
# SCIENTIFIC PROGRAM

# AND ABSTRACTS

**GLEACHER CENTER**

**UNIVERSITY OF CHICAGO**

**CHICAGO**

**ILLINOIS**

**ORGANIZING COMMITTEE**

**Rod Mackie (Chair)**

**Jane Leedle**

**Isaac Cann**

**Jeff Firkins**

**Congress Sponsors**

In addition, we sincerely appreciate other sources of financial support provided to CGIF 2015, as well as our earlier Conferences. Many of these financial supporters have had a long-standing relationship with the Conference. Sponsors of the 2013 Conference so far include:

**Philanthropic Level Sponsorship (>S10,000)**

Cargill®

**Platinum Level Sponsorship (>$2,000)**

agresearch

DUPONT®

KEMIN®

Elanco™

LALLEMAND

Diamond V®

Alltech®
...naturally

**Gold Level Sponsorship (>$1500)**

ABVista
Feed Ingredients

AGRI-KING
KEY TO PROFIT®

New Zealand Government

**Silver Level Sponsorship (>$1,000)**







**Bronze Level Sponsorship (>$500)**















If you would like additional information in terms of sponsorship or representation at the Conference, please contact us at cgif@assochq.org.

**MONDAY, APRIL 13:**

**Hungate 1000 Genome Workshop Program**
**Gleacher Center, First Floor, Tiered Classroom**

**08.45** Introduction –Chairperson: **Mark Morrison,** University of Queensland, Australia

**08.50** Opening - **Mark Powell**, USDA, USA: Global Research Alliance (GRA) on Agricultural Greenhouse Gases

**09.05** **Gemma Henderson**, AgResearch, New Zealand
A global census of rumen microbial diversity

**09.30** **Bill Kelly**, AgResearch, New Zealand
Towards a reference genome set of rumen microbes

**09.55** **Chris Creevey**, Aberystwyth University, Wales
Systems level investigation of microbial specialisation and activity in the rumen

**10.20** Tea Break

**10.45** **Bernard Henrissat**, CNRS, France
The carbohydrate-active enzymes database: application to genomes and metagenomes

**11.10** **Phil Pope**, Norwegian University of Life Science, Norway
Metagenomic tools to advance rumen microbiology: from genes to Genomes

**11.35** **Isaac Cann**, University of Illinois at Urbana-Champaign, USA
Mining rumen bacterial genomes for plant cell wall degrading Enzymes

**12.00** **Chairperson**, **Audience and Panel Discussion**

**12.30** Workshop close

**2015 CONGRESS ON GASTROINTESTINAL FUNCTION**
**PROGRAM AND SCHEDULE**
Full Program with podium speakers and times will be posted shortly

**MONDAY, APRIL 13:**
**8:00-14:00 REGISTRATION Gleacher Center, First Floor Foyer**
Please pick up your registration materials, name tag and mixer drink tickets
Mount posters on boards provided (6th floor)

**SPECIAL OPENING SESSION Gleacher Center, First Floor, Tiered Classroom**

**14:00-16:30** **SPECIAL OPENING SESSION**
Bryant Memorial Lecture and invited presentations

**14:00-14:10** **Rod Mackie**, Congress Chair, University of Illinois, USA
Welcome and introduction of the Marvin P. Bryant Memorial Lecture speaker

**14:10-15:00** **Dale Bauman,** Cornell University, USA
**Milk fat depression: A nutri-genomic view of how rumen fermentation products regulate dairy cow metabolism**
(Presentation of Honorary Plaque by Dr Kenneth Griswold Kemin Animal Nutrition and Health)

**15:00-15:45** **Sandrine Claus**, University of Reading, UK
**Host-gut microbial metabolic interactions: linking gut microbial ecology to metabolic health**

**15:45-16:30** **Eric Martens**, University of Michigan, USA
**Bacterial degradation of host and dietary polysaccharides in the human gut during health and disease**

**17:00-19:00** **WELCOME MIXER Gleacher Center, 6th floor (Room 621)**

Informal poster viewing session
All attendees please wear your name tags
Refreshments: Drink tickets, hors d'oeuvres and cash bar

**TUESDAY, APRIL 14:**

**08:30-09:00** **Continental breakfast** **First Floor, near Tiered Classroom**

**Morning Session** **Gleacher Center, First Floor, Tiered Classroom**

**09:00-10:30** **Podium presentations** **Session 1**

**09:00-09:45** **Cormac Gahan**, University College Cork, Ireland
**Regulation of host metabolism by bacterial bile acid modification in the gut: implications for rational selection of probiotics**

**09:45-10:05** **Bacteria and bile: it takes a lot of gall**
**Jason Ridlon**, Virginia Commonwealth University, USA

**10:05-10:25** **Diet–microbe co-metabolic interactions in wild primates reveal clues on human evolution**
**A. Gomez,** K. Petrzelkova, J. Rothman,C. Yeoman, K. Nelson, R. Stumpf, B. Wilson, D. Modry, B. White, R. Blekham and S. Leigh; University of Illinois and University of Minnesota

**10:30-11:00** **Coffee Break**

**11:00-12:00** **Podium presentations** **Session 1 continued**

**11:00-11:20** **The polysaccharide-degrading mechanisms of uncultured rumen Bacteroidetes**
Naas, A.K. Mackenzie, V.G.H Eijsink and **P.B. Pope**
Norwegian University of Life Sciences, Norway

**11:20-11:40** **Citrus pectin breakdown by *Bacteroides xylanisolvens* XB1A involves at least four large polysaccharide utilization loci**
**J. Despres,** E. Forano, P. Lepercq, S. Comtet-Marre, C.J. Yeoman, M.E. Berg-Miller, C.J. Fields, B. Henrissat, B.A. White and P. Mosoni; INRA, France and other institutions

**11:40-12:00** **New ways of looking at enteric phage populations: Viral metagenomes of cattle, sheep, and kangaroos**
**R. Gilbert,** S. Goodwin, L-M. Gulino, M. Kienzle, D. Ouwerkerk and A. Klieve; Department of Agriculture and Fisheries and University of Queensland, Australia

**12:00-13:00** **LUNCH** Please make your own arrangements

**Afternoon Session** **Gleacher Center, First Floor, Tiered Classroom**

**13:00-14:20** **Podium presentations** **Session 2**

**13:00-13:45** **Mark Morrison,** Diamantina Institute**,** University of Queensland, Australia
**Can we transform *Faecalibacterium prausnitzii* from a friend in need, to a friend in deed, for IBD patients?**

**13:45-14:05** **Development of an enteric inflammation model in broilers and methods to detect mucosal permeability**
L**.R. Bielke**, V.A. Kuttapan, J.C. Bielke, E.A. Vicuna, L.R. Berghman, R. Galarza-Seeber, X. Hernandez-Valasco, B.M. Hargis and G. Tellez; University of Arkansas, USA

**14:05-14:25** **Specific bacterial strains associated with high milk production and favorable milk profiles in lactating dairy cows: A case study using a novel platform for DFM product discovery**
**C. Belnap** and M. Ashby; Taxon Biosciences Inc, California, USA

**14:25-15:00** **Coffee Break**

**15:00-16:30** **Podium presentations** **Session 2 continued**

**15:00-15:20** **Metabolism pathway shifts by bacteria competing for hydrogen in vitro mixed rumen microbial fermentations**
**S. Denman,** L. Bragg, W. Smith, C. McSweeney and M. Morrison; CSIRO Agriculture Flagship and University of Queensland, Australia

**15:20-15:40** **Comparative genomic and transcriptomic analysis of *Ruminococcus albus* strains 7 and 8 grown on complex and defined substrates**
**I.H. Kwon,** I.K. Cann and R.I. Mackie; University of Illinois, USA

**15:40-16:00** **Transcriptome analysis of *Fibrobacter succinogenes* S85 in co-culture with non-fibrolytic ruminal bacteria**
N. Fukuma, **S. Koike** and Y. Kobayashi; Hokkaido University, Japan

**16:00-16:20** **Intake of lichens alters the rumen microbiome in Norwegian reindeer (*Rangifer tarandus tarandus*)**
A. **Salgado-Flores,** L. Heidal Hagen, P. B. Pope, S. Ishaq, and A.D. Wright; The Arctic University, Norway and University of Arizona, USA

**16:30-18:30** **POSTER SESSION & MIXER**
**Gleacher Center, 6th floor (Room 621)**

**WEDNESDAY, APRIL15**

**08:30-09:00** **Continental breakfast** **First Floor, near Tiered Classroom**

**Morning Session** **Gleacher Center, First Floor, Tiered Classroom**

**09:00-10:30** **Podium presentations** **Session 3**

**09:00-09:45** **Anne Salonen**, University of Helsinki, Finland:
**Toward understanding and predicting dietary responsiveness of the intestinal microbiome**

**09:45-10:05** Gene expression of *Streptococcus bovis* JB1 in response to monensin
**A.S. Biddle,** A. Klieve, I.K. Cann and R.I. Mackie; University of Illinois, USA

**10:05-10:25** **Exploring the transcriptome of rumen protozoa *Entodinium caudatum***
**L. Wang,** J. Plank, A. Abu-Doleh, U. Catalyurek, J. Firkins and Z. Yu; The Ohio State University

**10:30-11:00** **Coffee Break**

**11:00-12:00** **Podium presentations** **Session 3 continued**

**11:00-11:20** **Characterization along the porcine intestinal tract displays unique microbial differences in growing and finishing pigs**
**J.S. Thompson**, J.A. Benson, A.A. Hibberd, A. Owasu-Asiedo, M.C. Walsh and E.A Galbraith; DuPont Nutrition and Health, Danisco Animal Nutrition, USA

**11:20-11:40** **Cultivation studies on the gastrointestinal tract of an indigenous Peruvian community yields several novel bacterial taxa**
**N. Patel,** R. Tito, A. Obregon-Tito, O. Trujillo-Villreal, L. Martin-Reyes, L. Troncoso-Corzo, E. Guija-Poma, C. Lewis and P. Lawson; University of Oklahoma, USA and **U**niversídad Científica del Sur, Lima, Peru

**11:40-12:00** **Amylolytic bacteria in the equine hindgut: Effect of starch source and a case for antimicrobial-mediated competition**
**A.E. Harlow,** L.M. Lawrence and M.D. Flythe**;** Department of Animal and Food Sciences and USDA-ARS Lexington, USA

**12:00-13:00** **BUSINESS MEETING Open to all attendees**

**12:00-13:20** **LUNCH** Please make your own arrangements

**Afternoon Session** **Gleacher Center, First Floor, Tiered Classroom**

**13:20-14:20** **Podium presentations** **Session 4**

**13:20-13:40** **Metabolic networks for nitrogen utilization in *Prevotella ruminicola* 23**
**C. Mendez-Garcia,** J.N. Kim, I.K. Cann and R.I. Mackie; University of Illinois, USA

**13:40-14:00** **Pangenome analyses of *Methanosphaera* species**
**E. Hoedt,** P. O. Cuiv and M. Morrison: University of Queensland, Australia

**14:00-14:20** **Yeast with surface displayed xylanase as a new dual-purpose delivery vehicle of xylanase and yeast**
**J. Wang,** B. He, W. Du, Y. Luo, Z. Yu and J. Liu; Zhejiang University, China, and The Ohio State University, USA

**14:20-14:50** **Coffee Break**

**14:50-15:10** **Podium presentations** **Session 4 continued**

**15:10-15:30** **The effect of bovine fecal microbiome on *Escherichia coli* O157:H7 prevalence**
**M. Kim,** L. Kuehn, J. Bonio, E. Berry, N. Kalchayanand, H. Freetly, A. Benson and J. Wells; ARS-USDA, Clay Center and University of Nebraska, USA

**15:30-15:50** **A systematic review of the factors influencing methane reduction and toxicity with the use of nitrate salts in ruminants**
**E. A. Latham,** R. A. Anderson and W.E. Pinchak; Texas A&M University, ARS-USDA, USA

**15:50** **CLOSING REMARKS and INVITATION TO CGIF 2017**
**PRESENTATION OF RUSSELL AWARDS**

## POSTER PRESENTATIONS

1. **Cattle waste is an effective inoculum for anaerobic digestion of thin stillage under mesophilic conditions**
   **M.J. Oosterkamp,** C. Mendez-Garcia, C-H. Kim**,** I.K. Cann and R.I. Mackie; University of Illinois, USA
2. **Homoacetogenic activity in the sheep rumen**
   **P. Raju,** G. Henderson, M. Tavendale, J. Rakonjac and P. Janssen; AgResearch Ltd and Massey University, New Zealand

3. **The genome sequence of a member of the rumen Methanomassiliicoccales—Methanogenic archaeon isolate ISO4-H5**
   **Y. Li,** S. Leahy, J. Jeyanathan, F. Cox, E. Altermann, W. Kelly, S. Lambie, P. Janssen, J. Rakonjac and G. Attwood; Ag Research Ltd and Massey University, New Zealand

4. **Effect of different levels of potassium nitrate with or without sulfur on enteric methane production in Teddy goats at post-weaning age**
   **M. Nisa,** M. Arif, M. Ali and M. Sarwar; University of Agriculture, Faisalabad, Pakistan

5. **Early microbiome drives rumen papillae development via the production of volatile fatty acids in pre-weaned calves**
   **M. Malmuthuge,** G. Liang and L..L. Guan; University of Alberta, Canada

6. **A comparative study on rumen microbiome in beef cattle and bison fed different diets**
   **M. Zhou,** Y. Chen, M. Walpole, P. Gorka, M. Woodbury, G.B. Penner and L.L. Guan; University of Alberta, and University of Saskatchewan, Canada

7. **Fecal microbiome and non-typhoidal *Salmonella enterica*: Changes through the transition period of dairy cattle**
   **L.M. Munoz-Vargas,** R. Digianantonio, M. Williams, G. Schuenemann and G. Habing; The Ohio State University, Ohio

