in optimising two essential roles of the rumen microbiota: feed digestion and gut health. The stabilising effect of ADY on the rumen microbiota has been mainly highlighted when cattle are subjected to stressful conditions such as high concentrate diets, hot weather, or transportation. Transportation is an important stress factor for horses also, and can lead to intestinal disorders associated with upsets in the microbial balance of the horse hindgut. Therefore, ADY supplementation may be beneficial in preventing microbial disturbances in the equine gut when horses are transported. There is already some evidence for yeast culture efficacy on colonic pH stabilisation in case of starch overload, but the impact of an ADY supplementation on faecal microbial communities in horses facing transport stress has never been evaluated. In the present study, we compared the bacterial profiles generated from TTGE analysis using DNA extracted from faecal samples taken from 4 mature geldings included in a cross-over design (2 × 2). The treatment (ADY) and the control group (C) received daily 2×10^{10} cfu of Levucell SC (*S. cerevisiae* I-1077) and no additive, respectively. All horses were fed a hay:concentrate diet (50:50). On D_0 the horses were transported in a truck for 2 hours. Fecal samples were obtained by rectal collection at D_{-1} (the day before transportation), D_0 and D_{+3} (3 days after transportation). Fecal samples were equally pooled within each group, and total DNA was extracted from these pools. The V6-V8 region of the 16S rDNA gene was PCR-amplified and fragments separated on polyacrylamide gels following TTGE procedure. Results indicated that transportation had an impact on bacterial diversity, as TTGE profiles were clearly different before and 3 days after transportation. When ADY were fed, the richness of the bacterial population appeared greater (+13.5 to +25%) whatever the time of sampling, and the similarity of the banding pattern was higher between (D_{-1}/D_0) and D_{+3} than in the C group. These data suggest that the use of ADY may favour retention of microbial diversity of the equine intestinal microbiota, and limit the imbalance that can arise due to transportation stress

Key words: active dry yeast, equine microflora, transportation stress, TTGE

P-2.3

Effect Of Adding A Starter Culture Or Formic Acid To Fermented Liquid Feed On Gastrointestinal Ecology And Growth Performance Of Piglets

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A study was carried out to investigate whether adding a starter culture or a formic acid containing-product to fermented liquid feed of suboptimal microbial quality could improve the characteristics of the mixture and the gastrointestinal ecology and growth performance of piglets. Fermented liquid feed of good microbial quality was also prepared to be used as a positive control. Four experimental diets were prepared and the feed and water were mixed in the ratio 1:2.5 (w/w) in four closed tanks. Diet 1) Three times daily, 50% of the mixture stored in the tank was removed and then replaced with an equal amount of fresh feed and water, (FLF); Diet 2) Three times daily, 95% of the mixture stored in the tank was removed and then replaced with an equal amount of fresh feed and water. *Escherichia coli* K12 (1×10^6/g) was added to the tank every morning (+COLI); Diet 3) as the COLI treatment but added *Lactobacillus plantarum* VTT E-78076 (1×10^6/g) every morning (+PLANTARUM); and Diet 4) as the COLI treatment but added 0.26 g AIV/KemiSile 2 plus/100 g mixture three times daily (+ACID). One hundred and sixty weaners were used. On day 14, one littermate from each pen was sacrificed and the gastrointestinal tract was immediately removed. The remaining piglets were used to measure the effect of the experimental diets on growth performance during the first six weeks post-weaning. The +ACID diet had lower numbers of lactic acid bacteria and *Enterobacteriaceae*, higher numbers of yeasts, and lower concentration of lactic acid and acetic acid than the COLI and +PLANTARUM diets. Feeding the +PLANTARUM and the +ACID diets increased daily weight gain during the six weeks postweaning ($P \leq 0.02$) compared to feeding the COLI diet. The +ACID group showed the highest numerical daily feed intake in all periods. The gain/feed tended to be lower in the COLI group than in the other three dietary groups during the six weeks post-weaning ($P < 0.10$). The counts of lactic acid bacteria were lowest in the stomach ($P \leq 0.003$) and distal small intestine ($P \leq 0.06$) of the piglets fed the +ACID diet. The counts of yeasts in the proximal gastrointestinal tract was highest in the +ACID and FLF groups. This study showed that addition of *Lactobacillus plantarum* VTT E-78076 or AIV/KemiSile 2 plus to fermented liquid feed of suboptimal microbial quality improved growth performance without affecting the gastrointestinal ecology of piglets substantially

Keywords: formic acid, fermented liquid feed, gastrointestinal ecology, piglets, starter culture

P-2.4

Characteristics Of *Bifidobacterium bifidum* Strains As Probiotics And Survivability Of Microencapsuled Cells *In Vitro*

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The widespread interest in probiotic bacteria that can be observed nowadays results from their medical properties which have been reported both for humans as well as animals. However, it should be noted that the positive "probiotic" properties are observed only in case of certain strains. This study aimed to characterize *Bifidobacterium bifidum* DSM 20082, *Bifidobacterium bifidum* DSM 20215, *Bifidobacterium bifidum* DSM 20239 and *Bifidobacterium bifidum* DSM 20456 (DSMZ) strains and evaluate their biochemical properties, resistance to antibiotics, and antagonism towards selected pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Helicobacter pylori* DSM 4867, *Salmonella enteritidis* SL 5319). Moreover, we investigated the effect of selected *Bifidobacterium* strains in different environmental conditions with special emphasis on conditions prevailing in the human gastrointestinal tract (pH 2, 3, 7, 8; bile salts 3%, 4%). Experiments revealed significant differences between strains of the same species. All examined strains were characterized by diverse biochemical properties, resistance to antibiotics and showed antagonistic action against *Helicobacter pylori*. At pH 7

and pH 8 the number of viable *B. bifidum* cells decreased to 10^7 cfu/ml after 48 hours of cultivation. At pH 2 and pH 3, the number of viable *B. bifidum* cells decreased to 0 after 48 and 72 hours of cultivation, respectively. All the examined bacterial strains fulfill the basic criteria expected for probiotic strains, i.e. survival under *in vitro* conditions of the gastrointestinal tract, at low pH and in the presence of bile salts. Microencapsulation was shown to increase the resistance of *B. bifidum* DSM 20082 and *B. bifidum* DSM 20215 to pH 3. The examined strains are capable of utilising Raftilose and Raftiline (Orafti) preparations as a carbon source. The tested *Bifidobacterium* strains proved to have a good tolerance to low pH (stomach) and pH 7–8 (intestinal tract) as well as to bile and a number of antibiotics

Key words: probiotic, *Bifidobacterium bifidum*, microencapsulation

P-2.5

Intestinal Morphology And Nutrient Ileal Digestibilities In Broiler Chickens Fed With Mannan Oligosaccharide Plus A Yeast Culture And/Or A Live Microorganism

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Two experiments were done to evaluate changes in villi proliferation with age and its relationship with ileal digestibility of dry matter (DM), ash (A), nitrogen (N) and energy (E) in broiler chickens fed cell wall components and a culture from *Saccharomyces cerevisiae* (MOS+CSc) and/or *Bacillus subtilis* (Bs). In both experiments, birds were assigned to a complete randomized design with a factorial arrangement of 2 levels of MOS+CSc (0 and 1 kg/ton of feed) and 2 levels of Bs (0 and 125 g/ton of feed). In experiment 1, 160 chicks were used from 1 to 21 days and were allocated to battery pens in groups of ten. There were four replications per treatment. On days 7 and 21, duodenal samples were taken from two chicks per pen for villi morphology. In experiment 2, 72 broiler chickens were used from 29 to 42 days of age and were individually allocated in holding pens. There were 18 replications per treatment. On day 42, all birds were sacrificed and the ileal contents and duodenal samples were taken. On day 7 of experiment 1, the thickness of the mucosa and muscle ($P < 0.01$), and height of villi and depth of crypts ($P < 0.05$) were lower with MOS+CSc; the thickness of crypts were greater ($P < 0.10$) with MOS+CSc or Bs. On day 21, the thickness of the mucosa ($P < 0.05$), the height of villi ($P < 0.05$) and the number of crypts ($P < 0.10$) were lower and the thickness of the muscle was greater ($P < 0.05$) with Bs with or without MOS+CSc (interaction of MOS+CSc and Bs); the depth of crypts was greater ($P < 0.10$) with Bs. In experiment 2, the ileal digestibility of DM ($P < 0.10$), A and E ($P < 0.05$) were greatest with MOS+CSc without Bs (interaction of MOS+CSc and Bs); the ileal digestibility of N ($P < 0.05$) was higher with MOS+CSc. The thickness of the mucosa ($P < 0.01$), and height of villi ($P < 0.10$) were lower with Bs without MOS+CSc (interaction of MOS+CSc and Bs); the thickness of villi was greater with MOS+CSc and Bs (interaction of MOS+CSc and Bs); the depth and thickness of crypts were greater ($P < 0.10$) with MOS+CSc and the depth ($P < 0.05$) and crypt number ($P < .10$) were lower with Bs. In summary, on day 7 the addition of MOS+CSc and on day 21, the combination of MOS+CSc and Bs elicited greater villi proliferation. On day 42, there was not a clear relationship between the intestinal morphology and the ileal nutrient digestibilities

Keywords: broiler chickens, Saccharomyces cerevisiae, Bacillus subtilis

P-2.6

Effect Of Probiotic On Broiler Performance And Some Blood Factors

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The aim of this trial was to study the effect of different levels of a bacterial probiotic on broiler performance and some blood factors. This experiment was conducted in a randomized complete block design (RCBD) and included 600 Ross-308 broiler chicks (male and female) which were divided into four groups with five replicates. The experiment was conducted in two periods: starter (0–21) and grower (22–42) days. All of the diets were isocaloric and isonitrogenous. In this experiment, the birds received either 0, 800, 1000, 1200 g probiotic per ton DM in the starter period, and either 0, 320, 400, 480 gr probiotic per ton DM in the grower period, which were termed T_1, T_2, T_3 and T_4, respectively. The probiotic was a powder in which the active ingredient is an equal mixture of spray-dried spore-forming bacteria, *Bacillus licheniformis* and *B. subtilis*, at a minimum concentration of 3.2×10^9 viable spores/g. Weight gain during the starter period was significantly affected by dietary treatments ($P < 0.05$), but weight gain during the grower period was not affected by using the probiotic supplement ($P > 0.05$). At the end of trial, the T_4 had maximum weight gain, and T_1 had minimum weight gain. The feed intake and mortality were not affected by experimental diets ($P > 0.05$). Analysis of variance showed no significant difference among treatments for feed efficiency ($P > 0.05$), but there were significant differences among mean of treatments that were derived from Duncan's multiple range test ($P < 0.05$). Analysis of variance showed no significant difference among treatments for serum cholesterol content ($P > 0.05$), but significant difference among mean of treatments were derived from Duncan's multiple range test ($P < 0.05$). The blood hemoglobin content was not affected by experimental diets ($P > 0.05$). The number of white blood cells were significantly affected by dietary treatments ($P < 0.05$): the T_1 and T_1 had maximum and minimum numbers of white blood cells, respectively

Keywords: probiotic, broiler, cholesterol, immune system

P-2.7

Novel Source For Probiotic Bacteria Capable Of Metabolizing Ergovaline

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Fescue toxicosis and ergot alkaloids are estimated by USDA to cost U.S. livestock producers $860 million per year. The endophyte fungi *Neotyphodium coenophialum* found in tall fescue (*Festuca arundinacea*) produce toxic alkaloids (principally erogovaline) that cause deleterious effects in cattle and other livestock consuming endophyte-infected (E+) tall fescue as forage. The earthworm *Eisenia fetida* has been used as a model to determine the toxicity of chemicals such as munitions (TNT, RDX) as well as various herbicides and pesticides in soil. *E. fetida* has been judged a viable model for environmental detoxification through the process of verimicomposting. We hypothesize that anaerobic microbes of the worm's gut will break down ergovaline. Very little is known about the effects of ergovaline on *E. fetida* or its gut-microbes. For this study, *E. fetida* worms were kept in a vermicomposting bin where E+ tall fescue seed containing more than 10,000 ppb ergovaline was added. These worms were harvested to produce gut homogenates that were used as inoculums for culture media with added ergovaline. Compared to control treatments, a 60% decrease in detectable ergovaline was observed in less than 72 hours in worm-gut enrichment cultures

incubated at 38 C. Bacterial 16S profiles of the worm-gut and enrichment cultures were assayed by cloning and DGGE analysis. DGGE showed a detectable shift in the 16S profile during incubation. The 16S clone library represented sequences from multiple phyla, including *Plantomyces, Chloroflexi, Bacteroides*, and *Proteobacteria*. Through vermicomposting we have enriched for bacteria that were isolated from the gut of *E. fetida*. These bacteria have demonstrated the ability to convert ergovaline into unknown metabolites. This evidence suggests that the worm-gut microbiota exposed to E+ plant material has the ability to metabolize ergovaline

Keywords: ergovaline, 16S rRNA, DGGE, earthworms, probiotic development

P-2.8

The Variability Of *Oxalobacter formigenes* And Oxalate In Stool Samples

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Oxalobacter formigenes (OxF) inhabits the intestinal tract of a majority of individuals and is unique in utilizing oxalate as a primary carbon and energy source. Intestinal colonization with OxF may have clinical significance by decreasing intestinal oxalate and its absorption. This could lead to a decrease in the concentration of oxalate in plasma and urine. As the oxalate content of the diet varies considerably, we hypothesized that numbers of OxF would also vary in the intestine and in feces. To enumerate numbers of OxF in feces, a real-time PCR assay was developed which is both accurate and reproducible in quantifying OxF DNA. Stool samples were obtained from 10 individuals known to be colonized by OxF. The oxalate content of stool samples and fecal water was measured by ion chromatography. Concentrations of OxF ranged from lower than the limit of detection (LOD), 5×10^3 cells/g, to 1.04×10^9 cells/g stool. The total oxalate content of stool samples varied from 0.1–1.8 microgram/g, and fecal water oxalate varied from 60–600 micromoles. All parameters measured varied within each stool collection, between stool collections on different days, and between individuals. Notably, in 7 of 10 individuals at least one stool sample contained no detectable OxF. As oxalate was detected in all stool samples, factors other than substrate availability may influence the numbers of OxF shed in feces. The relationships between levels of OxF and oxalate, oxalate absorption, intestinal flora, and other intestinal environmental factors warrant further investigation

Keywords: oxalate, real-time PCR, urolithiasis

P-2.9

The Effect Of Distiller's Grain Products On The Prevalence And Growth Of *E. coli* O157

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Studies were conducted to examine the effects of feeding distiller's grains (DG) on the prevalence of *E. coli* O157 in feedlot cattle and on *in vitro* growth of *E. coli* O157. In the first study, cattle (n = 370) fed steam-flaked corn (SFC) finishing diets were allocated in a 2 × 3 factorial arrangement of treatments (9 replicates) with wet DG (WDG; 0 or 25% diet dry-matter) and antibiotic (none, monensin, or monensin and tylosin) as factors. Fecal samples were collected from each animal twice during the feeding period (d 122 and d 136) and cultured for *E. coli* O157. In the second study, cattle (n = 370) were allocated to 1 of 3 treatments: SFC with 5% silage, SFC with 15% silage, or SFC with 15% silage and 25% dried DG (DDG). Cattle were fed in pens each containing 14–16 animals, with 8 pen replicates per treatment. From each pen, 10 pen-floor fecal samples were collected weekly for 12 weeks and cultured for *E. coli* O157. In study 3, growth of *E. coli* O157 was examined using *in vitro* fermentations with ruminal and fecal microbial inocula. Ruminal fluid and feces were collected from twelve ruminally-cannulated steers adapted to one of four diets. The diets were SFC or dry-rolled corn (DRC) with 0 or 25% DDG. Fermentations, set up in duplicates with or without diet substrate, were repeated on two days. Each fermentation was inoculated with naladixic acid resistant (Nal^R) *E. coli* O157 and samples were removed at hour 0, 6, 12 and 24 to determine concentration of Nal^R *E. coli* O157 In study 1, the prevalence of *E. coli* O157 in cattle fed WDG was significantly higher than those fed SFC only on d 122 ($P < 0.01$), but not on d 136 ($P > 0.5$). The second study also showed a significant DG effect. Cattle fed 25% DDG had a higher prevalence of *E. coli* O157 when compared to cattle fed SFC with 5% silage ($P = 0.01$) or SFC with 15% silage ($P = 0.01$). Rumen microbial fermentations revealed no difference in 24-hr *E. coli* O157 growth with respect to DG when substrate was included; however, when no substrate was included, DDG had significantly higher growth than no DDG ($P < 0.05$) at 24-hr. Fermentations with fecal microbial inoculum showed no DG effect on *E. coli* O157 growth ($P > 0.7$). The results indicate that feeding distiller's grain to cattle may result in a higher prevalence of *E. coli* O157

Keywords: *E. coli* O157, distiller's grains, cattle

P-2.10

Stimulation Of *E. coli* O157 In Batch Culture Fermentation By Constituents Of Intestinal Mucus