8. **Correlation of microbiome populations with performance measurements and sex in broiler breeder**
   **S. Diaz-Sanchez,** R. Hawkins, R. Okimoto, A.Layton, A. Saxton, A. Blakeley-Ruiz and I. Hanning; University of Tennessee, USA

9. **An attempt to detoxify the leucaena mimosine in sheep using immobilized IBT-Goettinger bioreactor grown *Klebsiella pneumonia***
   **A. Aung,** F. Gesslar and H.B. Bohnel; University of Gottingen, Germany

10. **Transport of a fluorescent analog of glucose (2-NBDG) by rumen bacteria**
**J. Tao,** R. Diaz and T. Hackmann; University of Florida, USA

11. **Intake of lichens alters the rumen microbiome in Norwegian reindeer (*Rangifer tarandus tarandus*)**
**S. Qi,** B. Smiley, S. Huntlye, F.Owens, Y.Jiang, A. Adesogan and W. Rutherford; DuPont Pioneer, Iowa nd University of Florida, USA

12. **Incubation temperature affects the post-hatch response of broiler chicks to *Salmonella* Enteritidis**
P.E. N. Givisiez, L.B. Moreira-Filho, H.B. Oliveira, F.G.P.Costa, D.B. Campos and C.J. B. Oliviera; Universidadae Federal da Paraiba, Brazil and University of Illinois, USA

13. **Effects of the interaction between *Entodinium caudatum* and an amino acid-fermenting bacterial consortium on fermentation characteristics and protozoal population in vitro**
**T. Park** and Y. Zu; The Ohio State University, USA

14. **Carbohydrate utilization by *Clostridium scindens*, a key bile acid-dehydroxylating anaerobe of the human gut microbiome**
**A.L. Allen,** E.L. Springer and S.L. Daniel; Eastern Illinois University, USA

15. **Carbohydrate utilization by *Clostridium scindens*, a key bile acid-dehydroxylating anaerobe of the human gut microbiome**

16. **Isolation and Identification of Cellulolytic and Xylanolytic Bacteria from the Gut of Subterraneum Termites**
**I. Batool,** A. Biddle, R. Mackie and M. Gulfraz; PMAS Arid Agriculture University, Pakistan and University of Illinois, USA

17. **Neonatal treatment effects of garlic oil on the microbial ecosystem in the intestinal tract of Hanwoo calves**
**J. Song,** G-S Bae, J. Park, J. Manhiapig, J.N. Kim, E.J. Kim. C.H. Kim, T.Seo and J. Chang

18. **Gene expression of *Streptococcus bovis* JB1 in batch and continuous culture at high and low growth rates**
**A.S.Biddle,** A. Klieve, I.K. Cann and R.I. Mackie; Uinversity of Illinois, USA and University of Queensland, Australia

19. **Rational media design for the isolation of rare bacterial taxa from the Synergistetes phylum**
**B. Davis,** M. Bruschii, D. Ouwerkerk, R.G. Glibert and A. Klieve; Department of Agriculture and Fisheries, Brisbane, Australia

20. **Feeding urea molasses block on productive and reproductive performance of crossbred dairy cows under smallholder village farm conditions in Bangladesh**
    **M.A. S. Khan** and M.A. H. Sarker; Bangladesh Agricultural University, Bangladesh

21. **Informing ulcerative colitis pathophysiology and outcomes through metabolic profiling**
    **S.C. Fong**, C.I. Le Roy, S.P. Claus and J.D. Sanderson; Guy's and St Thomas NHS Foundation trust, London and University of Reading, United Kingdom

**22.** Effects of the dose and viability of *Saccharomyces cerevisiae* on the ruminal bacterial population and fermentation and the performance of lactating dairy cattle
    **Y. Jiang**, R.M. Martino, I.M. Ogunade, W. Rutherford, S. Qi, F. Owens, B. Smiley, K. Arriola, C. Staples and A.T. Adesogan

23. **Early supplementation of alfalfa to starter diets altere electrophysiological properties and permeability of the gastrointestinal tracts in growing lambs**
    **B, Yang,** S. Wang. B. He, J. Liu and J. Wang: Zheijiang University, China

24. **Early supplementation of alfalfa to starter diets altered electrophysiological properties and permeability of the gastrointestinal tracts in growing lambs**
    **A. Garcia,** C. Ariza, T. Rodriguez and O.L. Mayorgs: Corporacion Colombia de Investigacion Agropecuaria, Colombia

25. **Evaluation of different essential oils in modulating methane production, rumen fermentations, and microbial population in vitro**
    **G. Cobellis,** M. Trabaiza-Marinucci and Y. Zu: The Ohio State University, USA

26. **Induction of subacute ruminal acidosis affects the ruminal microbiome**
    **J. C. McCann,** S. Alqarni, S. Luan, F.C. Cardoso and J.J. Loor: University of Illinois, USA

27. **Effect of probiotics on the activity of intestinal carbohydrases in rats with lead intoxication**
    **L.S. Kuchkarova,** G.T. Kudeshova, I.I. Karimova, N. Sh. Nadzhmutdinova and R.O. Atoeva: Natioanal University of Uzbekistan, Uzbekistan

28. **Effect of varying levels of urea-molasses fermented wheat straw on ruminal characteristics, nutrient digestibility, blood urea nitrogen, and nitrogen balance**
    **M.Nisa,** M. Ali and M. Sarwar; and Government Colelge University, and University of Agriculture, Faislabd, Pakistan

29. **Fermentation of spent craft brewer's yeast by caprine rumen bacteria**
    B. E. Harlow, R. Bryant and M.D. Flythe; University of Kentucky and ARS-USDA, USA

30. **Utilization of prebiotic carbohydrates by the human gut acetogen *Blautia producta*: You are what your acetogens eat!**
    K. Ohseki and S. L. Daniel; Eastern Illinois University, USA

31. **Efficacy of butyric acid and monolaurate to combat bacterial enteritis problems in broilers**
    **F. Dias,** A. Schwartz, T. Rogge, J. de Gussen and H. van Meirhaeghe; Vetworks, Proviron and Poulpharm, Belgium

32. **Anti-*Salmonella* effect of thymol-β-D-glucopyranoside in porcine jejunal, cecal, and rectal gut contents**
    **G Levant,** G. Ciffcioglu, R. C. Anderson, R. C. Beier and D.J. Nisbet; ARS-USDA, Texas, USA and Istanbul University, Turkey

33. **Effect of *in ovo* and hatcher spray of probiotics on microbial properties of gastrointestinal tract and hatcher cabinets**
    **B.M. Hargis,** L.E. Graham, K.D. Teague, J.C. Bielke, R.E. Wolfenden, G. Tellez and L.R. Bielke; University of Arkansas and Pacific Vet Group, USA

34. **A novel yeast strain *Meyerozyma guilliermondii* isolated from native fruits from Colombian ecosystems as a prospective probiotic to be used in dairy systems**
    **T. Rodriguez,** M. Chaparro, M. Gomez, C. Castillo, A. Garcia, C. Ariza and O. Mayorga; Corporacion Colombia de Investigacion Agropecuaria, Colombia

35. **Isolation and identification of probiotic bacteria from the gut of yellow perch, *Perca flavescens*, and evaluation of their probiotic potential against *Vibrio anguillarum***
    **J. Stiverson,** Y. Zu and K. Dabrowski; The Ohio State University, USA

## ABSTRACTS

## INVITED PRESENTATIONS

### Milk fat depression: A nutri-genomic view of how rumen fermentation products regulate dairy cow metabolism

D. E. Bauman*,
Cornell University, Ithaca, NY USA.

Milk fat comprises many differ fatty acids (FA) and the most variable component of milk. Nutrition can have a major effect on milk fat output, the most striking example being the low-milk fat syndrome, more commonly referred to as diet-induced milk fat depression (MFD). Recognized for over a century, certain diets and dietary conditions cause a reduction in milk fat whereas yield of milk and other milk components are unaffected. It was clear that MFD involved a metabolic interaction between fermentation in the rumen and the metabolism of body tissues, but the cause remained elusive until it was recognized that it was associated with rumen biohydrogenation of dietary polyunsaturated FA. The biohydrogenation theory established that diet-induced MFD occurred when typical pathways of rumen biohydrogenation were altered to produce unique FA intermediates that inhibited milk fat synthesis. Through advances in analytical and chemical synthesis methods, *trans*-10,*cis*-12 conjugated linoleic acid (CLA) was the first of these unique intermediates identified. During MFD, mammary lipogenic capacity is reduced and the transcription of key mammary lipogenic enzymes is coordinately down regulated. Dose-response studies established *trans*-10,*cis*-12 CLA is a very potent inhibitor of FA synthesis, but the mechanism by which it causes this coordinated downregulation is not known, but evidence supports roles for sterol response element binding protein-1 (SREBP1) and Spot 14 as components in the mammary signaling pathway. Overall, the MFD story in dairy cows represents an elegant example of nutrigenomics, and investigations have provided novel mechanistic insight in the regulation of FA synthesis with potential applications in agriculture and human biology.

**Key Words:** rumen, milk fat, conjugated linoleic acid, milk fat depression, nutrigenomics

### Host-gut microbial metabolic interactions: Linking gut microbial ecology to metabolic health

S. P. Claus*,
Department of Food & Nutritional Sciences, The University of Reading, Whiteknights campus, PO Box 226, Reading, United Kingdom.

Gut microbiota are now recognized as fundamental partners of the host's health. As the environment changes, our overall metabolism adapts to maintain homeostasis within an optimal metabolic space, and so do our microbiota. Normally, the host-microbiota symbiosis triggers a healthy metabolic phenotype. But how does this interplay result in an optimal metabolic state? And how can this be measured? Nutrimetabonomics is a useful tool to assess the metabolic state of the host in

response to a gut microbial modulation. This method was successfully applied in mouse models to examine how the progressive colonization of the gut affects the host metabolism. In particular, it was revealed that gut bacteria triggered a specific hepatic response to modulate energy metabolism during the early colonization process. In humans, we applied a similar nutrimetabonomics approach to investigate the effects of an inulin-type fructans prebiotic on obese patients. The prebiotic treatment resulted in specific modulations of the gut microbiota, such as increased levels of *Collinsella* that were positively correlated with urinary hippuric acid, a marker of gut microbial degradation of polyphenols. In addition, the prebiotic treatment induced a decrease level of *Propionibacterium,* which were found positively correlated with circulating levels of VLDL, lactate and phosphatidylcholine, suggesting a potential positive effect of the treatment on energy metabolism. Finally, we also applied such approach in a clinical setting to decipher the metabolic perturbations associated with the degree of disease severity in patients suffering from ulcerative colitis. Altogether, these data indicate that nutrimetabonomics can be a powerful approach to study host-gut microbial interactions in a clinical context where patient stratification is key to further develop personalized medicine.

**Key Words:** gut microbiota, nutrimetabonomics, metabolic profiling

**Bacterial degradation of host and dietary polysaccharides in the human gut during health and disease**

Eric C. Martens
University of Michigan

The trillions of symbiotic microorganisms in the human gut expand our digestive physiology by providing an armament of polysaccharide-degrading enzymes that are absent in the human genome. Dietary polysaccharides, mixed with endogenous mucosal secretions, present a diverse menu of complex carbohydrates that our gut symbionts have adapted strategies to sense, triage and degrade. Understanding which species consume each nutrient, how abilities vary among taxa and what the molecular mechanisms involved are, represent central problems in defining the relationship between diet, microbiota and health.

We are taking microbiological, genomic, genetic and biochemical approaches to address these problems. Our results have revealed that members of the phylum Bacteroidetes are major contributors to carbohydrate digestion and rely on expression of discrete gene clusters that each encodes the requisite proteins to catabolize a particular polysaccharide. Expression of each gene cluster is activated by a locally encoded transcription factor that participates in carbohydrate sensing and metabolism until its supply is exhausted. In the context of a single bacterium, many dozen individual gene clusters may simultaneously be triggered to respond to available nutrients. Yet, in experimental conditions in which such complex nutrient environments are modeled, there is an ordered progression of carbohydrate utilization that is reminiscent of catabolite repression.

Of central focus in our studies is the interplay between dietary fiber polysaccharides and mucosal glycans. Some bacteria possess mechanisms to suppress utilization of mucosal glycans when dietary alternatives are present, while

others exhibit opposite behavior. Our results reveal that sensing and triaging of glycans is a complex process that varies among species, underscoring the idea that these phenomena are likely to be hidden drivers of microbiota community dynamics and may dictate which microorganisms commit to various niches in a constantly changing nutritional environment.

**Bile Acid Modifications at the Microbe-Host Interface: Implications for the Rational Selection of Probiotics**

**C.G.M. Gahan**[1,2,3] and S.A. Joyce[1,4]
[1] Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland
[2] School of Microbiology, University College Cork, Cork, Ireland
[3] School of Pharmacy, University College Cork, Cork, Ireland
[4] School of Biochemistry & Cell Biology, University College Cork, Cork, Ireland

Bile acids act as key signalling molecules that have the capacity to alter systemic endocrine functions in the host. Individual bile acids are capable of interacting with host cell receptors (including FXR and TGR5 receptors) to induce cellular responses in the intestine and other tissues (including the liver and adipose tissue). As gut microorganisms have the capacity to significantly alter the signalling properties of bile acids we, and others, have investigated the impact of altered microbial bile acid signatures upon host physiological processes. In particular we have focused upon microbial bile salt hydrolase (BSH) activity as a gut microbial activity that has the capacity to profoundly alter both local (gastrointestinal) and systemic (hepatic) host functions. Using a functional metagenomics approach we demonstrated that BSH activity is widely distributed amongst gut bacteria and may contribute to microbial colonisation in the gut. Using both germ free and conventionally-raised mouse models we showed that gastrointestinal expression of BSH results in local bile acid deconjugation with concomitant alterations in lipid and cholesterol metabolism, signalling functions and weight gain. Key mediators of cholesterol homeostasis (*Abcg5/8*), gut homeostasis (*RegIII*□) and circadian rhythm (*Dbp*) were influenced by elevated BSH in our study. The implications of this work for the rational development of probiotics with the potential to modulate host weight gain will be discussed.

**Can we transform *Faecalibacterium prausnitzii* from a friend in need, to a friend in deed, for IBD patients?**

**M. Morrison** University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, Queensland, Australia

*Faecalibacterium prausnitzii* is a Gram-positive “commensal” bacterium that produces anti-inflammatory factors and also enhances intestinal barrier function in murine models of chemically induced colitis. Langella and colleagues have shown that peptides, as well as other fermentation products arising during *F. prausnitzii* growth *in vivo*, possess anti-inflammatory effects. These findings have prompted widespread interest and optimism that *F. prausnitzii* can be used therapeutically to attenuate inflammation and/or promote gut homeostasis. However, microbiota profiling studies commonly show that *F. prausnitzii* is present in high abundance in

healthy subjects, but depleted in most patients suffering from Crohn's disease, ulcerative colitis, or other gastrointestinal disorders with associated inflammation. Furthermore, two longitudinal studies of CD patients suggest that the restoration of *F. prausnitzii* populations is variable across patients after disease episodes, and their sustained low numbers are predictive of poor health outcomes, including recurrent disease. This poses the question as to whether depletion of *F. prausnitzii* is a cause, or consequence, of inflammation. In this context, the host- and environmental cues affecting *F. prausnitzii* colonisation, persistence and adaptive capacity in the human gut remain poorly defined, as does the degree of genome variation across strains and its effects on their ecological fitness. In collaboration with French colleagues we have so far examined the genomes of five *F. prausnitzii* strains, and found some key differences between the phylogroups in terms of their glycoside hydrolase and glycosyltransferase profiles, suggesting possible differences in carbohydrate utilization and bacterial surface decoration relevant to host-microbe interactions. Interestingly, all five genomes appear to lack the common pathway for tryptophan biosynthesis, and a candidate tryptophan uptake system was assigned to the core genome. Even at this early stage, our comparative genomic analyses have provided new opportunities for interventions that can be evaluated to enhance the restoration and/or persistence of *F. prausnitzii* in the human gut, which may be critical for translating this bacterium from a "friend in need" to a "friend in deed", that counteracts dysbiosis with clinical benefits.

Keywords: IBD, *Faecalibacterium prausnitzii,* genomics, carbohydrate active enzymes, ecological fitness

**Towards understanding and predicting dietary responsiveness of the gut microbiota and the host**

Anne Salonen, University of Helsinki, Finland

There is growing interest to understand how diet affects the intestinal microbiota and how this translates to host health. One of the major challenges in the field is the high individuality of the microbiota composition and its individual-specific dietary responses. Similarly, the high variation of host responses is a challenge especially in human nutritional research and practise. We have started to study the microbiological basis of the individual dietary responses by using a deep community-wide microbiota analysis before and after dietary interventions. We and others have shown that categorization of the study subjects to dietary responders and non-responders allows identification of baseline microbiota features that are specific to responders, both in terms of the microbiota and most importantly, of the anticipated host parameters, such as metabolic health markers or gastrointestinal symptoms. If such predictive microbiota signatures can be validated in further studies, they will provide a radically new way to understand diet-microbiota-health trialogue and further use this knowledge as a basis for tailored nutrition.

## SUBMITTED ABSTRACTS

### Session: Environmental impacts (including livestock waste, GHGs, and antibiotic resistance)

### A systematic review of the factors influencing methane reduction and toxicity with the use of nitrate salts in ruminants

E. A. Latham*[1,2], R. A. Anderson[3], and W. E. Pinchak[2],
[1]Texas A&M University, College Station, TX, USA,
[2]Texas A&M AgriLife Research, Vernon, TX, USA,
[3]United States Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX, USA.

Methane production is an energetic inefficiency associated with ruminant digestion, constituting a 2 to 12% loss in energy consumed. Methane is also a potent greenhouse gas. Feeding nitrate can decrease ruminal methanogenesis, but accumulation of nitrite, a toxic intermediate, must be managed. This review discusses causes of variation in methane reduction efficiency and toxicity with the use of nitrate salts. Nitrate levels were converted to mol/kg of BW for ease of comparison. A systematic review of 36 peer-reviewed articles revealed that sodium nitrate more effectively reduced methanogenesis, but was more toxic than potassium and calcium salts, possibly due to differences in affinity, dissociability, or microbial uptake. It was also found that peak methemoglobin levels were 56% lower for adapted animals as compared with unadapted animals across all nitrate types ($P = 0.065$). Selection and enrichment of nitrate-metabolizing bacteria may contribute to adaptation, but little is known about how the different salt types affect adaptation. Less is known about the sensitivity of animals having been deadapted to dietary nitrate via missed meals or changes in diet, which hypothetically could result in an imbalance in rates of nitrite conversion and detoxification. Animal type (sheep, goat, dairy, or beef) did not influence methane reduction or methemoglobin formation; however, younger animals were more prone to intoxication. Intracannula administration resulted in higher methemoglobin levels than feeding ($P < 0.05$). Standards for safe and efficacious nitrate feeding regimens that account for salt type, adaptation status, age, and mode of delivery are needed to avoid toxicity, facilitate comparison across studies, and to improve methane mitigation.

**Key Words:** nitrate poisoning, methane reduction, ruminants

### Cattle waste is an effective inoculum for anaerobic digestion of thin stillage under mesophilic conditions

M. J. Oosterkamp*, C. Méndez García, C.-H. Kim, I. K. Cann, and R. I. Mackie, Energy Biosciences Institute, Institute for Genomic Biology, University of Illinois, Urbana, IL, USA.

For every liter of bioethanol that is formed, up to 20 L of high chemical oxygen demand (COD) thin stillage is generated. Reactor start up is a common problem so

we used cattle waste as the source of inoculum and analyzed if residual thin stillage is a suitable substrate for anaerobic digestion. We used high rate hybrid 1.25-L laboratory-scale anaerobic bioreactors maintained at 40°C, and analyzed bioreactor performance, as well as microbial diversity and predicted metabolic potential using *in silico* omics-derived data analyses. Thin stillage contained 30 to 43 g/L COD and had a low pH (pH 4.3). Optimal removal of organic compounds was reached at an organic loading rate of 15 to 21 g COD/L/day for stillage derived from sugar and energy cane, respectively, with 90 to 93% COD removal and suitable for environmental discharge. The optimal specific methane production was reached at higher organic loading rates of 19 and 39 g COD/L/day for sugar and energy cane stillage respectively, and this was equivalent to 0.3 to 0.4 L $CH_4$/g COD utilized. The microbial community of the hybrid reactors comprised members of domains Bacteria, Archaea and Eukarya. Major bacterial phyla included Firmicutes, Bacteroidetes and Synergistetes. Archaea (phylum Euryarchaeota) were significantly ($P < 0.05$) more abundant in the biofilm retained on the annular rings in the upper part of the reactor compared with the lower part containing upflow sludge, while bacterial phyla Lentisphaerae, NKB19, Synergistetes and WPS-2 were significantly more abundant in the sludge. Fungi (chiefly Saccharomycetes) and protozoans represented the eukaryotic life in the reactors. The main predicted functional categories include carbohydrate, protein and nucleic acid metabolism, primarily attributed to Euryarchaeota, Firmicutes, Bacteroidetes and Proteobacteria. Complete pathways for carbohydrate conversion and methane production have been detected and attributed to Firmicutes/Bacteroidetes and Euryarchaeota, respectively. Altogether, our data reveal that highly efficient bioconversion of stillage occurs in anaerobic, high rate hybrid-type mesophilic digesters by the synergistic activity of selected communities of gut methanogens, as well as Firmicutes and Bacteroidetes, that are the major contributors to rumen fermentation and metabolism.

**Key Words:** thin stillage, hybrid reactors, anaerobic digestion, methane

**Homoacetogenic activity in the sheep rumen**

P. Raju*[1], G. Henderson[1], M. Tavendale[1], J. Rakonjac[2], and P. Janssen[1],
[1]AgResearch Ltd., Grasslands Research Centre, Palmerston North, New Zealand,
[2]Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand.

Ruminant livestock contribute significantly to global greenhouse gas emissions. This is due to rumen microorganisms, known as methanogens, that generate methane from hydrogen and carbon dioxide during the microbial fermentation of feed. Various methane mitigation strategies (vaccines, inhibitors, etc.) are being developed to reduce methane emissions from ruminants. However, inhibiting methane production may cause accumulation of unused hydrogen in the rumen, which may slow down rumen fermentation and thereby affect animal productivity. Homoacetogens, a group of microbes known to reside in the rumen, can use hydrogen and carbon dioxide to form acetate. Homoacetogens could therefore take over the role of ruminal hydrogen disposal following the inhibition of methanogens. The aims of this study were to identify alternative hydrogen utilizers, such as homoacetogens, and to quantify their involvement in hydrogen utilization. Chemical compounds were screened to identify

specific inhibitors of methanogens and homoacetogens. Then, the effects of these inhibitors on rumen fermentation and homoacetogenic acetate formation were studied in sheep. Homoacetogenesis was measured via incorporation of $^{13}CO_2$ into $^{13}$C-acetate and microbial community structures followed over time. Homoacetogenic activity increased when methanogenesis was inhibited using chemical inhibitors. An increase in propionate, a further ruminal hydrogen sink, was also observed, and there were changes in the archaeal and bacterial communities. These results indicate that homoacetogenesis occurs in the ovine rumen, even when methanogenesis is not inhibited and that this increases following inhibition of methane formation. In the future, knowledge of these hydrogen-utilizing microorganisms could facilitate the transition from a normal methane-producing rumen to an equally or even more productive low methane one.

**Key Words:** rumen fermentation, methane inhibition, homoacetogenesis

**The genome sequence of a member of the rumen Methanomassiliicoccales—Methanogenic archaeon isolate ISO4-H5**

Y. Li*[1,3], S. Leahy[1,2], J. Jeyanathan[1,4], F. Cox[1], E. Altermann[1], W. Kelly[1], S. Lambie[1], P. Janssen[1,2], J. Rakonjac[3], and G. Attwood[1,2],
[1]AgResearch Limited, Grassland Research Centre, Palmerston North, Manawatu, New Zealand,
[2]New Zealand Agricultural Greenhouse Gas Research Centre, Palmerston North, Manawatu, New Zealand,
[3]Massey University, Palmerston North, Manawatu, New Zealand,
[4]INRA, Saint-Genès, Champanelle, France.

Methane emissions from agriculture represent around 9% of the emissions produced by human-related activities, the single largest source being animal enteric fermentation, predominantly from ruminant livestock. Ruminant methane is produced mainly in their fermentative forestomach (or reticulo-rumen) by a group of archaea known as methanogens. To reduce methane emissions from agriculture, it is necessary to understand the role of methanogenic archaea in the rumen, and to identify their distinguishing characteristics that may be used to develop methane mitigation technologies for ruminants. To gain insight into the role of methylotrophic methanogens in the rumen environment, a methanogenic archaeon (ISO4-H5) isolated from the ovine rumen and designated as a member of the seventh order of methanogens, Methanomassiliicoccales, has been sequenced and compared with the genomes of other members of this order. Genomic analysis suggests ISO4-H5 is an obligate hydrogen-dependent methylotrophic methanogen, and is able to utilize methanol and methylamines as substrates for methanogenesis. Similar to genomes of other methanogens from this order, the genes required for the first 6 steps of hydrogenotrophic methanogenesis are absent. Genomic comparison between members of the Methanomassiliicoccales revealed strong conservation in energy metabolism, particularly in methanogenesis genes, as well as in biosynthesis and utilization of pyrrolysine. However, the cysteate synthase, cysteate aminotransferase and sulfopyruvate decarboxylase genes required for the synthesis of coenzyme M (CoM) are absent from the ISO4-H5 genome, suggesting it cannot synthesize CoM itself and requires an exogenous source to survive within the rumen. The

sequencing and analysis of this ovine isolate expands our knowledge of the Methanomassiliicoccales order in the rumen, and contributes to the methanogen genome resources available for ruminant methane mitigation research.

**Key Words:** methanogen, methane, ruminant, Methanomassiliicoccales

**Effect of different levels of potassium nitrate with or without sulfur on enteric methane production in Teddy goats at post-weaning age**

M. Nisa*[1], M. Arif[2], M. Ali[3], and M. Sarwar[3],
[1]Department of Food Science, Nutrition and Home Economics, Govt. College University, Faisalabad, Pakistan,
[2]Department of Animal Sciences, University College of Agriculture, University of Sargodha, Sargodha, Pakistan,
[3]Institute of Animal Science, University of Agriculture, Faisalabad, Pakistan.

Efforts are being made to reduce enteric methane ($CH_4$) production from livestock. Nitrate and sulfate supplementation could be used to mitigate enteric $CH_4$ production. The main objective of present study was to determine the effect of different levels of potassium nitrate ($KNO_3$) with or without added sulfur (S) on the enteric $CH_4$ production and growth performance in weaned Teddy male goats. The experiment lasted for 3 mo. Twenty-four male goats approximately 3 mo of age were randomly divided into 6 groups, 4 animals in each group. Six isonitrogenous and isocaloric diets were formulated. Nonprotein nitrogen was same across all diets. The control diet (C) contained only urea as non-protein nitrogen without $KNO_3$ and S. However, K0-S4, K3-S0, K3-S4, K6-S0, and K6-S4 diets had 0% $KNO_3$ and 0.4% S, 3% $KNO_3$ and 0% S, 3% $KNO_3$ and 0.4% S, 6% $KNO_3$ and 0% S, and 6% $KNO_3$ and 0.4% S, respectively, on a dry matter basis. Feed intake was recorded daily, and the animals were weighed fortnightly. Digestibility and nitrogen balance trials were conducted during the last week of the experiment. Enteric $CH_4$ was analyzed at the end of the experiment using a GASMET infrared $CH_4$ analyzer. There were no differences ($P > 0.05$) in dry matter intake, nutrient digestibility, and nitrogen balance in goats fed all diets. The enteric $CH_4$ was 56% decreased ($P < 0.05$) in goats fed the K6-S4 diet compared with those fed the C diet. Daily live weight gain of goats fed the K6-S4 diet was the highest (66.0 g/d), and goats fed the K3-S0 diet had the lowest weight gain (61.25 g/d). The feed conversion ratio was better in animals fed the K6-S4 diet. In conclusion, animals fed diet containing $KNO_3$ and S not only grew at a faster rate, but enteric $CH_4$ production was also decreased.