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In cattle, *E. coli* O157 generally persists in the hindgut (cecum, colon, rectum). Uronic acids are monocarboxylic acids often contained within mucoid secretions of the gut. Bacterial species, including *E. coli* O157, with the capacity to metabolize uronic acids via the Entner-Doudoroff pathway may have a selective advantage in the hindgut where these acids are present. Therefore, our objective was to test the effects of mucin and carbohydrate constituents of mucin on growth of *E. coli* O157 in *in vitro* ruminal or fecal microbial fermentation. Ruminal contents and feces were collected from a ruminally-cannulated steer fed a high-grain diet. Ruminal contents and feces suspended in physiologic saline to increase fluidity, were strained through 2 layers of cheesecloth, and used as microbial inocula. Fermentations (50 ml) were set up in serum bottles with a 2:1 McDougall's buffer to inoculum ratio. Nine substrates (500 mg/bottle) were evaluated with 2 bottles designated to each substrate/inoculum combination. A mixture of 5 strains of nalidixic acid resistant (Nal^R) *E. coli* O157 was added to each bottle at 2.8 log CFU/ml. Concentration of Nal^R *E. coli* O157 was determined at 0, 6, 12 and 24 hr by serial dilution and plating. The experiment was performed 3 times and O157 concentrations were analyzed using a statistical model with main effects of treatment and hour, the treatment × hour interaction, and repetition as a random effect. In ruminal fermentation, galactose reduced ($P < 0.01$) *E. coli* O157 concentration at 24 hr compared to control (no substrate). Fucose, mannose, glucuronic acid, galacturonic acid, glucosamine, galactosamine and porcine gastric mucin had no impact on *E. coli* O157 compared to control. However, gluconic acid increased ($P < 0.01$) *E. coli* O157 compared to control at 24 hr. In fecal fermentation, mannose, galactose, gluconic acid, glucuronic acid, galacturonic acid, glucosamine and porcine gastric mucin

increased (P <0.01) *E. coli* O157 compared to control at 24 h, while galactosamine and fucose did not elicit significant responses (P >0.05). In summary, some constituents of mucus stimulate growth of *E. coli* O157, especially in fecal fermentation. We identified gluconic acid as the most stimulatory substrate, increasing *E. coli* O157 by more than 1.0 log in ruminal fermentation and 2.0 log in fecal fermentation

Keywords: *Escherichia coli* O157, *in vitro*, mucus, carbohydrates

P-2.11

Association Of *Providencia alcalifaciens* With Diarrhea In Dogs

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Providencia alcalifaciens has been implicated as a primary enteric pathogen in humans, associated with traveler's diarrhea and diarrhea in children from developing countries. Strains of *P. alcalifaciens* have previously been shown to be invasive in cell studies, but other virulence mechanisms have not been detected. In September 2005 a rectal swab of a dog with bloody diarrhea grew *P. alcalifaciens* as the predominant organism. Subsequently, this was also seen in 13 additional faecal specimens from dogs with severe diarrhea. Two of the dogs were from the same household, but no connections are known between the other dogs. The aim of this study was to investigate the 14 isolates of *P. alcalifaciens* from these dogs. Pulsed field gel electrophoresis (PFGE) was performed on genomic DNA from all isolates after digestion with *Sma*I restriction enzyme. Representative isolates were tested for enterotoxin production and cell invasive ability in Vero cells. PFGE showed that ten of the isolates were closely related, nine of these were shown to be indistinguishable by visual examination of the PFGE banding patterns, while one isolate differed by the lack of one band. No secreted cytotoxic activity was detected in cell culture assays of culture supernatants but the bacteria were able to invade Vero cells. The clonal appearance of the majority of these isolates indicates that *P. alcalifaciens* may have a predilection for the intestine of dogs

Keywords: *Providencia alcalifaciens*, dogs, diarrhea, PFGE

P-2.12

Alternatives To Antibiotics-Grasping Bacteria By Their Achilles Heels

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The EU precautionary approach to antibiotic use and the increased interest for gut health significantly impacted the food/feed additives market in recent years. An alternative and optional extra to stimulating beneficial bacteria in the intestine is to tackle the weak spots (=Achilles heels) of pathogenic bacteria. General strategies for warding off different pathogenic bacteria include the supply of specific milk components, antagonistic bacteria for competitive exclusion, or the alternative adhesion approach. Different food and feed ingredients have the potential to bind pathogens and thus form an alternative binding-site to the gut epithelium, interfering with the first step of infection. For instance, type1-fimbriated bacteria attach to mannan-rich plant or fungal substances. To study the binding capacity of different fibrous edibles for bacterial cells, a microtitration plate-based adhesion test was developed. The test principle is based on an inverse relationship between initial cell densities and the appearance of growth: the detection times of bacterial growth will be shorter for those bacteria with high adhesion values, because more cells remain adherent to the test matrix. Growth was measured in an automated way as turbidity against time with a microplate reader. Employing a sigmoidal curve model, it was shown that four time-coordinates were highly separating for different initial cell numbers, and as a consequence also for different food and feed ingredients. For evaluation of the method, Bio-Mos (Alltech Inc.), the fermented soya bean product tempe, palm kernel extract and carrot pomace (from "SAFEWASTES" EU project) were tested. The tested substances effectively bound *Salmonella enterica* sv. Typhimurium ATCC 13311. Significant differences were shown for the test strains *Salmonella enterica* sv. Typhimurium ATCC 13311 and *Salmonella enterica* sv. enteritidis ATCC 13076; in agreement with the fact that the former bacterial strain does agglutinate mannan-oligosaccharide preparations and the latter one does not. As most pathogens can express more than one type of adhesin and natural products can diverge due to biological variation or processing, this high-throughput method offers the opportunity to screen a large variety of food and feed ingredients with various strains of bacteria

Keywords: gut health, pathogenic bacteria, *Salmonella*, adhesion test, food and feed

P-2.13

Improved RNA Extraction Procedures To Increase The Sensitivity Of Real Time Reverse Transcription-PCR Detection Of *Escherichia coli* O157:H7

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The detection of small numbers of pathogenic bacteria in biological samples has been a difficult problem over the years, but molecular microbiological methods have increased the accuracy and sensitivity of their detection, as well as the rapidity of the process. However, most of these molecular methods for detection still require a culture-based enrichment step prior to PCR. Another disadvantage is that genomic DNA from viable as well as non-viable organisms can be amplified by PCR. Our goal is to develop and use real-time Reverse Transcription-PCR (qRT-PCR) with mRNA as the template, which should improve sensitivity of detection of viable bacteria without pre-enrichment. Because the sensitivity of qRT-PCR can be hampered by inefficient RNA extraction, or failure to remove inhibiting substances that interfere with downstream applications, the aim of this study was to develop spin column-based RNA extraction procedures for environmental samples containing either low or high numbers of background micro-organisms (such as carcass and faecal samples). Faecal samples were washed in the presence of CTAB prior to the lysis procedure, and to try and improve total RNA recovery from samples with low numbers of bacteria, we added non-target bacterial cells to these samples. RNA synthesis and degradation was halted by placing the samples into of a mixture of Phenol-Ethanol (pH 4.3). The samples were subjected to bead beating in the presence of TE buffer, SDS and Phenol-Chloroform, prior to total RNA recovery using spin columns. The sensitivity of the extraction procedures were assessed by qRT-PCR detection of the *rfbE* gene after known numbers of a target organism (*E. coli* O157:H7) were seeded into the samples. The modified RNA extraction procedures increased the recovery of amplifiable RNA target without pre-enrichment to approximately 63 CFU/mL in the starting material for carcass samples, 4×10^3 CFU/ml for rumen and 4×10^4 CFU/ml for faecal material. This method represents a significant improvement in sensitivity of detection of micro-organisms in environmental samples without pre-enrichment of the sample

Keywords: RNA extraction, pathogen detection, environmental samples

P-2.14

The Digestion Of Newborn Lambs With Various Body Mass

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The purpose of the research was to compare activity of alpha-amylase and complex proteases in the pancreas of newborn lambs with different body mass, and in colostrum of their nursing mothers. The experiments were done on sheep bred only for astrakhan skin. Enzyme activities were determined for pancreatic homogenates from newborns and in colostrum of nursing mothers on the first day of lactation. The results showed that pancreas mass, as well as the activity of pancreatic enzymes in lambs with low body mass at birth (2.3 ± 0.2 kg), was much less than similar parameters in newborns with normal body mass (3.6 ± 0.4 kg). Specific alpha-amylase activity and activity of protease complex of pancreas in lambs with small body mass was 110.4 ± 10.0 microgram/min/g protein and 9.1 ± 0.6 microgram/min/g protein respectively, compared to 144.3 ± 7.0 microgram/min/g protein and 17.4 ± 0.3 microgram/min/g protein in normal newborns. The daily average yield of colostrum in mothers of small mass lambs was 247.8 ± 30.3 ml/day, whereas in mothers of normal mass lambs was 411.2 ± 27.4 ml/day. Despite of a difference in colostrum quantity in lactating sheep between the two groups, the activity of amylolytic and proteolytic enzymes in their colostrum did not differ. Thus, in lambs with low body mass at birth, a delay in development intestinaldigestion was observed. This is shown by reduction of pancreatic mass and decreasing specific enzyme activity of the initial stages of carbohydrate and protein hydrolysis. However, the specific activity of these hydrolytic enzymes in colostrum of their nursing mothers did not differ. Hypofunction of intestinal digestion in newborns with small body mass, obviously, is a consequence of infringement of prenatal feeding. Hydrolytic enzymes of colostrum, hence, play a more significant role in intestinal digestion in lambs with low bodymass, since their own digestive ability is not developed enough