**Key Words:** Teddy goats, potassium nitrates, sulfur, enteric methane

**Session: Immunology (including host-microbe interactions)**

**Development of an enteric inflammation model in broilers and methods to detect mucosal permeability**

L. R. Bielke*[1], V. A. Kuttappan[1], J. C. Bielke[1], E. A. Vicuña[1], L. R. Berghman[3], R. Galarza-Seeber[1], X. Hernandez-Valasco[2], B. M. Hargis[1], and G. Tellez[1],
[1]Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA,
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de

México, México,
[3]Departments of Veterinary Pathobiology and Poultry Science, Texas A&M University, College Station, TX, USA.

A hypothesis for one mechanism by which antibiotic growth promoters increase performance parameters in poultry may be through control of enteric inflammation. With decreasing acceptance of growth promoters in poultry, alternatives must be sought to sustain growth and health of flocks, and research models capable of inducing and measuring changes in enteric inflammation are urgently needed. Multiple induction models and markers of reduced enteric integrity have been investigated in this laboratory. Markers of inflammation such as fluorescein isothiocyanate dextran (FITC-D; 3–5kDa), xanthophyll absorption, bacterial translocation (BT) to liver, and serum opacity have been investigated as markers of decreased enteric integrity. Although some markers have shown promise, FITC-D and BT have proven the most consistent and reliable for detecting tight junction leakage, and optimization experiments have investigated parameters such as molecule size and dose of FITC-D, as well as timing of BT detection. Furthermore, induction methods have included dextran sodium sulfate (DSS), 24-h feed restriction (FR), rye-based diet (replacing corn with rye), and oral dexamethasone (1 mg/kg in feed for 6 d). Although DSS clearly caused intestinal inflammation (0.75% in drinking water for 3 d), lesions were mostly limited to the cecum and treatment effects were generally noted only when severe illness was induced (3% in drinking water for 72 h). Presently, 24-h FR and rye-based diet have consistently resulted in increased tight junction leakage ($P < 0.05$), though oral dexamethasone has shown promise, with increased serum FITC-D and bacterial translocation ($P < 0.05$) in 2 experiments. These enteric inflammation research models provide a means by which alternative antibiotic growth promoters and interventions after intestinal insult can be investigated. Additionally, there is interest in determining if enteric inflammation markers can predict flock health and well-being.

**Key Words:** chicken, enteric inflammation, mucosal integrity, fluorescein isothiocyanate dextran, bacterial translocation

**Session: Microbiology (including ecology, (meta)genomics, physiology, and proteomics)**

### Early microbiome drives rumen papillae development via the production of volatile fatty acids in pre-weaned calves

N. Malmuthuge*, G. Liang, and L. L. Guan,
University of Alberta, Edmonton, Alberta, Canada.

Ruminants have evolved to utilize indigestible forage using its symbiotic microbiome and to produce meat and milk that serve as rich protein sources in human diet. It has been commonly accepted that this organ is fully developed after weaning of ruminants and capable of microbial fermentation. However, the establishment of rumen microbiome and effect of early microbial fermentation on rumen papillae development of pre-ruminant calves are largely unknown. The present study collected rumen content and tissue samples from calves (n = 24) within 30 min. after birth (0D), 1 week (1W), 3 week (3W) and 6 week (6W) to explore taxonomical and functional composition of rumen microbiome, production of volatile fatty acids (VFA) as well as papillae development. At birth (0D), calf rumen was colonized with dense ($9.1 \pm 3.1 \times 10^8$ 16S copy/g of content) and diverse (47 genera) bacterial population, which gradually increased with calf age and dietary changes. Pre-ruminant calf rumen at 1W was colonized with dynamic microbial population dominated by *Prevotella* (58.3 ± 21.4%) and capable of producing acetate (21.1 ± 2.2 m*M*/mL of rumen fluid), propionate (10.6 ± 2.0 m*M*/mL) and butyrate (5.6 ± 1.8 mM/mL), even when they were only fed milk. In total, 3443 microbial genes, 167 bacterial genera, and 31 archaeal genera were observed from the rumen content of all the tested calves. The increasing concentration of acetate, propionate and butyrate was positively correlated with rumen papillae length and width. Although archaea were observed in rumen from 1W, methyl coenzyme-M reductase only appeared after 3W with starter intake; alternatively, 1W rumen microbiome contained archaeal-specific glycolysis enzymes. The present study revealed drastic temporal changes in the microbiome, VFA concentration, papillae length and width within the first 6 weeks of life, suggesting a rapid colonization of the rumen immediately postpartum, and this early microbiome actively involved in the fermentation of dietary substrates to produce VFA that provide energy for the developing rumen epithelium.

**Key Words:** pre-weaned calves, rumen microbiome

### The polysaccharide-degrading mechanisms of uncultured rumen bacteroidetes

A. E. Naas, A. K. Mackenzie, V. G. H. Eijsink, and P. B. Pope*,
Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Aas-1430, Norway.

Polysaccharide utilization loci (PULs) are ubiquitous in Bacteroidetes-affiliated bacteria inherent to the gastrointestinal tract of mammals where they contribute toward plant cell wall degradation. Current perceptions regarding the enzymatic machineries encoded in PULs have been largely developed from cultivated

Bacteroidetes strains that utilize starch and other dietary fibers within the human distal gut. However, uncultured and therefore uncharacterized Bacteroidetes lineages dominate other digestive ecosystems whose fibrolytic proficiency expands beyond that of the human digestive system. Here we discuss the genomes of 2 uncultured dominant Bacteroidetes phylotypes that have been reconstructed from the rumen microbiome of 2 herbivores that specialize in lignocellulose utilization, albeit with contrasting habitats and diets. We demonstrate correlations between the plant cell-wall polymers available to the phylotypes and the enzymatic capabilities of PUL structures. In particular, functional characterization of 2 new PULs reveals unprecedented substrate versatility within a single PUL as well as the first evidence for PUL-catalyzed cellulose conversion. These results expand the Bacteroidetes PUL paradigm while generating new insight into the essential contributions made by the irrecoverable microbiota. Given that a plethora of novel uncultured Bacteroidetes lineages exist in digestive ecosystems and that one species alone can harbor scores of individual PULs, the enormity of the PUL paradigm is only beginning to emerge.

**Key Words:** polysaccharide utilization loci, cellulases, metagenomics, rumen, microbiome

**Citrus pectin breakdown by *Bacteroides xylanisolvens* XB1A involves at least four large polysaccharide utilization loci**

J. Despres*[1], E. Forano[1], P. Lepercq[1], S. Comtet-Marre[1], C. J. Yeoman[2], M. E. Berg Miller[3], C. J. Fields[4], B. Henrissat[5], B. A. White[3,4], and P. Mosoni[1],
[1]INRA, Saint-Genès Champanelle, France,
[2]Montana State University, Bozeman, Montana, USA,
[3]University of Illinois, Department of Animal Sciences, Urbana-Champaign, IL, USA,
[4]University of Illinois, Institute for Genomic Biology, Urbana-Champaign, IL, USA,
[5]Aix-Marseille University and Centre National de la Recherche Scientifique (CNRS), Marseille, France.

Symbiotic microorganisms that reside in the human intestine are able to utilize dietary fibers mainly composed of resistant starches, cellulose, hemicelluloses and pectins. Pectins are diverse in composition and structure, and represent one of the most complex plant cell wall polysaccharides. Very few pectinolytic bacteria have been described in the human colon to date and little is known about the enzyme systems involved in pectin degradation. Therefore our goal was to characterize by transcriptomic and mutagenesis approaches the pectinolytic enzyme system of *Bacteroides xylanisolvens* XB1A, a species belonging to the human core microbiome initially described as xylanolytic. *In silico* analyses of the strain XB1A genome revealed a large array of genes encoding CAZymes potentially involved in pectinolysis. We first showed that the bacterium grew with a high growth rate on citrus pectin. Then, RNaseq data (Illumina/HiSeq2000) were obtained from strain XB1A grown on citrus pectin and compared with growth on glucose at mid- and late-log phase (biological triplicates in each condition). RNaseq revealed that 207 and 140 genes were significantly overexpressed (Log2 fold-change >3) on pectin at mid- and late-log phase, respectively. Among them, 4 large polysaccharide utilization loci (PULs) were identified and shown to be overexpressed depending on the growth phase. The CAZyme composition of these PULs suggests that they are specific to

different pectin polymers; that is, homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. Induction of one of the most overexpressed PUL (potentially targeting homogalacturonan) was confirmed by RT-qPCR. We further studied the importance of this PUL in pectin utilization by directed mutagenesis. Gene disruption by plasmid insertion into the unique *susC* gene of this PUL strongly altered the growth rate of the bacterium on citrus pectin although growth was not totally abolished. In conclusion, this study shows the existence of several new PULs involved in pectin degradation by *B. xylanisolvens* XB1A, one of them being particularly important in this function. Our findings highlight the metabolic plasticity of *B. xylanisolvens* toward non-starch dietary polysaccharides, which contributes to its competitive fitness within the human gut ecosystem.

**Key Words:** pectin degradation, human gut, *Bacteroides xylanisolvens*, RNAseq

**A comparative study on rumen microbiome in beef cattle and bison fed different diets**

M. Zhou*[1], Y. Chen[1], M. Walpole[2], P. Gorka[2], M. Woodbury[3], G. B. Penner[2], and L. L. Guan[1],
[1]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada,
[2]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,
[3]Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Beef and bison bulls display varied capability in feed digestion, especially the fiber component. The current study aimed to examine and compare the rumen microbiome in bovine and bison bulls fed either a backgrounding high forage diet (BCK) or finishing high grain diet (HG), to understand the roles of microbes in such variation. Rumen digesta samples were collected at slaughter and subjected to DNA extraction. Amplicon based 454 pyrosequencing was applied to identify the bacterial, archaeal, and protozoal communities; and qPCR was used to quantify the microbial population. In total, each bison hosted 109 to 289 bacterial OTUs at genus level, 4 to 9 archaeal OTUs and 17 to 53 protozoal OTUs, respectively, at species level, whereas each cattle hosted 55 to 252 bacterial OTUs at genus level, 3 to 12 archaeal OTUs at species level, and 15 to 55 protozoal OTUs at species level. None of the identified microbial phylotypes were specific for particular host species or diet (BCK vs. HG). Firmicutes and Bacteroidetes dominated the bacterial communities; *Methanobrevibacter gottschalkii* was the predominant archaeal species identified; and Entodinimorphida was the predominant protozoal order. Bacterial communities were distinctive between bull cattle and bison ($P < 0.01$), while archaeal and protozoal communities were not different between the 2 species. Diet had a strong effect on the bacterial communities for both cattle and bison ($P < 0.01$) as well as the protozoal community in cattle ($P < 0.05$), but did not affect the protozoa in bison or the archaeal communities in the 2 species. qPCR showed that the bacteria population was significantly higher ($P < 0.01$) in bovine than that in bison, whereas the archaeal and protozoal population were similar between bison and bovine. For both 2 species, animals fed HG diet hosted more abundant of bacteria than animals

fed BCK diet. The compositional and quantitative variation of the rumen microbiome warrants further studies investigating the functional variation between these 2 ruminant species using metagenomics, so that interpretations can be assigned to their different capabilities in digesting different fiber components.

**Key Words:** bison, bovine, rumen microbiome

**Fecal microbiome and non-typhoidal *Salmonella enterica*: Changes through the transition period of dairy cattle**

L. M. Munoz-Vargas*[1], R. Digianantonio[1], M. Williams[2], G. Schuenemann[1], and G. Habing[1],
[1]The Ohio State University, Columbus, Ohio, USA,
[2]Ohio Agricultural Research and Development Center, Wooster, Ohio, USA.

Stressors in livestock have been associated with shedding of non-typhoidal *Salmonella* (NTS) in multiple studies and species. However, there is a lack of understanding of how stress can influence the stability and diversity of the gut microbiome and the patterns of colonization and shedding of NTS. The objective of the study was to determine the association between changes in the fecal microbial community, metabolic stress, and shedding of NTS through the calving period of dairy cattle. This longitudinal trial included 48 cows from 4 commercial dairy farms in Ohio. In total, 192 fecal and blood samples (48 cows × 4 time points) were collected at 3 wk and 1 wk pre-calving, and 1 wk and 3 wk post-calving. Culture of fecal samples was used to determine the prevalence of NTS at each time point. Serum concentrations of nonesterified fatty acids (NEFA) were used to measure metabolic stress. Extracted total genomic DNA of the 192 fecal samples was used as a template for conventional PCR, and subsequent sequencing of the V4 region of the 16S rRNA gene using the Illumina platform. A logistic regression model was used for the statistical analysis, where NTS prevalence, NEFA, days relative to calving, day temperature, body condition score, and antibiotic administration were considered for inclusion. Descriptive analysis of the structure of the microbial community was generated by the Base Space software, and the changes overtime were assessed by a principal coordinate analysis using SAS 9.4 software. *Salmonella* shedding across sampling time points was found to be statistically different ($P = 0.01$). Preliminary results demonstrate that a substantial proportion of cows began shedding *Salmonella* closer to the calving day. Overall, 38% positive cultures were detected in the 3 wk pre-calving, 48%, 52%, and 34% during −1, +1 and +3 weeks relative to parturition, respectively. Metabolic stress indicators increased from pre to post parturition as expected, with a mean NEFA concentration of 0.35 and 0.56 mEq/L, respectively. This study shows that metabolic stress and microbiome perturbations during the calving period can influence the shedding of NTS in fecal samples. Data generated from this study could lead to specific farm management strategies to decrease the prevalence of NTS on dairy productions.