Keywords: newborns lambs, digestion, colostrum, hydrolytic enzymes

P-2.15

Changes In Serum Gastrin Level, Gastric Motility And Acidity After Roux-En-Y Biliary-Entereic Reconstruction In Patients With Obstructive Jaundice

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Roux-en-Y biliary enteric reconstruction procedures have been widely used in the treatment of many patients with obstructive jaundice. However several studies showed that there is increased incidence of peptic ulceration after such procedures. The objective of this study was to evaluate the effect of Roux-en-Y biliary-enteric reconstruction on serum gastrin level, gastric acidity and motility in patients with obstructive jaundice. Twenty patients with confirmed biliary obstruction who underwent biliary enteric reconstruction were studied. Pre- and postoperative serum gastrin level, gastric motility and acidity were assessed by radioimmunoassay, gamma scintillation camera and basal acid output (BAO), and pentagastrin stimulated maximum acid output (MAO), respectively. The results showed a significant rise in postoperative serum gastrin levels when compared with preoperative levels (percent of change: $29.53 \pm$SD 7.82 pg/ml, and $p = 0.000$). Postoperative BAO and MAO increased significantly when compared with preoperative values (percent of change: $32.99 \pm$SD 25.91, $p = 0.000$ and percent of change: $18.64 \pm$SD 11.86 $p = 0.000$ respectively). On the other hand, there was significant delay in gastric emptying postoperatively (percent of change: $19.45 \pm$SD 8.91 and $p = 0.000$). It is our conclusion that Roux-en-Y biliary enteric reconstruction results in increased levels of serum gastrin, gastric acidity, and delayed gastric emptying

Keywords: gastrin, motility, physiology, enteric reconstruction

P-2.16

Buffer Concentration Influences the Effects of Supplemental Yeast on pH in Rumen-Simulating Continuous Cultures

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A recent literature review and meta-analysis comprising 52 references indicates that supplementation with *Saccharomyces cerevisiae* live yeast culture (Yea-Sacc® 1026, Alltech Inc.) increases ruminal pH. Nonetheless the magnitude of this increase may depend on dietary factors and preceding ruminal conditions including basal ruminal buffering capacity. This study was designed to evaluate if the magnitude of the increase in ruminal pH resulting from Yea-Sacc® 1026 supplementation is dependent on the ability of the rumen to resist changes in ruminal pH. This work was conducted *in vitro* using twelve single-flow one-liter fermentation vessels. Each of two experiments included seven adaptation and three collection days. The same diet (corn grain (43.4%), grass hay (30.0%), alfalfa hay (10.0%), soybean meal (9.5%), distiller's grains (3.3%) and mineral supplement) and dilution rate ($0.03\ h^{-1}$) were used in all cultures in both experiments. Differences in basal ruminal buffering capacity were induced by diluting the McDougall's artificial saliva solution used for buffering continuous cultures. Thus, undiluted buffer (100%) and buffer diluted at 60% were used in experiment 1 while undiluted buffer and buffer diluted at 80% were used in experiment 2. Presence or absence of Yea-Sacc® 1026 were also included resulting in a 2×2 factorial design in each experiment. Buffer concentration induced differences in the basal culture pH as expected. Culture pH were 6.50 for undiluted buffer, 6.30 for buffer diluted at 80% and 5.67 for buffer diluted at 60% on average. Yea-Sacc® 1026 supplementation increased culture pH with undiluted buffer by 0.14 ($P < 0.05$) and 0.11 ($P = 0.08$) pH units in experiments 1 and 2, respectively. This effect was unapparent with the diluted buffers in both experiments. Several studies have demonstrated the ability of Yea-Sacc® 1026 to stimulate the growth of specific ruminal lactate-utilizing species. This study suggests that these stimulatory effects may be influenced by the buffering capacity in the rumen

Keywords: yeast, buffer concentration, pH

P-2.17

Nordihydroguaiaretic Acid Causes A Decrease In Rumen Bacterial Growth Yield

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Creosote bush, *Larrea tridentata* is a source of the antioxidant nordihydroguaiaretic acid (NDGA). Nordihydroguaiaretic acid is antimicrobial in nature. Criticisms against the use antimicrobial drugs for growth promotion in livestock have caused an interest in the use of plant compounds as rumen modifiers. The effect of the consumption of creosote bush and NDGA on rumen bacteria and rumen fermentation has not been documented. An *in vitro* experiment was conducted to evaluate the effect of NDGA on viability of pure cultures of rumen bacteria and microbes found in

whole rumen fluid from a cow. All cultures were grown in 5 ml of complex growth medium with 40% clarified rumen fluid. Varying levels of NDGA were dissolved in ethanol and added to culture medium at 0, 5, 10, 50, and 100 microgram/ml prior to inoculation with pure cultures. Similiarly, NDGA was added to 5 ml of growth medium prior to addition of microbes from whole rumen fluid at 0, 10, 20, 30, 40, 50, 60, 70, 80, 100, 150, 200, 250, 300, 400, 500 microgram/ml. Growth yield was monitored by turbidity at 600 nm at 0, 1, 2, 3, 4, 6, 8 and 24 h. Independent of species, additions of NDGA (0 vs 100 microgram/ml) to pure cultures of rumen bacteria caused a 35 fold decrease in turbidity (0.71 vs 0.02; $P < 0.001$). Additions of 10 microgram/ml NDGA caused a 62% decrease in turbidity compared with 0 microgram/ml. Ethanol control and 0 microgram/ml NDGA were similar (0.57 vs 0.71) to 5 microgram/ml NDGA (0.51; $P > 0.10$). Growth yield of *Butyrivibrio fibrisolvens* H17E, *Prevotella ruminicola* GA33, and *Ruminococcus albus* 8 was most affected by NDGA compared to *Prevotella ruminicola* 118B, *R. flavefaciens* FD1, and *Streptococcus bovis* JB1 (0.01, 0.20, 0.10 vs 0.59, 0.58, 0.65, respectively). *In vitro* growth yield of microbes in whole rumen fluid was not affected until 100 microgram/ml of NDGA was added to the growth medium ($P > 0.05$). There was a 133% reduction in growth yield between 0 vs 100 microgram/ml NDGA (0.60 vs 0.08) and a 50% reduction when comparing 50 vs 100 microgram/ml NDGA (0.6 and 0.3 respectively; $P < 0.001$). Further additions of NDGA did not result in a decrease in yield (100, 150, 200, 250, 300, 400 and 500 microgram/ml NDGA; 0.08, 0.07, 0.11, 0.12, 0.08. 0.10 and 0.02, respectively). These data suggest that NDGA is toxic to rumen bacteria. Level of toxicity is dependent upon a species and mixture of bacteria present. The bioconversion of NDGA by rumen bacteria is currently under investigation

Keywords: Nordihydroguaiaretic acid, bacteria, growth

P-2.18

Phenolic Compounds Correlation With *in vitro* Gas Production And Degradability Of Selected Tibetan Shrub Species

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The occurrence of phenolic compounds, particularly condensed tannins (CT), and their correlations with *in vitro* gas production and degradability of selected species of Tibetan alpine plants *Dasiphora fruticosa* (*DF*), *Spiraea alpine* (*SA*), *Hippophae tibetica* (*HT*) and *Caragana jubata* (*CJ*) were evaluated. Chemical analysis showed the average CP content of the four tested species was over 100 g kg^{-1} DM, regardless of species. The CP contents declined with increasing age of maturity (from June to October), with the mean values varying from 51 g kg^{-1} DM in *SA*, to 143 g kg^{-1} DM in *HT*, and the concentrations of CP and fiber did not show any significant differences among the species ($P > 0.05$). However, differences were observed for total phenolics (TP), total tannins (TT), CT and extractable CT (ECT). The highest TP content was record for *HT*, followed by *SA*, *DF* and *CJ* (68.43, 40.59, 38.34 and 36.01 g kg^{-1} DM, respectively). Potential *in vitro* gas production at 24 h and 48 h incubations for *DF* was higher than those for *SA*, *CJ* and *HT*. Rates of fermentation at 48 h were 0.070, 0.052, 0.025 and 0.036 for *DF*, *SA*, *CJ* and *HT* ($P < 0.001$). Species had a significant ($P < 0.05$) effect on apparent DM degradability and *in vitro* N degradability. After 24 h incubation, the highest mean value for apparent DM degradability and *in vitro* N degradability was in *DF*, and *CJ* had the lowest values for the above parameters. Negative correlations were observed between CT and apparent DM degradability, *in vitro* N degradability, as well as phenolic compounds and *in vitro* gas production after 24 h incubation. However, this was not observed when the samples of different ages were mixed, suggesting that CT may still be impeding digestion; and concentration of tannins may not be the exclusive factor influencing degradability and nutrient utilization from these plants

Keywords: Tibetan shrubs, gas production; in vitro fermentation, tannins, phenolic compounds

P-2.19

Effects Of Rumen Undegradable Protein And *Yucca schidigera* Extract On Metabolism By Ruminal Microbes Maintained In Dual Flow Continuous Culture Fermenters

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An experiment was conducted to determine the effects of rumen undegradable protein (RUP) and supplementation of *Yucca schidigera* extract on fermentation by ruminal microbes maintained in dual flow continuous culture fermenters. Eight fermenter flasks (1.03 L in volume) were inoculated with ruminal fluid from a cannulated cow during two 10-d experimental periods. Isoenergetic and isonitrogenous diets were formulated with two sources of supplemental protein, solvent-extracted soybean meal and mechanical-extracted soybean meal processed with fresh soy gums (Soy Best®) to attain low RUP (LRUP) and high RUP (HRUP) diets. In addition, there were two concentrations of *Yucca schidigera* extract added to the LRUP and HRUP diets at 0 (0YS) and 50 (50YS) microgram/kg of DM. The latter concentration would be equivalent to supplementing 1 g/d of *Yucca schidigera* extract to a dairy cow. The LRUP (28.2% RUP) and HRUP (41.1% RUP) diets, along with the two concentrations of *Yucca schidigera* extract were randomly assigned in a 2×2 factorial arrangement of treatments with two replicates per period. After a 7 day adaptation period, the last 3 days of each experimental period were used to collect effluents for chemical analyses. Differences in digestion ($P < 0.05$) between LRUP and HRUP diets, respectively, were observed for apparent OM (29.5 vs 24.4%), true OM (43.4 vs 38.3%) and NDF (33.0 vs 22.2%). There was a tendency ($P < 0.10$) for ADF digestion to also be higher with the LRUP diets (40.9 vs 34.2%). Because of greater digestion with the LRUP diets, total VFA concentration (mM) was greater ($P < 0.05$) at 142.3 vs 126.0. Ammonia-N (microgram/100 ml) was greater ($P < 0.05$) with the LRUP diets (8.9) compared with the HRUP diets (5.4) while dietary N flow (g/d) was less ($P < 0.05$) with the LRUP diets (1.61 vs 1.88). There were no differences in bacterial N flow between RUP treatments or *Yucca schidigera* extract treatments. In this experiment, there were no interactions between RUP and *Yucca schidigera* extract supplementation. In general, RUP elicited various responses, while the dose of *Yucca schidigera* extract had minimal effect on microbial fermentation. Previous experiments have shown some effects of *Yucca schidigera* extract on fermentation by rumen microbes at a dose equivalent to 2 g/d. Therefore, responses to *Yucca schidigera* extract may be dose dependent

Keywords: rumen, undegradable protein, *Yucca schidigera*

P-2.20

Effects Of Urea Or Optigen On Fermentation, Digestion, And N Flow In Rumen-Simulating Fermenters

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The effects of two NPN sources, urea or Optigen (controlled-release NPN) on ruminal fermentation, digestion, and N flow

were investigated in single-flow rumen-simulating fermenter cultures. Data from 17 experiments were included in this meta-analysis (urea: 59 cultures; Optigen: 59 cultures). Cultures were fed diets with a forage base of corn silage and alfalfa hay, 45 to 50% forage (DM basis), and equal NPN from urea or Optigen at 0.44 to 0.66% dietary DM. NPN from urea or Optigen replaced 6.0 to 9.8% of dietary N. Cultures were fed 12.5 g as fed of experimental diets twice daily for six days. Target dilution rate with McDougall's artificial saliva solution diluted 70:30 with tap water was 0.045 h^{-1}. Samples were collected from all cultures immediately prior to morning feeding during the last 3 days of the experiment for fermentation analysis. Effluent weights were recorded each day and a composite sample for each fermenter was used for DM, OM, and NDF disappearance determination. Nitrogen flow measures were estimated by using purine to N ratios for effluent DM and bacteria. Data were analyzed using the PROC MIXED Model of SAS. Culture fluid pH was not affected by diet (6.37 vs. 6.36; $P > 0.10$). Cultures fed Optigen diets had higher acetate:propionate ratio (3.21 vs. 3.40, $P < 0.01$) and tended to have higher total VFA concentrations (73.01 vs. 76.36 mM, $P < 0.10$) than urea-fed cultures. Ammonia was greater in cultures fed Optigen (6.24 vs. 7.34 microgram/dl, $P < 0.0001$). True DM digestion tended to be greater in Optigen-fed cultures ($P < 0.10$). Cultures receiving Optigen had higher protein degradability (70.0 vs. 74.4, $P < 0.05$), higher bacterial N yields (0.342 vs. 0.353 g, $P < 0.05$), and were more efficient in synthesizing bacterial N (33.3 vs. 34.3 g bacterial N/kg fermentable carbohydrate, $P < 0.05$) than urea diet cultures. We conclude that providing dietary N to rumen-simulating fermenter cultures in the form of Optigen, a controlled-release NPN source, is superior to providing NPN from urea in terms of improving bacterial N yield. Higher ammonia concentrations at 16 h post-feeding in cultures fed Optigen diets (+17.6% compared to urea) is indirect evidence of the controlled release of N from Optigen

Keywords: non-protein nitrogen, urea, Optigen, ruminal metabolism

P-2.21

Effect Of Different Sources Of Nitrogen On Ruminal Microbial Growth And Fermentation of Pretreated Rice Straw *in vitro*

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The objective of this study was to investigate the effect of N forms on microbial growth and fermentation of untreated (URS), ammonium bicarbonate-treated (ABRS) and sodium hydroxide-treated rice straw (SHRS) using *in vitro* gas production technique (Reading Pressure Technique). Ammonium bicarbonate (AB) and casein were used as N sources isonitrogenously. After 24 h of incubation, gas production, concentration of ammonia N (NH_3-N), volatile fatty acid (VFA) and microbial protein (MCP) in incubation medium were determined. Gas production of URS, ABRS and SHRS increased when N was added compared with non-addition control ($P < 0.05$), with the highest gas value (93.1 ml/g DM) in SRS added with casein. The NH_3-N concentration was significantly higher N was added for all straws, with little difference between two N-added treatments but lowest NH_3-N content in the SHRS. The presence of casein increased total VFA for three substrates, with greater value for SHRS than for ABRS and URS ($P < 0.05$). Casein and AB increased the concentration of acetate, propionate and butyrate for SHRS, but no significant differences were observed in total VFA, acetate, propionate and butyrate between AB addition and non-addition of N for URS and ABRS ($P > 0.05$). Casein decreased the ratio of acetate to propionate for both treated straws ($P < 0.05$), while the acetate to propionate ratio for URS and SHRS was not influenced by addition of AB ($P > 0.05$). Casein significantly increased MCP for all straws compared with non-addition of N ($P < 0.05$). Addition of AB also increased MCP for all straws, with little difference for ABRS between AB-added and no-added treatments. From the results in this study, it is inferred that rumen microorganisms can use different N sources for their growth, and ammonia could replace protein as the only N sources for microbial growth

Keywords: ammonia bicarbonate, casein, ruminal fermentation, microbial growth, *in vitro*

P-2.22

Effect Of Protein Level In The Diet On Activity Of Tannins During Ruminal Breakdown Of Structural Polysaccharides