**Key Words:** *Salmonella*, microbiome, calving, stress, dairy

**Correlation of microbiome populations with performance measurements and sex in broiler breeders**

S. Diaz-Sanchez*[1], R. Hawkins[2], R. Okimoto[2], A. Layton[3], A. Saxton[4,5], A. Blakeley-Ruiz[5], and I. Hanning[1,5],
[1]University of Tennessee, Department of Food Science and Technology, Knoxville, TN, USA,
[2]Research and Development, Cobb-Vantress Inc., Siloam Springs, AR, USA,
[3]University of Tennessee, Department of Microbiology, Knoxville, TN, USA,
[4]University of Tennessee, Animal Science, Knoxville, TN, USA,
[5]University of Tennessee, Department of Genome Science and Technology, Knoxville, TN, USA.

Two experiments were conducted where fecal samples from 2 genetically distinct lines of broilers (Lines A and B) were analyzed using Illumina sequencing to identify the bacterial populations. The experiments evaluated (1) bacterial profiles that affected weight gain; and (2) correlation of feed efficiency (FE) with microbiome profiles. In the weight experiment, bacterial populations were obtained and compared from males and females with relatively high weight and low weights, while in the FE experiments, microbiomes were obtained only from females. 16SrRNA gene sequences were processed with Qiime and statistical analyses were conducted in R. Variables assessed included, sex, weight gain, feed conversion, and genetic line. Data from each line were analyzed separately. Microbial populations that correlated with weight gain were specific to lines and sex. In Line A, heavy males possessed a lactobacilli prevalence of 42% or greater, conversely, the lactobacilli population of heavy females was less than 2%. In Line B, lactobacilli prevalence negatively affected weight gain for males. In line B, low weight for females correlated with a prevalence of >30% *Enterobacteriaceace*, while a prevalence of >12% *Ruminococcaceae* was associated with low weight males. For the feed conversion experiments, 51 microbial families were present in Line A and most of the families (41) negatively correlated with feed conversion. The opposite was true for Line B where 42 out of the 54 total number of families positively correlated with feed conversion. The data indicate microbial populations in broiler breeders are associated with factors including weight, feed efficiency, sex, and genetics.

**Key Words:** feed efficiency, weight, broilers, breeder, microbiome

**An attempt to detoxify the leucaena mimosine in sheep using immobilized IBT-Goettinger bioreactor grown *Klebsiella pneumoniae***

A. Aung*[1], F. Gessler[2], and H. Böhnel[3],

[1]Department of Physiology and Biochemistry,University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar,
[2]Miprolab GmBH, Göttingen, Lower Saxony, Germany,
[3]Institute of Biotechnology in the Tropics, University of Göttingen, Göttingen, Lower Saxony, Germany.

*Klebsiella pneumoniae*, a bacterial strain that can degrade mimosine, was enumerated by using IBT- Goettinger Bioreactor. Then the bacteria were immobilized in sodium alginate beads. Nine sheep locally available in Myanmar were allocated in 3 groups for feeding trial to investigate the ability of immobilized *K. pneumoniae* on the detoxification of leucaena mimosine. The animals from group I were fed on the diet without leucaena (control) while the animals from group II and III were fed a diet containing 40% of dried leucaena leaves. One gram of alginate beads with mimosine degrading *K. pneumoniae* ($5.0 \times 10^{10}$/g) was fed to the animals from group III. After one week of experimental period, the animals from group II commenced to show toxic signs while there was no toxic symptom in the animals from group III for the whole experimental period. The digestibilities of nutrients of animals from group III were higher than those of animals from group II and the same as the animals from group I. Therefore, immobilization of *K. pneumoniae* in sodium alginate beads did not disturb mimosine degradability of those bacteria.

**Key Words:** *Klebsiella pneumoniae*, leucaena, mimosine

**Transport of a fluorescent analog of glucose (2-NBDG) by rumen bacteria**

J. Tao, R. Diaz, and T. Hackmann*,
University of Florida.

Substrate preferences of uncultured bacteria have been difficult to identify, but fluorescent substrates may have potential to reveal those preferences. Our aim was to determine if cultured strains of rumen bacteria would transport a fluorescent analog of glucose (2-NBDG) with the same specificity and kinetics as glucose. Transport was measured at intervals as short as 2 s by employing a novel method. Transport was initiated by adding 2-NBDG (0 to 100 μ*M*) to washed cells; transport was stopped by adding −5°C buffer and filtering through a polycarbonate membrane; the membrane was dissolved; and fluorescence intensity was read in a fluorometer. Transport of 2-NBDG could be detected for *Streptococcus bovis* and *Selenomonas ruminantium* (2 strains each) within as little as 2 s of 2-NBDG addition, but transport could not be detected for 6 other glucose-fermenting species. Genomes of *S. bovis* and *S. ruminantium* strains are predicted to contain genes for the mannose phosphotransferase system, whereas the other species had genes for other glucose transporters. For *S. bovis* JB1, the Michaelis constant ($K_m$) for 2-NBDG transport was 10.6-fold lower than that for [$^{14}$C]-glucose transport ($P = 0.006$). The maximum velocity ($V_{max}$) was 2.9-fold lower than that for [$^{14}$C]-glucose, but this difference was not significant ($P = 0.100$). Based on these results with cultured bacteria, 2-NBDG could be used to identify some uncultured bacteria that take up glucose. It would identify only those bacteria with a mannose phosphotransferase system (not other glucose transporters), and its transport would not occur at the same velocity as glucose over all concentrations.

**Key Words:** rumen bacteria, 2-NBDG, transport

**Metabolic networks for nitrogen utilization in *Prevotella ruminicola* 23**

C. Méndez-García*, J. N. Kim, I. K. Cann, and R. I. Mackie,
University of Illinois at Urbana-Champaign, Urbana, IL USA.

Bacterial nitrogen assimilation constitutes a key process in the rumen because digested bacterial cells constitute the main source of protein for the host animal. Nitrogen metabolism in the predominant, metabolically versatile ruminal bacterium *Prevotella ruminicola* (phylum Bacteroidetes) is poorly understood. To gain insight into the mechanism underlying ammonium uptake and assimilation in this bacterium, changes in global gene expression profiles of *P. ruminicola* 23 in response to variations in the available nitrogen source (ammonia or peptides) and in response to environmental ammonia concentrations (excess or growth-limiting) were analyzed by qPCR and microarray-based transcriptomics, and were complemented using enzymatic assays and differential proteome analysis. *P. ruminicola* 23 grew well on ammonia and peptides, but not on amino acids, as the sole nitrogen source. During growth with excess concentrations of ammonia, functional categories including amino acid and protein biosynthesis, as well as genes involved in ammonium transport and its regulation were highly induced; genes involved in protein fate, energy metabolism, DNA metabolism, and signal transduction and regulation were more highly expressed when *P. ruminicola* 23 was grown on limiting ammonium concentrations. *P. ruminicola* 23 showed differential gene expression patterns during growth on ammonia or peptides. Genes induced by growth on peptides largely encoded enzymes implicated in DNA metabolism or protein biosynthesis (ribosomal proteins, essentially). These patterns of transcript abundance for genes involved in ammonia uptake and metabolism differ from the classical “enteric paradigm” and are likely more representative of those occurring in predominant commensal gut bacteria.

**Key Words:** *Prevotella ruminicola*, ammonia assimilation, microarray, enteric paradigm

### The effect of orange peels on ammonia and VFA production and growth in *Entodinium caudatum*

B. Denton* and Z. Yu,
The Ohio State University, Columbus, Ohio, USA.

Rumen protozoa, through predation of rumen bacteria, contribute significantly to increased ammonia ($NH_3$) production in the rumen, decreased protein efficiency, and increased nitrogen excretion into the environment. The aims of this in vitro study was to determine if orange peels compared with yucca and quillaja would (1) decrease *Entodinium caudatum* growth, (2) decrease $NH_3$ concentrations, and (3) affect VFA concentrations and the ratio of acetate:propionate (a:p). For experiment 1 treatments were control; orange peel at 50, 100, 200, 400, and 800 mg/L; and yucca schidigera or quillaja saponaria at 300, 600, and 900 mg/L in duplicate. For experiment 2, treatments were control and orange peel 1600 mg/L in triplicate. For both experiments, culture tubes were sampled at 0, 24, and 48 h and analyzed for *E. caudatum* counts and concentrations of $NH_3$ and VFA. All treatments with increasing dose appeared to lower *E. caudatum* growth at 24 h, but only orange peel at 1600 mg/L significantly ($P < 0.05$) decreased *E. caudatum* growth. At 48 h the difference between the control and the treatments was less profound and quillaja at 300 mg/L even significantly increased the *E. caudatum* count relative to the control; however, orange peel at 1600 mg/L still significantly decreased *E. caudatum* growth. At both

24 and 48 h, orange peel at 1600 mg/L resulted in a significantly lower $NH_3$ concentration, whereas yucca saponin at 900 mg/L only decreased ammonia concentrations at 48 h. The a:p ratio was significantly lower in all the yucca treatments at 24 and 48 h except yucca at 300 mg/L which still tended to lower the a:p ratio. Yucca, orange peels, and possibly quillaja are capable of reducing $NH_3$, and orange peel at 1600 mg/L was effective at decreasing the growth of *E. caudatum,* but it is unknown whether the culture is capable of adapting to the treatments after 48 h. Yucca has the ability to decrease the a:p ratio, and thus might lower methane production. In conclusion, orange peels, yucca, and quillaja might decrease the growth of *E. caudatum*, which is the most predominant rumen protozoa, and decrease $NH_3$ production in the rumen leading to increased protein efficiency and decreased nitrogen excretions, whereas yucca might shift the fermentation pathways in the rumen decreasing methane emission.

**Key Words:** rumen, *Entodinium*, protozoa, ammonia, orange peels

**Transcriptome analysis of *Fibrobacter succinogenes* S85 in co-culture with nonfibrolytic ruminal bacteria**

N. Fukuma, S. Koike*, and Y. Kobayashi,
Hokkaido University, Sapporo, Hokkaido, Japan.

We previously reported that fiber digestion was enhanced by the ruminal fibrolytic bacterium *Fibrobacter succinogenes* S85 when cocultured with non-fibrolytic strains R-25 and *Selenomonas ruminantium* S137. In the present study, transcriptome (RNA-Seq) analysis was performed in a coculture setup comprising these 3 microorganisms. Within the genome of *F. succinogenes* S85, genes related to signal transduction and transcriptional regulator showed increased expression in the 3-member coculture when compared with monocultures of *F. succinogenes* S85. The expression level of 16S rRNA was also greater, whereas that of fibrolytic enzyme genes did not differ between the mono- and the coculture. On the other hand, the expression level of chaperone protein genes was markedly decreased in the 3-member coculture. Accumulation of fiber digestion/fermentation products of *F. succinogenes* S85 may lead to enzyme inhibition and ATP loss due to the induced expression of chaperone proteins, which in turn results in limited fiber digestion in monoculture of *F. succinogenes* S85. In the 3-member coculture conditions, these accumulated products could be consumed by non-fibrolytic strains, thus leading to overall activation of transcription and protein synthesis of *F. succinogenes* S85. The enhancement of fiber digestion by *F. succinogenes* S85 in co-culture with non-fibrolytics may thus be attributed to the activation of overall protein synthesis rather than the induction of specific fibrolytic enzymes.

**Key Words:** fiber digestion, *Fibrobacter succinogenes,* RNA-Seq, rumen bacteria, co-culture

**Intake of lichens alters the rumen microbiome in Norwegian reindeer (*Rangifer tarandus tarandus*)**

A. Salgado-Flores*[1], L. Heldal Hagen[2], P. B. Pope[2], S. Ishaq[3], and A. D. Wright[4],

[1]Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway,
[2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway,
[3]Department of Animal Sciences, University of Vermont, Burlington, VT, USA,
[4]School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA.

Reindeer are large Holarctic herbivores whose heterogeneous diet has led to the development of a unique rumen microbiome. This consortium of anaerobic microbes is essential for the digestion of plant cell wall carbohydrates from these arctic plants, including lichens. Lichens are reported to be high in plant secondary metabolites that may directly or indirectly affect members of this microbial consortium, such as methanogenic archaea. Methanogens produce methane mainly from carbon dioxide and hydrogen. Little is known about the factors affecting methanogenesis in these Arctic ruminants. Here, we examined the effect of lichen ingestion on the reindeer rumen microbiome, mainly focused on methanogens. Samples from rumen were collected from 2 groups of Norwegian reindeer (*Rangifer tarandus tarandus*), 1 group (n = 4) fed on a lichen-based diet and 1 fed a standard pelleted reindeer feed (n = 3). Population densities for methanogens, protozoa and bacteria were estimated by Real-time PCR experiments. Microbial diversity was assessed by amplicon sequencing (454 pyrosequencing) of the 16S rRNA gene from methanogens and bacteria. In general, the concentration of methanogens was not significantly affected ($P > 0.05$) in the rumen of reindeers with lichens as the only sustenance. *Methanobrevibacter* (= 97% similarity) constituted the main archaeal genus (>95% of reads) in both groups, with *M. thaueri* as the dominant species. Remarkably, *M. ruminantium* increased up to 23.8% with a lichen-based diet (3.6% to 27.4% of reads). *Firmicutes* and *Bacteroidetes* were the main bacterial phyla in all the samples, with increased *Clostridia*-related constituents in reindeers fed lichens (12.8%). Permutational multivariate analysis (vegan:adonis, $F < 0.05$) demonstrated that at least the bacterial microbiome was significantly different between both groups. Accordingly, methanogenesis can be stronger influenced by altered diversity than density. A low *M. thaueri*:*M. ruminantium* ratio in the rumen has been reported to be linked with lower methane output in cattle and alpaca (*Vicugna pacos*). Therefore, reduced methane emissions in reindeer fed lichens may be accounted for either by a lower methane-yielding efficiency by *M. ruminantium* or an alteration on the other microbial groups by lichen compounds, leading to low methanogenesis.