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Tannins in plants can have both positive and negative effects on forage digestibility, depending upon amount and type of tannins present. Additionally, protein availability is often decreased by many types of tannins, which can have negative effects on animal development and performance. The purpose this work was to evaluate the relationship between dietary protein and tannins on the ruminal digestion of structural polysaccharides. Tannins were obtained from grape marc (*Vitis vinifera*), and cell wall polysaccharides were prepared from rye grass (*Lolium perenne* L.), lucerne (*Medicago sativa* L.), and other purified energy sources. Both soybean meal and ammonium sulphate were used as nitrogen sources. Four levels of tannins and additions of non protein nitrogen were studied on a basal diet of soybean meal. Diet with grape marc containing 5.11 g/kg DM (or 1.24 g "CE/L", catechin equivalents per liter) of tannins showed an effect on ruminal digestibility of plant cell wall preparations, and the intensity of this effect was closely related with tannin concentration and protein content in the diet. With diets containing a low protein content (4.8 microgram of nitrogen), the degradability of plant wall cell decreased from 59 to 25% for rye grass, and from 55 to 13% for lucerne, as levels of grape marc tannins increased. The latter suggests that tannins complexed with the protein substrate of the diet; and (or) they have an inhibitory effect on polysaccharidase activity, either because of the binding effect on polysaccharides or because of the inhibition of the microbial enzymes. When nitrogen content was increased in the diet, a slight increase was observed in cell wall digestibility, from 5 to 2.5% percentage units for rye grass and lucerne, respectively. Both situations have a direct impact on the ruminal fermentation rate, lag time and volume of gas produced. When polyethylene glycol (PEG) was added of the diet, the effect of the grape marc tannins was partially overcome: being 40% of the *in vitro* gas production measured from the control incubations. We conclude the negative effects of tannins on microbial enzymes and rumen digestibility is more accentuated when the dietary protein content is low

Keywords: tannins, rumen, protein, gas production, digestibility, polysaccharides, cell wall

P-2.23

Attachment, Colonization And Formation Of Ruminal Microbial Biofilms *In Vitro* On Inert Surfaces

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A biofilm is an association between microorganisms, surface and a polysaccharide matrix, and it changes according to the

roughness of the surface, nutrients, temperature and pH of environment. Relatively little information about the ability of ruminal microorganisms to adhere on inert surfaces to form a biofilm exists, therefore it was decided to evaluate if adhesion, colonization and formation of biofilms by the ruminal microorganisms on inert surfaces, depends on survival and (or) enzymatic secretion during the degradation process of the substrate. In vitro incubation with PVDF membranes was conducted. Ruminal fluid was pre-incubated for 2 hours with sugars and starch, and the membranes were incubated for 6, 12, 24 and 48 hours. The membranes were fixed with glutaraldehyde to 4% in 0.05M sodium cacodylate buffer for 8 hours to 4 °C. The membranes were freeze dried and coated with gold palladium and later observed on a scanning electron microscope. Other membranes were soaked in 0.01% (w/v) acridine orange in acetate buffer (pH 4.0) and they were observed under a light microscope with UV illumination. Both forms of microscopy showed the presence of microorganisms attached on the membrane surface, mainly cocci, bacilli and protozoa, with heterogeneous microbial colonies and the appearance of flagellum-like structures mediating adhesion. Some bacillus-like cells also had protuberances on their surface, similar to cellulosomes. Biofilms presented superficial and submerged colonies in a matrix, separated from each the other by interstitial spaces that allow the flow of nutrients and gases. On the other hand, both microscopic approaches demonstrated that the ruminal microorganisms were metabolically active

Keywords: attachment, biofilm, surface, flagella, ruminal microorganisms

P-2.24

Effects Of Forage To Concentrate Ratio On Intestinal Absorbable Amino Acid In Goats

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Four young goats fitted with rumen fistulae and T-type cannulae in proximal duodenum and terminal ileum were used to investigate the effects of forage to concentrate ratio on intestinal absorbable amino acid (AAA) in a 4 × 4 latin square design. Four ratios of forage to concentrate were 10:0 (Diet A), 8:2 (Diet B), 6:4 (Diet C) and 4:6 (Diet D), respectively. The rumen bacteria and protozoa were separated from rumen content. The flows of duodenal and ileal digesta were determined by collecting whole digesta. With diaminopimelic acid as rumen bacterial marker and phosphatidyl choline as the protozoal marker, the output of AAA from rumen bacteria, protozoa and bypass protein to intestinal total AAA (TAAA) was measured. The output of AAA from bacteria in diet B was $1.54\ g\ d^{-1}$, significantly higher than that in diet A ($0.45\ g\ d^{-1}$), C ($0.36\ g\ d^{-1}$) and D ($0.39\ g\ d^{-1}$) ($P < 0.01$). The contribution of bacterial AAA to the intestinal TAAA in diet B was 34.8%, which was higher than that in diet A (13.25%), C (14.81%) and (D (24.84%) ($P < 0.05$). The output of AAA from rumen protozoa in diet A was $1.22\ g\ d^{-1}$, significantly higher than that in diet B ($0.63\ g\ d^{-1}$), C ($0.57\ g\ d^{-1}$) and D ($0.42\ g\ d^{-1}$) ($P < 0.01$). The contribution of protozoal AAA to the TAAA in diet A was 36.74%, while in diet B, C and D was 15.71%, 23.45% and 26.75%, respectively. The output of AAA from by-pass rumen protein in diet A and B was $1.65\ g\ d^{-1}$ and $1.84\ g \cdot d^{-1}$, respectively, significantly higher than that in diet C ($1.51\ g\ d^{-1}$) and D ($0.76\ g\ d^{-1}$). The highest contribution of protozoal AAA to the TAAA was observed in diet C (61.37%), significantly higher than that in diet A (49.13%), B (45.88%) and D (48.41%). The content of intestinal TAAA in diet A, B, C and D was 3.32, 4.01, 2.43, $1.57\ g\ d^{-1}$, respectively. It was concluded that ratio of forage to concentrate can affect the intestinal AAA of goats and diet B (8:2) has the highest efficiency of providing intestinal AAA to goats

Keywords: forage to concentrate ratio, absorbable amino acid, goats

P-2.25

An Investigation Of The Relationship Between Rumen And Fecal Bacterial Concentrations

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With no acceptable method for collecting fresh rumen fluid from exotic zoo animals, while fecal matter is easily obtained, it was proposed that fecal bacterial concentrations may be correlated with rumen bacteria. If so, fecal bacterial concentrations could be used to study both the effects of diet on rumen bacteria as well as rumen abnormalities. In a Latin square design, four crossbred ewes were fed diets of 100% long or chopped orchardgrass hay (OH) and 60% ground or whole shelled corn plus 40% chopped OH. Diets were fed at 0800 and 1700 hours. In a second trial, the sheep were fed a pelleted complete feed at varying levels of intake, i.e., control at 2.0% of body weight, and at a 10%, 20% and 40% reduction. Each period lasted three weeks, two for adjustment and the third for sampling. Samples were collected from each animal on two days during the sampling week, the first just prior to the 0800 hr feeding and the second just prior to the 1700 hr feeding. Total, cellulolytic, and amylolytic bacterial concentrations were determined with MPN assays. Significantly higher total rumen bacterial concentrations were found on the high concentrate diets as compared with the high forage diets ($P < 0.01$). Grinding the corn also resulted in significantly higher total bacterial concentrations ($P < 0.05$). Fecal concentrations of total and amylolytic bacteria were higher with the high concentrate diets ($P \leq 0.01$), while chopping the forage resulted in a decrease in concentration of fecal cellulolytic bacteria ($P \leq 0.05$). Correlation coefficients between the concentrations of rumen and fecal bacteria were non-significant. Concentrations of total, cellulolytic, and amylolytic bacteria in the rumen of sheep on varying intakes were not significantly different from one another. However, a linear increase in fecal bacterial concentrations was observed as intake decreased ($P < 0.01$). Correlation coefficients between rumen and fecal bacterial concentrations were again non-significant. In conclusion, fecal bacterial concentrations reflected both extreme diet changes and differences in particle size. Fecal bacterial concentrations may also reflect a change in diet intake. However, the data from this study clearly indicate that rumen bacterial populations cannot be predicted from fecal bacterial populations

Keywords: amylolytic, bacteria, cellulolytic, feces, rumen

P-2.26

Temporal Stability And Effect Of Preceding Diet On The Rumen Bacterial Population Structure In Sheep