**Key Words:** reindeer, lichens, rumen, metagenomics, methanogens

**Intake of lichens alters the rumen microbiome in Norwegian reindeer (*Rangifer tarandus tarandus*)**

S. Qi*[1], B. Smiley[1], S. Huntley[1], F. Owens[1], Y. Jiang[2], A. Adesogan[2], and W. Rutherford[1],
[1]Forage Additive Research, DuPont-Pioneer, Johnston, IA, USA,
[2]Department of Animal Science, University of Florida, Gainesville, FL, USA.

Rapid advances in sequencing technologies enable high-resolution population profiling of microbial communities such as the rumen. Until now, the majority of rumen amplicon sequencing studies have been performed using 454-pyrosequencing technology. However, the Illumina-based platform, particularly MiSeq 16S rRNA gene sequencing, is gaining popularity due to its lower cost and higher throughput. To assess the suitability of the MiSeq platform for analysis of the rumen microbial community, we evaluated 2 non-overlapping regions of the 16S rRNA gene that vary in length (i.e., V1~3 and V4). qPCR of selected rumen species also was used to validate the sequencing results from both regions. Microbial groups identified from V4 amplicon sequences showed a strong positive correlation with those identified from V1~3 sequences. The correlation was positive between amplicon sequencing and qPCR analysis for microbial groups with high abundance but not for microbial groups with low abundance. Although V1~3 region sequencing slightly increased the number of species identified, the majority of the MiSeq sequence fragments could not be classified down to the species level. Although care must be taken to minimize the introduction of artifacts during sample preparation and data processing, our results demonstrated that MiSeq sequencing can provide useful information to help understand the highly diverse and complex microbial ecosystem of the rumen.

**Key Words:** MiSeq, rumen, microbial population, 16S rRNA

**Incubation temperature affects the post-hatch response of broiler chicks to *Salmonella* Enteritidis**

P. E. N. Givisiez*[1,2], A. L. B. Moreira Filho[1], H. B. Oliveira[1], F. G. P. Costa[1], D. B. Campos[1], and C. J. B. Oliveira[1,2],
[1]Universidade Federal da Paraiba, Areia, PB, Brazil,
[2]University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, USA.

This study evaluated how incubation temperature affects the response of chicks challenged with *Salmonella* Enteritidis (SE) after hatch. Fertile Cobb500 eggs were incubated at 37.7°C and 60% relative humidity from one to 11 d of incubation. Afterward, the eggs were divided into 3 treatments until hatch: standard (37.7°C), continuous high temperature (38.7°C) and continuous low temperature (36.7°C). At 2 d of age, birds of the high and low temperature treatments were inoculated with SE, while the standard temperature group was sham-inoculated (20 chicks each group). SE-inoculated birds were slaughtered at 8 d post-inoculation (10 d of age) to assess *Salmonella* cecal counts. Semiquantitative real time polymerase chain reaction (sqPCR) was used to assess relative Hsp70 mRNA expression in the ileum mucosa in all groups (SE- and sham-inoculated) using the $2^{-\Delta\Delta}$Ct method; Ct values of each sample were standardized for β-actin RNA. High-temperature birds showed lower SE counts than low-temperature birds (2.7 vs 5.2 $\log_{10}$ cfu/g cecal content). Relative Hsp70 mRNA expression was similar in high-temperature (0.15 Hsp70 mRNA expression ratio) and sham-inoculated (0.17) birds, and both were lower than cold-temperature chicks (2.59). High temperature during incubation apparently improves resistance of birds to *Salmonella* Enteritidis colonization in the first days of age and this might be associated with lower induction of stress response.

**Key Words:** chicks, embryo, Hsp70, *Salmonella* Enteritidis

**Metabolism pathway shifts by bacteria competing for hydrogen in in vitro mixed rumen microbial fermentations**

S. Denman*[1], L. Bragg[2], W. Smith[1], C. McSweeney[1], and M. Morrison[3],
[1]CSIRO Agriculture flagship, Brisbane, Qld, Australia,
[2]CSIRO Digital productivity flagship, Brisbane, Qld, Australia,
[3]University of Queensland Diamantina Institute, Brisbane, Qld, Australia.

The rumen microbiota are responsible for the bioconversion of feedstuffs into various fermentation end products such as short chain fatty acids (SCFA). This is achieved initially through hydrolysis steps using pathways involving both hydrogen-producing and hydrogen-consuming reactions. Although hydrogen in the rumen is incorporated in fermentation end products by many bacteria, methanogenic archaea consume the majority of metabolic [H] and produce methane. To understand these metabolic pathways and the microbial interactions, in vitro enrichment cultures with defined consortia of bacteria and methanogens were developed that resulted in both “low” and “high” methanogenic systems. The differences in methane production were associated with shifts in the profile of SCFAs produced. Metatranscriptomic sequencing allowed for the quantifying of functional pathways to the relevant bacteria in the consortium. Within the 2 systems there was no significant change to the expression pattern of enzymes for the breakdown of plant fibers from the fibrolytic species, indicating no alteration to the method that bacterial species employ to degrade plant material. However, significant changes in fermentation pathways were observed that correlated with changes to the SCFA profiles. The inclusion of the acetogenic bacteria TWA4 in the consortium resulted in upregulation of mixotrophic pathways that reduced the amount of available hydrogen for *Methanobrevibacter smithii,* resulting in an increased acetate production with a concurrent decrease in methane production. Furthermore, the reduction in hydrogen partial pressure altered the fermentative pathways for *R. albus* with a reduction in ethanol production and redirection to acetate. *Methanobrevibacter smithii* showed an increase in expression for the methanol specific methyl-transferases of the methylotrophic methanogenesis pathway and an increase in a NADP-dependent alcohol dehydrogenase (*adh*) and a $F_{420}$-dependent NADP oxidoreductase (*fno*). This was countered with a reduction in genes involved in the transfer of $CO_2$ to the formyl-methanofuran and subsequent transfer to the methanopterin. Results here demonstrate the conditions under which the acetogenic bacteria TWA4 can compete for hydrogen even under low partial pressures without the addition of methanogen inhibitors.

**Key Words:** rumen, methane, hydrogen, metatranscriptomics, acetogenesis

**Characterization along the porcine intestinal tract displays unique microbial differences in growing and finishing pigs**

J. S. Thompson*[1], J. A. Benson[1], A. A. Hibberd[1], A. Owusu-Asiedu[2], M. C. Walsh[2], and E. A. Galbraith[1],

[1]DuPont Nutrition and Health, Waukesha, WI, USA,
[2]Danisco Animal Nutrition - DuPont Industrial Biosciences, Marlborough, Wiltshire, UK.

With the continuous growth of the world's population, questions arise if sufficient food sources will be available for future generations. Optimization of the intestinal microbiota has been a feeding strategy used to maintain intestinal health leading to improved growth performance of livestock. To gain a better understanding of the porcine intestine, the current study characterized the microbiota along the intestine of growing and finishing pigs. One hundred 7-wk-old pigs of uniform genetic stock were fed similar corn-soybean meal based diets formulated to meet requirements from d 0–34 (P1) and 35–89 (P2). Mucosal samples from the stomach, duodenum, jejunum, ileum, cecum, colon of 12 pigs were extracted at the end of each phase as well as digesta from the cecum and colon. DNA was extracted from the samples and the V4 region of the 16S rRNA gene was amplified and characterized by 454 pyrosequencing. Firmicutes were found in higher relative abundance in P1 compared with P2 in the cecum ($P < 0.05$) and colon ($P < 0.001$) mucosa, and were more abundant along the proximal intestine, decreasing in the colon ($P < 0.001$). Bacteriodetes had a lower abundance in P1 compared with P2 in the cecum ($P < 0.05$) and colon ($P < 0.001$) and along the proximal to distal intestine, increasing in the colon ($P < 0.001$). Digesta samples did not differ in abundances of Firmicutes and Bacteriodetes. High abundance genera, *Turicibacter, Lactobacillus* and *Prevotella,* differed in abundance among mucosa locations along the tract ($P < 0.05$), and tended to differ by phase ($P < 0.10$). *Lactobacillus* and *Turicibacter* also differed when comparing P1 to P2 in the digesta ($P < 0.05$). *Clostridium* and *Rosburia* had a higher relative abundance in P2 compared with P1 in the cecum and colon digesta ($P < 0.05$). No sex differences were observed in the porcine microbiota. This characterization of the microbiota along the porcine intestine revealed differences in highly abundant populations, likely attributed to by factors including nutrient availability, pH and bile salts of the mucosal environment. Dietary phase, but not sex, significantly affected the composition of the porcine intestinal microbiome. Understanding bacterial distribution along the tract could lead to development of feeding strategies to maximize nutritional output with the aid of a balanced microbiota.

**Key Words:** pyrosequencing, microbiota

**Effects of the interaction between *Entodinium caudatum* and an amino acid-fermenting bacterial consortium on fermentation characteristics and protozoal population in vitro**

T. Park* and Z. Yu,
The Ohio State University, Columbus, Ohio, USA.

Sitting at the top of the food chain in the rumen, protozoa, especially the entodiniomorphs, engulf other members of the rumen microbiome in large numbers, releasing small peptides and amino acids. Results from studies using defaunated sheep suggest that such bacterivory considerably contributes to the excessive intraruminal nitrogen turnover because the peptides/amino acids released from

protozoa can be fermented to ammonia by amino acid-fermenting (AAF) bacteria. However, direct interaction between rumen protozoa and AAF bacteria has not been demonstrated because of the inability to establish axenic cultures of any rumen protozoan. The objective of this study was to evaluate the interaction between *Entodinium caudatum*, the most predominant rumen ciliate species, and AAF bacteria with respect to feed degradation and ammonia production. An *En. caudatum* culture has been maintained by daily feeding with a suspension containing 1.5% ground wheat, 1.0% each of ground alfalfa and grass and transfers every 3 or 4 d. The bacteria and methanogens associated with *En. caudatum* cells were removed by filtration and washed. An AAF bacterial consortium was established by repeated transfer and enrichment on casamino acids as the sole substrate. *En. caudatum* alone (Ec), AAF bacteria alone (AAF), and coculture of *En. caudatum* and AAF bacteria (Ec-AAF) were set up in 3 replicates and incubated at 39°C for 24 h. Digestibility of dry matter (DM) and fiber (NDF), concentrations of VFA and ammonia, pH, and microscopic counts of *En. caudatum* were compared among the 3 cultures. No interaction was noted with respect to DM degradation or *En.* counts, with respect to increased NDF degradation, ammonia production, pH values, and all concentration of all volatile fatty acids except propionic acid. Our results indicate that *En. caudatum* and AAF bacteria could have mutualistic interaction that benefited each other. Research is underway to examine how the interaction affects the population dynamics of the AAF bacteria.

**Key Words:** *Entodinium caudatum*, amino acid-fermenting bacteria, nitrogen turnover, co-culture

**Exploring the transcriptome of rumen protozoa *Entodinium caudatum***

L. Wang*, J. Plank, A. Abu-Doleh, Ü. Çatalyürek, J. Firkins, and Z. Yu,
The Ohio State University.

Rumen protozoa play important roles in degradation of carbohydrates, excessive nitrogen excretion, and $CH_4$ production. However, the physiology and metabolism of rumen protozoa are poorly defined due to lack of axenic cultures of any rumen protozoa. In this study, we performed a transcriptomic study on *Entodinium caudatum* to elucidate its metabolism and physiological features using RNA-seq. The sequences passing quality control were assembled using different k-mers and assembler programs, resulting in 123,490 contigs with an average length of 855 bases. The sequences were annotated using tblastx against GenBank non-redundant protein (NR) database and then using MEGAN5 against the KEGG and COG databases. More than 45,700 transcripts were identified to have homologous hits in the NR database with an average length of 1390 bases. Based on COG classification, genes involved in replication, recombination and repair (category L); posttranslational modification, protein turnover, and chaperones (category O); and translation, ribosomal structure and biogenesis (category J) were the most predominant. Bioinformatic analysis of the contigs revealed 4,788 transcripts involved in metabolism of several substrates, including starch, chitin, xylan, and protein as well as sugar (e.g., glucose, mannose, galactose, and maltose), suggesting that *En. caudatum* probably utilizes starch, chitin, and xylan as primary energy source. Most of the genes encoding the enzymes of the Embden–Meyerhof–

Parnas (EMP) pathway were found. Iron hydrogenase genes were well represented in the transcriptome. Interestingly, gene coding for Cytochrome *b* and c oxidases and superoxide dismutase were also found. About 900 genes were involved in signal transduction pathways, including the mitogen-activated protein kinases (MAPK) pathway, calcium-signaling pathway, phosphatidylinositol signaling system, and PI3K-Akt signaling pathway. Nearly 700 transcripts represent genes involved in amino acid transport and metabolism, with many representing genes encoding protease, peptidase, and aminotransferase. These results provided a better understanding of the basic metabolism and physiological features of *En. caudatum*. The data may be used to identify potential target that can be exploited to manipulate the growth of *En. caudatum.*

**Key Words:** *Entodinium caudatum*, RNA-Seq, metatranscriptome, metabolism, rumen protozoa

**The effect of bovine fecal microbiome on *Escherichia coli* O157:H7 prevalence**

M. Kim[1], L. Kuehn[1], J. Bono[1], E. Berry[1], N. Kalchayanand[1], H. Freetly[1], A. Benson[2], and J. Wells*[1],
[1]ARS/USDA US MARC, Clay Center, NE, USA,
[2]Univ. Nebraska-Lincoln, Lincoln, NE, USA.

The objective of this study was to determine if fecal microbiome would have an association on *E. coli* O157:H7 prevalence. Pyrosequencing analysis of fecal microbiome was performed from feedlot cattle fed 1 of 3 diets: (1) 94 heifers fed low concentrate (LC) diet, (2) 142 steers fed moderate concentrate (MC) diet, and (3) 132 steers fed high concentrate (HC) diet. A total of 2,411,122 high-quality sequences were obtained from 368 samples. In the LC diet group, 402,080 of the total sequences were recovered from 94 fecal samples and classified to 29 phyla. *Firmicutes*, candidate division TM7, *Bacteroidetes*, *Tenericutes*, *Proteobacteria*, *Verrucomicrobia* and *Actinobacteria* were core measurable phyla detected across almost all the 94 fecal samples. In the MC diet group, 1,035,186 of the total sequences were recovered from 142 fecal samples and classified to 22 phyla. *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Tenericutes*, TM7, *Cyanobacteria* and *Actinobacteria* were core measurable phyla detected across almost all the 142 samples. In the HC group, 973,856 of the total sequences were recovered from 132 fecal samples and assigned to 26 phyla. *Firmicutes*, *Bacteroidetes*, *Tenericutes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria* and TM7 were core measurable phyla detected across almost all the 132 samples. The 2,411,122 sequences were clustered into 322,585 OTUs at 97% sequence similarity. Stepwise regression with backward elimination indicated that 6, 15 and 10 OTUs brought significant information to each model ($P < 0.0001$) and explained 29, 51 and 33% of *E. coli* O157:H7 prevalence for each diet group, respectively. Research efforts would need to be made to isolate and characterize species corresponding to these OTUs correlated with *E. coli* O157:H7 prevalence. USDA is an equal opportunity provider and employer.

**Key Words:** cattle, *Escherichia coli* O157:H7, microbiome, diet

**Carbohydrate utilization by *Clostridium scindens*, a key bile acid-dehydroxylating anaerobe of the human gut microbiome**

A. L. Allen*, E. L. Springer, and S. L. Daniel,
Eastern Illinois University, Charleston, IL, USA.

The primary bile acids chenodeoxycholic acid and cholic acid are converted, via 7α-dehydroxylation, to the toxic secondary bile acids lithocholic acid and deoxycholic acid, respectively, by *Clostridium scindens*, an anaerobe that resides in the human gut. Interestingly, other than bile acid dehydroxylation, little is known about the physiology of *C. scindens*. The goal of our study was to determine the types of carbohydrates that support growth and bile acid-dehydroxylation by *C. scindens. C. scindens* ATCC 35704 was maintained in an anaerobic defined medium (DM; minerals, vitamin mix [*p*-aminobenzoate, biotin, cyanocobalamin, folate, lipoate, nicotinate, pantothenate, pyridoxal, riboflavin, thiamine], amino acid mix [alanine, arginine, asparagine, aspartate, cystine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine], metals, bicarbonate, 100% $CO_2$ gas phase, cysteine) at 37°C. Anoxic, sterile (autoclaved) stock solutions of monosaccharides (glucose, fructose, mannose, galactose, rhamnose, sorbose, arabinose, ribose, xylose), sugar alcohols (adonitol, dulcitol, erythritol, glycerol, inositol, lactitol, mannitol, sorbitol, xylitol), disaccharides (cellobiose, lactose, lactulose, maltose, melibiose, sucrose, trehalose), trisaccharides (melezitose, raffinose), and complex polysaccharides (glycogen, dextrin, inulin, mucin, pectin, polydextrose [Litesse], stachyose, starch), glycosides (esculin, salicin), or artificial sweeteners (saccharin, sucralose) were added to DM to final concentrations of 0.1 or 0.25%. No growth was observed in DM alone. Of the 39 substrates tested, 6 monosaccharides (glucose, fructose, mannose, galactose, ribose, xylose), 2 sugar alcohols (dulcitol, sorbitol) and one disaccharide (lactose) supported the growth of *C. scindens*. None of the trisaccharides, complex carbohydrates, glycosides, or artificial sweeteners examined were growth supportive. These findings suggest that *C. scindens* is limited in scope relative to its saccharolytic activities. Future studies will address the types of products formed during sugar metabolism and how fermentation is energetically coupled to bile acid dehydroxylation by this important gut anaerobe.

**Key Words:** dehydroxylation, secondary bile acids, gallstones, colon cancer

**Isolation and Identification of Cellulolytic and Xylanolytic Bacteria from the Gut of Subterraneum Termites**

I. Batool*[1], A. Biddle[2], R. Mackie[2], and M. Gulfraz[1],
[1]PMAS Arid Agriculture University, Rawalpindi, Pakistan,
[2]University of Illinois, Urbana-Champaign, USA.

The world faces an energy crisis in relation to global warming and energy security so there is a need to develop renewable energy and next-generation biofuels as a partial solution to the problem. Some of the challenges for the commercial application of the advanced technologies include pretreatment methods and the development of low cost enzyme production. Discovery of novel enzymes with high catalytic efficiency could be provided by microorganisms from the termite gut with

enzyme systems for key physiological functions such as cellulose and hemicelluloses digestion that make up the plant cell wall. In the present study, we investigated the aerobic and facultative anaerobic bacteria in the subterranean (*Coptotermes* sp.) termite gut native to Pakistan. It is an aggressive, invasive termite genus that causes a lot of damage to houses and wooden structures. The bacteria were isolated from termite gut on selective media with carboxymethyl cellulose (CMC) or xylan as the sole carbon source incubated at 30°C. After purification, the isolates were identified based on phylogenetic analysis of full-length 16S rRNA gene sequences. The results showed that the bacteria belonged to the families *Bacillaceae*, *Enterobacteriaceae*, and *Micrococcaceae*. Their cellulolytic and xylanolytic activity on CMC or xylan-containing medium was also studied. Bacteria from the family *Bacillaceae* showed higher digestion of natural fiber substrates compared with the other bacteria tested. Enzymes from isolates with the highest cellulolytic and xylanolytic activities will be purified and assessed in future studies for their use in hydrolysis of agricultural waste.

**Key Words:** termite, cellulose, 16S rRNA, *Bacillaceae*

**Diet–microbe co-metabolic interactions in wild primates reveal clues on human evolution**

A. Gomez*[1,6], K. Petrzelkova[3], J. Rothman[2], C. Yeoman[4], K. Nelson[5], R. Stumpf[6], B. Wilson[6], D. Modry[3], B. White[6], R. Blekhman[1], and S. Leigh[7],
[1]University of Minnesota, St. Paul, Minnesota, USA,
[2]Hunter College of CUNY, New York, New York, USA,
[3]University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic,
[4]Montana State University, Bozeman, Montana, USA,
[5]The J. Craig Venter Institute, Rockville, Maryland, USA,
[6]University of Illinois, Urbana, Illinois, USA,
[7]University of Colorado, Boulder, Colorado, USA.

Recent advances in microbiome studies and meta-“omics” have offered new molecular insights into how host-microbe systems affect mammalian physiology. Here, we test the hypothesis that the molecular characterization of diet–gut microbe interactions in wild primates also provides valuable information on the factors that triggered human evolution. Thus, we use a longitudinal approach, along with high throughput sequencing and metabolomics to characterize the gut microbiome and metabolomes in 356 fecal samples of *Cercocebus agilis, Gorilla beringei*, *Gorilla gorilla*, *Pan troglodytes schweinfurthii*, and *Homo sapiens*. Our results show that the diet–microbe co-metabolic landscape of wild primates converges with that of humans when foraging is focused on increased energy harvest; specifically, as far as predominance of microbes and metabolites involved in simple sugar processing and lipid turnover. As such, we present evidence that primitive dietary shifts to foods with high contents of readily usable energy not only prompted the acquisition of specific morphological and anatomical adaptations in human ancestors, but also of a colonic micro-ecosystem with increased capacity to harvest energy from food. Accordingly, these results offer a novel perspective on the ecological and dietary triggers behind the origin of early humans, showing that specific gut microbiome arrangements could have contributed significantly to this process.

**Key Words:** evolution, microbiome, metabolomics, diet, primates

**Pangenome analyses of *Methanosphaera* species**

E. Hoedt*, P. Ó. Cuív, and M. Morrison,
University of Queensland, Brisbane, QLD, Australia.

The genus *Methanosphaera* comprises a relatively small number of cultured isolates from the gastrointestinal tracts of mammals, which has been augmented with *rrs* gene sequences, principally derived from surveys of the gut microbiota from other animals. This limited biotic representation of the genus justified our isolation of strains from the macropodid (strain WGK6) and the bovine (strain BMS) for comparison with the type strain *Methanosphaera stadtmanae* DSMZ3091, isolated from human stool. The WGK6 and BMS genomes have been sequenced using the 454 GS-FLX and PacBio RS2 “continuous long read” platforms, respectively; which affords us an opportunity to compare these 3 genomes, coupled with verification of any differences via biochemical and molecular-based assays. We have used MAUVE to show there is a very high level of gene organization (synteny) across the 3 isolates. Our annotations further confirm that all 3 strains have retained similar repertoires of genes to support respiratory and transport functions, in addition to methanol utilization and its conversion to methane. Similarly, our analysis of “predicted highly expressed” (PHX) genes also suggests the strains are very similar and for these reasons, we believe the “core genome” of *Methanosphaera* spp. is close to being fully determined. However, each strain does possess an “accessory genome” of varying size and content, which might separate the membership of this genus into no less than 2 different “phylogroups.” As an example, neither the BMS nor the DSMZ3091 genomes possess homologs of the 2 dehydrogenase genes now shown to support ethanol utilization by WGK6 (for methanol reduction to methane) and this has been confirmed by culture-based studies. Interestingly, the strains also appear to possess only a limited collection of genes annotated to encode functions that might support lateral gene transfer, suggesting the genomic differences might not be the result of such events.

**Key Words:** rumen, bovine, pangenome, *Methanosphaera*

**Neonatal treatment effects of garlic oil on the microbial ecosystem in the intestinal tract of Hanwoo calves**

J. Song[1,6], G.-S. Bae[2], J. Park[3], J. Manhlapig[1,5], J. N. Kim[4], E. J. Kim[6], C. H. Kim[7], S. Seo[5], and J. Chang*[1],
[1]Korea National Open University, Seoul, Korea,
[2]Chung-Ang University, Ansung, Korea,
[3]Stronghold Tech. Inc, Seoul, Korea,
[4]Easyfarm Inc., Cheonan, Korea,
[5]Chungnam National University, Daejeon, Korea,
[6]Kyungpook National University, Sangju, Korea,
[7]Hankyong National University, Ansung, Korea.

Microbial ecosystems in the gastrointestinal tract of ruminant are known to be established within 10 d after birth. After establishment, it is difficult to change the

microbial population to increase productivity of production animals due to its resistance to the long-term exposure of environmental stressors such as chemicals or phytochemicals. For this reason, early control of microbial ecosystem during the establishment stage of microbial ecosystem has been suggested as an effective option. In this study, the treatment effect of garlic during neonatal stage on the establishment of rumen and intestinal microbial ecosystem in Hanwoo calves was tested. Nineteen Hanwoo cows were synchronized for estrous and artificially inseminated using semen from one bull to minimize genetic variation. From 2 d after delivery, 9 calves were orally drenched with 20 mL of milk containing 200 μL of garlic oil for 7 d. Ten, 20, 40, 70, 110, 180 d after the first feeding of garlic oil, fecal samples were collected from the rectum of calves. Rumen fluid from 19 calves was collected after 6 mo of age by stomach tube. The microbial genomic DNAs were extracted both from rumen and fecal samples. For quantification of individual microbes real time PCR was performed and copy numbers of DNA were absolutely quantified. For detailed profiling of microbial population, 16S rDNA regions of bacterial genomes were amplified and analyzed using a GS-FLX next-generation sequencer. The microbial profile differences between cow and calf were detected with differences in ruminal microbial populations being more affected by animal-to-animal variation more than by garlic treatment. In addition, the population of methanogens in the rumen was not changed by garlic treatment during early in life. In this study, garlic oil was mixed with milk and orally administered to calf and it most likely flowed directly into abomasums through reticulo-omasal groove. Moreover, only 7 d of administration might not be long enough to eliminate methanogen from rumen ecosystem. For those reasons, the microbial population in the rumen was not greatly affected by garlic oil. However, some microbial populations in hindgut were changed with garlic treatment and the change was maintained for 6 mo after birth.

**Key Words:** neonatal, Hanwoo, microbial ecosystem, rumen

**Comparative genomic and transcriptomic analysis of *Ruminococcus albus* strains 7 and 8 grown on complex and defined substrates**

I. H. Kwon*[1], I. K. Cann[2,3], and R. I. Mackie[1,3],
[1]Department of Animal Sciences, University of Illinois, Urbana, IL, USA,
[2]Department of Animal and Microbiology, University of Illinois, Urbana, IL, USA,
[3]Institute for Genomic Biology, University of Illinois, Urbana, IL, USA.

*Ruminococcus albus* specializes in the ruminal degradation of both cellulose and hemicellulose. However, depending on strain, their phenotypic and fibrolytic abilities are variable and little information is available on genomic and transcriptional differences that underlie this phenotypic variation between strains. The regulation of genes involved in degradation of complex polysaccharides in both strains grown on either alkaline hydrogen peroxide treated corn stover (AHPCS), cellobiose, phosphoric acid-swollen cellulose (PASC), or wheat arabinoxylan (WAX) were compared using RNA sequencing approaches. Both strains demonstrated similar growth rates, substrate degradation, and accumulation of fermentation end products when grown on defined substrates. Although both strains harbor a similar repertoire of putative GH genes, both strains had different transcriptional profiles of GH genes on complex substrate as well as defined substrates. *R. albus* 8 had more

upregulated GH genes than *R. albus* 7 when grown with WAX relative to cellobiose, whereas *R. albus* 7 had more upregulated GHs genes than *R. albus* 8 when grown with PASC relative to cellobiose. However, when grown on AHPCS, *R. albus* 7 degraded cellulose and xylan faster than *R. albus* 8. During growth on AHPCS, both strains exhibited a different pattern, depending on growth stage, of hemicellulose and cellulose utilization genes, including glycoside hydrolase (GH), sugar transporter, and central metabolic pathway genes. Consistently, both strains had different expression levels of orthologous GH genes during growth on AHPCS, suggesting that these 2 strains have different strategies of substrate utilization and indicate that each strain has different niche in the same ecosystem.