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The importance of the structure of the microbiota on rumen function and ruminant nutrition is well recognized. However, the temporal stability and capacity of the microbial population to return to its initial state following a challenge (e.g., diet change or additive treatment) is not known. In this work the composition of the rumen bacterial population was qualitatively char-

acterized in vivo during a 5-month period. Two groups of four 2-year-old rumen-cannulated wethers without previous exposure to a concentrate-rich diet were used. One group was fed alfalfa hay throughout the experimental period, while the second group started with the same diet, switched to a wheat/alfalfa hay diet (65:35 ratio) during month 2 and 3, and changed back to the original alfalfa diet until the end of the study. Samples from the liquid and solid rumen phase as well as the rumen epithelium were taken each month and analyzed by PCR-DGGE. A correlation between sampling times was established using a moving window analysis. The bacterial populations in the alfalfa-fed group were highly similar from month to month. The percentage of similarity was about 60 to 65% for the populations attached to the epithelium or associated to the liquid phase. Although the structure of the bacterial populations were highly correlated from consecutive sampling times, comparisons of bacterial profiles at the start and end of the 5-month experimental period decreased by as much as 20% in this group of animals, indicating a variability over time. For the concentrate-fed group, the similarity dropped following the change in diet: from month 1 to 2 and from 3 to 4 to an average of 30% for all three bacterial populations. However, the percentage of similarity between month 2 and 3 was also low (35 to 50%), suggesting that the populations were not stable after 4 or 8 weeks after feeding the cereal-rich diet. Known differences induced by diet on the rumen populations were confirmed by hierarchical clustering analysis and biodiversity indexes. This study shows that rumen bacterial communities evolve over time in young adult sheep and that variations caused by a change in diet may take longer than previously though to stabilize

Keywords: PCR-DGGE, rumen bacteria, effect of preceding diet

P-2.27

Composition Of The Rumen Microbial Populations Of Colombian Wild Herbivores: Capibara (*Hydrochaeris hydrochaeris*), Danta (*Tapirus terrestris*) And Aguti (*Agouti taczanowskii*)

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Rumen microbial diversity of domesticated species of cattle has been widely explored, but the microbial composition of the gut of most wild herbivores remains unknown. Scientific and commercially promising microbial species potentially inhabit the gut of wild herbivores from the savannas of Colombia. We have initiated studies of the microbial diversity resident in the digestive tracts of the Capibara, the Danta, and the Aguti, based on real-time PCR (qRT-PCR). A set of primers developed and recommended by the International Atomic Energy Agency study group in rumen ecology were validated and used for the detection of cellulolytic and methanogenic bacteria in fecal samples from the herbivores listed above. By using these primers and conventional and qRT-PCR, both qualitative and quantitative changes in the digestive microbial populations were monitored. For the three herbivores studied, PCR products corresponding to total bacteria, fungi, archaea, and the cellulolytic degraders *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* were detected by qRT-PCR. In Aguti, *F. succinogenes* was detected, however, no signals were detected for *R. flavefaciens*. In Capibara and Danta, *F. succinogenes* outnumbered *R. flavefaciens*. Among the herbivores studied, the fecal samples from the Danta contained a larger proportion of methanogens and anaerobic fungi. When bacterial isolates from the herbivores were tested in their capacity to degrade the tropical grass *Brachiaria decumbens*, only 25% of this substrate was degraded. In recent years the farming of wild herbivores such as the Capibara, which is considered the largest rodent in the world, has increased in the natural environments of the East Savannas of Colombia. Knowledge of the digestive microbial composition of indigenous herbivores will allow a greater understanding of the relationship between the animal and its ecosystem, and to set recommendations to increase feed efficiency and animal productivity in low-input livestock systems

Keywords: quantitative PCR, wild herbivores, Colombia, gut bacteria, methanogens

P-2.28

Comparative Metagenomics Of The Chicken Cecal Microbiome Using 16S rDNA Terminal-Fragment Length Polymorphism And Random Sampling Pyrosequencing

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We have been investigating the intestinal microbiome diversity/similarity and relative concentrations within the bacterial microbiome in the ceca of chickens using a combined T-RFLP and pyrosequencing approach. Fresh cecal samples from two 45-day-old chickens were analyzed. One of the chickens was *Campylobacter* free, while the other was artificially colonized with *Campylobacter jejuni* NCTC11168 on day 14. Terminal-fragment length polymorphism (T-RFLP) analysis of the 16S rDNA was used to assess intestinal microbiome diversity of these two samples. Samples were PCR amplified using the 8F and 926R primers, and the microbiome amplicons were then digested with *Alu*I and *Msp*I. Resulting samples were then analyzed using a capillary electrophoresis sequencer and the ABI GeneScan software. T-RFLP profiles were then analyzed using the MiCA 3 T-RFLP Analysis software package (http://mica.ibest.uidaho.edu/trflp.php). Phylogroup analysis was done at the Order level. The *Campylobacter* free cecal sample showed less overall diversity with eight Orders represented, whereas the cecal sample from the *C. jejuni* NCTC11168 colonized chicken had 12 Orders represented. The distribution of phylotyopes fell predominantly into the *Clostridiales*, *Sphingobacteriales*, *Bacteroidales* and unclassified *Bacteroidales* Orders. Members of the *Campylobacterales* were not detected by T-RFLP. Moreover, the distribution of phylotypes between the two samples differed as well, with the *Sphingobacteriales* being more dominant in the *Campylobacter* free cecal sample, and the *Clostridiales* dominating in the cecal sample from the *C. jejuni* NCTC11168 colonized chicken. In order to gain a greater understanding of the ecology of these microbiomes, we used comparative metagenomic pyrosequencing to examine 19,166,161 bases of random sampled 454 pyrosequence data from these two cecal samples. These sequence reads were compared using the BLASTX algorithm (E value $<1 \times 10^{-5}$) for coding sequences and the subsystems-based annotations available in the SEED database to gain a better understanding of the metabolic potential of these microbiomes. The distribution of coding sequences detected within each cecal sample was very similar. This analysis has provided a baseline understanding of the metabolic potential of the chicken cecum

Keywords: pyrosequencing, metagenome, chicken, cecum

P-2.29

Differential Regulation By Nitrogen Source Of Gene Expression, Enzyme Activity And Growth In *Ruminococcus albus* 8

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Nitrogen source plays a major role in the regulation of nitrogen metabolism and growth rate of rumen bacteria. To examine the effect of nitrogen source, *R. albus* 8 was cultured with equimolar N concentrations of ammonium sulfate, urea, peptides, or amino acids as N source in the medium. As predicted, *R. albus* 8 responded differently to the different nitrogen sources. Both growth rate and yield were high with $(NH_4)_2SO_4$ and urea as N source. Growth yield and growth rate on peptides was decreased compared to ammonia while very low growth yield and growth rate was obtained with amino acids as N source indicating that *R. albus* 8 is able to obtain intracellular amino acids from peptide uptake and hydrolysis rather than free amino acid transport. To identify gene regulation, expression of *gdhA*, *glnA*, *glnN*, *gltB*, *gltD*, *amtB*, *glnK*, *nifH*, and *ureC* was analyzed by harvesting RNA from pelleted cells at early, mid, and late exponential phase of growth on different nitrogen sources. Gene expression level was differentially regulated by nitrogen sources and growth phase. The *gdhA* gene showed up-regulation patterns on all nitrogen sources. The *glnA* gene was up-regulated with peptides according to growth phase. Interestingly, the *gltD* gene was not expressed during growth on any of the N sources while *gltD* was highly expressed on all N sources. Enzyme activities of glutamate dehydrogenase (GDH), glutamine synthetase (GS), and glutamate synthase (GOGAT) were differentially regulated by nitrogen source. For example, NADH-dependent GDH specific activity on $(NH_4)_2SO_4$ was high initially and then decreased as ammonia concentration decreased while GDH specific activity with urea and peptide peaked during the exponentially growth phase. These results show that *R. albus* 8 regulates genes of nitrogen metabolism in response to different N sources at the transcriptional level, resulting in changes in the activity of enzymes of ammonia assimilation and subsequent growth rate

Keywords: rumen, *Ruminococcus albus*, nitrogen metabolism, ammonia assimilation, regulation of gene expression

P-2.30

Differential Translocation Of Green Fluorescent Protein Fused To Signal Sequences Of *Ruminococcus albus* Cellulases By The Tat And Sec Pathways Of *Escherichia coli*

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Ruminococcus albus is a cellulolytic bacterial species considered today to play a major role in fibre breakdown in the rumen of herbivores. In order to ensure this function of degradation, *R. albus* synthesizes a high number of glycoside-hydrolases, which are active after they have been exported and anchored, for some of them, at the cell-surface. In bacteria, the proteins destined to cross the cytoplasmic membrane are synthesized as precursors and possess a signal sequence (SS) directing them to the "Sec" or "Tat" translocation pathways. The composition of the signal sequence of the two major cellulases of *R. albus* strain 20, Cel9D and Cel48B, suggests that each enzyme translocates using one of the two pathways. In order to confirm this hypothesis, the translocation of the two enzymes was studied by expressing SS-GFP fusions in *E. coli*. The cytoplasmic or periplasmic localization of the recombinant proteins was monitored by Western blot and fluorescence microscopy in a wild type *E. coli* strain and in Sec and Tat isogenic mutants. This study shows that the SS of Cel9D directs the pre-protein to the "Tat" translocation pathway while the GFP fused to the SS of Cel48B is exported through the "Sec" machinery. These observations suggest that genes encoding components homologous to the Tat and Sec systems exist in *R. albus* and should be looked for once the genome sequence of *R. albus* strain 8 is complete

Keywords: secretion, *Ruminococcus albus*, cellulases, SS-GFP fusions, rumen

P-2.31

Xylanolytic System Of Two New Xylan-Degrading Species From The Human Colon: *Bacteroides* sp. And *Roseburia intestinalis*.

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In humans, plant cell wall polysaccharides (mainly cellulose and hemicelluloses) are degraded and fermented by the fibrolytic bacteria of the colon. However, the predominant fibrolytic bacteria of the human gut have not been extensively studied. As xylan is one of the main polysaccharides of the plant cell wall, we recently isolated bacterial strains with high xylanolytic activities from the faeces of healthy volunteers using xylan growth medium. Phylogenetic analysis of these strains showed that they belonged to *Roseburia* and *Bacteroides* genera. Isolates related to *Bacteroides* corresponded to four new species of this genus, whereas most of the *Roseburia* strains could be assigned to the *R. intestinalis*. The xylanase activity of *Bacteroides* and *Roseburia* isolates was quantified and compared with that of xylanolytic bacteria already isolated from human gut, and closely related to our strains. Most of our new xylanolytic isolates showed higher xylanase activity than that of *Roseburia intestinalis* (L1-82) and *Bacteroides ovatus* (0038). The end-products of xylan fermentation were also determined and zymogram analyses revealed the presence of both cell-free and bacterial-associated xylanases for the *Bacteroides* and *Roseburia* isolates grown on different xylan sources (wheat, cabbage, and oat spelt). The synthesis and localization of xylanases is probably regulated by the nature of the carbon substrate present in the culture medium. PCR primers were designed to amplify family 5, 10 and 43 xylanase genes present in other *Bacteroides* spp. available in the databases. Entire xylanase genes or 500bp PCR fragments of xylanase genes were obtained by using genomic DNA of the *Bacteroides* isolates. Bioinformatic analyses revealed that most of the xylanase sequences were closely related to those present in *Bacteroides thetaiotaomicron* VPI 5482. Our results provide new information on the diversity of the xylanolytic flora in the human colon and revealed several characteristics of their xylanolytic system

Keywords: human colon, *Bacteroides*, *Roseburia*, phylogeny, xylanase

P-2.32

A Novel Clamp Loader Complex, A Molecular Switch In DNA Replication, From Methanogenic Archaea

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Free-living organisms can be divided into Bacteria, Archaea and Eukarya. In each of these domains, multiple forms of proteins known as DNA polymerases synthesize DNA in either a dis-

tributive or a processive fashion. In distributive DNA synthesis, the polymerase binds and dissociates from the DNA template frequently, leading to synthesis of short stretches of DNA (e.g. 10 nucleotides) per binding. In processive DNA synthesis, the DNA polymerase binds to the template and synthesizes as long as 50,000 nucleotides without dissociation. The different DNA polymerases that replicate genomes use processive DNA synthesis to perform their function. To achieve this feat, replicative polymerases depend on interaction with a ring-shaped clamp protein loaded onto DNA by its clamp loader. We have discovered a novel form of clamp loader in mesophilic methanogens, including the genera *Methanosarcina* and *Methanospirillum*. Unlike most archaea that have a clamp loader that is a heteropentameric complex of two different proteins, the novel clamp loader is a heteropentameric complex of three different proteins. The process of clamp loading requires energy derived from ATP hydrolysis. The *M. acetivorans* homolog of the novel clamp loader exhibited low inherent ATPase activity that was stimulated >50X by a partial duplex DNA. None of the subunits was capable of stimulating *M. acetivorans* clamp-dependent DNA synthesis by a cognate DNA polymerase. However, the complex of the three subunits stimulated the polymerase in the presence of its clamp to completely replicate the 7.2 kb genome of M13mp18 phage. The stoichiometry of the clamp loader was determined by a densitometric method to be one mole of each of the large subunit and small subunit 2, and three moles of small subunit 1. Through site-directed mutagenesis, we determined that while mutations in the ATP-binding site of the other subunits have little influence on clamp loading activity, similar mutations in small subunit 1 abrogated clamp loader function. Truncation analysis showed that the C-terminal regions of the small subunits are essential for complex formation and that of the large subunit is dispensable. Biophysical analysis through fluorescence resonance energy transfer and evolutionary implications of our discovery will be discussed. This unique protein may serve as a target for controlling methanogenesis

Keywords: methanogens, DNA replication, clamp, clamp loader, evolution

P-2.33

DNA Replication Proteins Required For Origin Recognition In The Methanogenic Archaeon *Methanosarcina acetivorans* C2A

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In each domain of life (Archaea, Bacteria, and Eukarya), DNA replication starts from specific regions in the genome. Whereas bacteria are known to have a single origin of replication, the archaea have either a single or multiple origin/s of replication. In contrast, all investigated eukaryotes have multiple replication origins. Although archaea are phenotypically different from eukaryotes, their information processing machinery (transcription, translation and replication) is strikingly similar to that of eukaryotes, and very different from that of bacteria. The replication factor called origin recognition complex (Orc1-6) binds to origins of replication to initiate replication, and genetic studies in eukaryotes have shown that the cell division control (Cdc) 6 protein interacts with Orc1-6 to recruit and load the replicative DNA helicase (minichromosome maintenance or MCM proteins) that begins unwinding the DNA double helix. *M. acetivorans* possesses two genes encoding proteins with homology to both Orc1-6 and Cdc6 proteins. The recombinant gene products, designated MacCdc6-1 and MacCdc6-2, from the *M. acetivorans orc/cdc6* genes have been purified. Although, eukaryotic Orc1-6 is a complex of six proteins, our gel filtration analysis suggested that MacCdc6-1 and MacCdc6-2 exist as monomers in solution. Furthermore, both MacCdc-6 proteins have higher affinity for double-stranded origin-like DNA than for single-stranded origin-like DNA. Both MacCdc6 proteins possess ATPase activity and they are auto-phosphorylated *in vitro*. A similar auto-phosphorylation activity has been reported for the yeast Cdc18 homolog. As an extension to this work, recombinant proteins from two genes encoding MCM-like proteins in *M. acetivorans* have been produced, and although eukaryotic MCM is a hexameric complex of six different proteins, *M. acetivorans* MCM-1 and MCM-2 both form homohexameric complexes. Similar to Cdc6, the MacMCM proteins are members of the AAA+ ATPases known to couple ATP hydrolysis to various DNA transactions. Both MacMCM proteins exhibited ATPase activities that were stimulated in the presence of DNA. Protein-protein interactions are underway to determine which Cdc6 homolog interacts with the MCM proteins in order to determine which homolog likely loads the MCM helicase in *M. acetivorans* cells

Keywords: *Methanosarcina acetivorans*, origin recognition complex (ORC), cell division control 6 protein (Cdc6), minichromosome maintenance protein (MCM), AAA+ ATPase

P-2.34

Proteomic Analysis Of Proteins Expressed By *Bifidobacterium longum* S3 When Grown On Galactooligosaccharides Or Glucose

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Galactooligosaccharides (GOS) have been shown to stimulate the growth of beneficial intestinal bacteria, especially bifidobacteria. It is believed that GOS can be selectively fermented by these bacteria to produce short chain fatty acids. Although the genomes of several bifidobacterial species, including *B. longum*, have been sequenced, little published research has been carried out on these strains at the proteomic level. In this study, we examined the proteomic profiles of *B. longum* S3 (ATCC 15708) following growth on GOS or glucose as the sole substrate. Cytoplasmic proteins were separated by 2D electrophoresis. Many more proteins were detectable from the GOS-grown culture than the glucose-grown culture (144 vs. 52). Nine proteins (pI, 4.49–5.69; MW, 32.4–71.33 kDa) present in both proteomes were more highly expressed in the GOS-cultured cells than the glucose-cultured cells, whereas another 12 proteins with a pI ranging from 4.47–5.83 and a MW ranging from 23.6–60.5 kDa showed the opposite trend. These results suggest that a different set of genes and metabolic network is involved in the fermentation of GOS than fermentation of glucose. Analyses using mass spectrometry and protein sequencing are being carried out to identify these proteins pertaining to GOS metabolism. Completed analyses of these protein spots will advance our understanding of the genetic and biochemical mechanism underpinning the fermentation of GOS mixture by *B. longum*, and such knowledge may be useful to devise means to effectively stimulation the growth of bifidobacteria

Keywords: *Bifidobacterium longum*, GOS, proteomics, prebiotics