**Key Words:** *Ruminococcus albus*, cornstalk, cellulose, hemicellulose, transcriptome

**Gene expression of *Streptococcus bovis* JB1 in response to monensin**

A. S. Biddle*[1], A. Klieve[2], I. K. O. Cann[1], and R. I. Mackie[1],
[1]University of Illinois, Urbana, Illinois, USA,
[2]University of Queensland, Queensland, Australia.

The ionophore monensin has been used worldwide since 1971 as a highly effective means to increase feed efficiency of cattle. It is known to enhance propionate production and prevent conditions such as bloat and lactic acidosis. Its use as a growth promoter has been banned in the European Union since 2006, prompting interest in the development of alternatives. In bacteria with a gram-positive cell wall such as *Streptococcus bovis* JB1, monensin has been shown to dissipate ion gradients (particularly $Na^+$). Previous work has shown that although treated cells do not grow, they are still able to ferment glucose and produce ATP, an adaptation in which growth is decoupled from energy generation. The only known resistance mechanism for an ionophore antibiotic thus far is an ATP-dependent efflux system found in *Streptomyces longisporoflavus* against tetronasin, which it produces. To understand the metabolic response of *S. bovis* JB1 to monensin, continuous cultures grown in glucose-limited medium at either 2 or 4 turnovers per day were switched to monensin-treated medium at the same retention times. Metabolic products measured at time points by HPLC demonstrated that glucose fermentation to lactate continued unabated despite a rapid decline in cell growth. RNA-Seq analysis identified a disruption of purine metabolism in particular as a potential source of growth related damage. Most interestingly was the upregulation of several clusters of multidrug ABC transporters, metal ion transporters, penicillin-binding proteins, bacteriocin and bacitracin transporters, and genes related to peptidoglycan synthesis. These responses to monensin could provide clues for mechanisms of adaptation and potential resistance to ionophores, but also provide useful targets for the design of alternatives to monensin.

**Key Words:** *Streptococcus bovis* JB1, RNA-Seq, monensin

**Gene expression of *Streptococcus bovis* JB1 in batch and continuous culture at high and low growth rates**

A. S. Biddle*[1], A. Klieve[2], I. K. O. Cann[1], and R. I. Mackie[1],

[1]University of Illinois, Urbana, Illinois, USA,
[2]University of Queensland, Queensland, Australia.

*Streptococcus bovis* is a starch-degrading ruminal bacterium important in the formation of lactic acid during the onset of lactic acidosis. In batch culture, this anaerobe rivals most aerobes with a maximum specific growth rate of 1.47 generations/h, yet in the rumen under normal conditions, *S. bovis* JB1 maintains a moderate, constant density of about $10^7$ cells/mL. Under conditions of excess glucose, during maximal growth, *S. bovis* JB1 produces primarily lactate, despite the gain of less ATP per glucose, maximizing ATP yield per unit of time. During slower growth, *S. bovis* JB1 produces more formate, acetate, and ethanol, and gains more ATP from these fermentations, maximizing ATP yield per unit of glucose. To understand the metabolic differences of *S. bovis* JB1 at different growth rates, continuous cultures were grown in glucose-limited medium at either 2 or 4 turnovers per day in a crossover study design and in comparison with batch cultures. Metabolic products measured at time points by HPLC demonstrated the shift to higher lactate and lower formate production at the higher growth rates. RNA-Seq analysis confirms previous in vivo studies to measure the expression of key metabolic genes such as lactate dehydrogenase and pyruvate formate lyase under batch and chemostat conditions, and identifies differences in specific ABC transporters and transcriptional regulators such as phosphotransferase system HPr, *DoeR* and *LacI* that may be responding to the change in growth conditions. Identifying the mechanisms behind the shift in metabolism for *S. bovis* JB1 between slow and fast growth conditions may enable the design of more targeted strategies for the prevention and treatment of lactic acidosis.

**Key Words:** *Streptococcus bovis* JB1, RNA-Seq, lactic acidosis

**Cultivation studies on the gastrointestinal tract of an indigenous Peruvian community yields several novel bacterial taxa**

N. Patel*[1], R. Tito[2], A. Obregón-Tito[2], O. Trujillo-Villaroel[3], L. Marin-Reyes[4], L. Troncoso-Corzo[2], E. Guija-Poma[2], C. Lewis Jr.[5], and P. Lawson[1],
[1]University of Oklahoma-Department of Microbiology & Plant Biology, Norman, Oklahoma, USA,
[2]Universídad Científica del Sur, Lima, Peru,
[3]Centro Nacional de Salud Intercultural, Instiuto Nacional de Salud, Lima, Peru,
[4]Centro Nacional de Salud Publica, Instituto Nacional de Salud, Lima, Peru,
[5]University of Oklahoma-Department of Anthropology, Norman, Oklahoma, USA.

Although there are examples of studies focused on the human gut microbiome of individuals from Western populations, indigenous populations with “non-Western” diet and lifestyles are underrepresented. To truly determine if there is a core human microbiome, individuals with a variety of diets and geographic regions also need to be included in these investigations. The primary purpose of this study was to test the hypothesis that traditional communities from remote regions harbor novel microorganisms influenced by diet, health, and environmental conditions. We used rRNA-based road maps generated in our laboratories to target previously uncultivated bacterial groups to investigate their phylogenetic, physiological,

biochemical, and chemotaxonomic properties. Freshly voided fecal samples were collected from members of the Afro-Peruvian community of Cruz Verde in Tambo de Mora, region Ica, in Peru. Multiple enrichments using an array of substrates were constructed and inoculated into a fecal slurry. All isolates recovered from the enrichments were maintained on blood agar plates and were screened using 16S rRNA gene sequence analysis. Several isolates yielded relatively low sequence similarity values to those in DNA databases; phylogenetic tree topologies demonstrated that several isolates belonged to a group of organisms known as the anaerobic gram-positive cocci. The nearest relatives included members of the genera *Peptoniphilus*, *Finegoldia*, *Gallicola*, and *Parvimonas*. To date, our studies have identified 2 novel genera and a new species belonging to the genus *Peptoniphilus* recovered from a single individual. Our investigations demonstrate that remote indigenous communities harbor novel microbial taxa and further studies employing culture-based approaches of human gut microbiomes of diverse communities are encouraged to augment the insights provided by molecular investigations. Cultivation and characterization of novel organisms from these unique communities will help to gain a deeper understanding of ecological and functional diversity of the gastrointestinal tract of indigenous communities.

**Key Words:** microbiome, taxonomy, anaerobe, gastrointestinal, indigenous communities

**New ways of looking at enteric phage populations: Viral metagenomes of cattle, sheep, and kangaroos**

R. Gilbert*[1,2], S. Godwin[4], L.-M. Gulino[1,2], M. Kienzle[1], D. Ouwerkerk[1,2], and A. Klieve[1,3],
[1]Department of Agriculture and Fisheries, Brisbane, Queensland, Australia,
[2]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane, Queensland, Australia,
[3]School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia,
[4]Department of Primary Industries, Parks, Water and Environment, Launceston, Tasmania, Australia.

Viruses of prokaryotes (phages) are known to naturally occur in dense populations in the intestinal tract of herbivores, infecting and actively replicating within the enteric bacterial populations. Developments in high throughput sequencing have enabled these viral populations to be characterized on an unprecedented scale, revealing extensive viral genetic diversity. In this study the viral fraction of rumen fluid was prepared from cattle (*Bos taurus* fed a high grain diet and *Bos indicus* × *Bos taurus* grazing a pasture diet), sheep (Merino and Merino cross grazing an improved pasture diet) and native Eastern Grey kangaroos (*Macropus giganteus* grazing native and improved pasture). Viral DNA prepared from a total of 18 animals was sequenced using either 454-pyrosequencing or Illumina MiSeq sequencing platforms. Analysis of sequencing data revealed that although all of the viral metagenomes were dominated by extensive populations of tailed phages, classified within the viral order Caudovirales, the relative abundance of the 3 tailed phage families, the Siphoviridae, Myoviridae, and Podoviridae differed between individual viral

metagenomes. The majority of sequences that could be assigned taxonomically were related to phages known to infect bacterial species normally found in fecal and other gut-associated microbial ecosystems, such as *Lactobacillus*, *Lactococcus*, *Clostridium*, and *Bacteroides*. The animal host species appeared to have the greatest effect on the composition and relative abundance of the phage populations. Differences in the composition and relative abundance of phage populations occurred to a lesser extent between individual host animals, with pasture-fed cattle having the most similar populations. Comparisons with previously published viral metagenomes sourced from Holstein dairy cows also indicated that herbivore host species was a factor in determining phage community composition. The effect of diet and microbial population was also explored, with major differences in the gut microbial populations corresponding to differences in the observed phage populations. This study has utilized new technologies to provide novel insights into the nature of the enteric phage populations of domesticated ruminants (cattle and sheep) and native Australian macropods.

**Key Words:** ruminant, phage, viral, metagenome, kangaroo

**Rational media design for the isolation of rare bacterial taxa from the Synergistetes phylum**

C. Davis[1], M. Bruschi[1], D. Ouwerkerk[1,2], R. Gilbert*[1,2], and A. Klieve[3,1],
[1]Department of Agriculture and Fisheries, Brisbane, Queensland, Australia,
[2]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane, Queensland, Australia,
[3]School of Agriculture and Food Science, University of Queensland, Gatton, Queensland, Australia.

Much of what we currently know about the microorganisms that inhabit the gut has come from studies on pure cultures. However, with the rapid expansion of next-generation sequencing, organisms that were once termed “uncultured” have become synonymous with “unculturable.” Nonetheless, many of the current molecular techniques cannot detect rare or minor microbial populations, and these can unarguably play important roles in any gut ecosystem. *Synergistes jonesii* is a member of the widely distributed phylum, Synergistetes, which is ubiquitous to gastrointestinal ecosystems. *S. jonesii* was isolated over 20 years ago and found to degrade toxic plant-derived alkaloids. In Australia, it is introduced into ruminants to allow them to graze the fodder tree *Leucaena leucocephala*, which would otherwise be toxic to them. In this study, we used rational media design to develop a selective medium for the isolation of novel isolates from the phylum Synergistetes. Several antimicrobial-resistance and metabolic traits were identified from in-house genomic data and published literature sources. A complex medium containing protein hydrolysates was developed to select for indicative traits, and various antimicrobials added to suppress the growth of non-target organisms. Plating of a rumen-derived fermentor inoculum directly onto selective medium, under anaerobic conditions, led to the exclusive isolation of novel strains representing 3 Synergistetes taxa. These included a new strain of *Synergistes jonesii*, and novel strains of *Cloacibacillus* sp. and *Pyramidobacter* sp. Combining genome sequence data and rational media

design enabled the development of new methodology for isolating members of this under-represented taxa.

**Key Words:** culturomics, *Synergistes*, Synergistetes, rumen

**Session: Nutrition and metabolism of livestock, humans, and companion animals**

## Feeding urea molasses block on productive and reproductive performance of crossbred dairy cows under smallholder village farm conditions in Bangladesh

M. A. S. Khan* and M. A. H. Sarker,
Department of Dairy Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

The smallholder farmers of Bangladesh do not feed their dairy cattle adequately. Most fibrous feeds available to animals are deficient in some nutrients and are poor in digestibility. Straw is the most important crop residue contributing more than 90% of the basal dry matter to the dairy cattle. The nutritional limitation of straw could be overcome by physical and chemical treatments or supplementation with specific nutrients to provide an optimum ruminal condition for rumen microorganisms. Urea molasses block (UMB) supplementation with rice straw based diet, increases microbial growth by altering in rumen ammonia concentration. Ammonia together with other microbial growth factors (sulfur and trace elements) in the blocks promotes rumen microbial growth. UMB provides additional N and $NH_3$ level in the rumen, which makes a favorable condition for growth of rumen microbes; as a result, it helps in the improvement of digestibility of straw. The increased microbial population in the rumen of animal supply increased microbial protein in the lower part of the digestive system of the animal. Feeding of UMB together with rice straw has been found to be a satisfactory method of improving digestibility of straw, which in turn improves the productive and reproductive performance of crossbred dairy cows. Accordingly, UMB were given to 108 Holstein Friesian crossbred cows at the rate of 0 ($T_0$), 350 ($T_1$), 500 ($T_2$), 650 ($T_3$) g/h/d under small holder farm condition. The difference of milk yield between $T_2$ (5.95 kg/h/d) and $T_3$ (5.99 kg/h/d) was insignificant ($P > 0.05$). Milk fat increased linearly with the increasing levels of UMB, but it was same in $T_2$ and $T_3$ (47.1 g/kg). Reproductive intervals were decreased with the increasing levels of UMB and insignificant ($P > 0.05$) differences between $T_2$ and $T_3$ for all parameters were found. Feed intake, milk yield, body weight gain of cows, and growth rate of calves increased linearly with the increasing level of UMB. These increased productions were due to the better manipulation of rumen microbes. From the above results, the $T_2$ group cows, those that received 500 g/h/d UMB, considered as the best among 3 supplemented groups. Therefore, 500 g/d/h UMB was the optimum amount for crossbred dairy cows to earn maximum benefit.

**Key Words:** UMB, rumen microorganisms, milk, body weight, nitrogen

**Informing ulcerative colitis pathophysiology and outcomes through metabolic profiling**

S. C. M. Fong[1], C. I. Le Roy[2], S. P. Claus*[2], and J. D. Sanderson[1,3],
[1]IBD Centre, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom,
[2]Department of Food and Nutritional Sciences, University of Reading, Reading, United Kingdom,
[3]Diabetes and Nutritional Sciences Division, King's College London, London, United Kingdom.

Ulcerative colitis (UC) is a chronic gastrointestinal disorder involving a complex interplay between host genetics, mucosal immunology, gut microbiome, and the environment. A complete understanding of etiology and biomarkers predicting outcome are lacking. We used high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy to generate metabolic profiles from intact colonic biopsies from UC patients and controls to inform us about disease pathophysiology and severity. Colonic tissue biopsies were acquired from 20 individuals (10 UC patients and 10 controls who had normal mucosa on colonoscopy) at the time of routine colonoscopy. Metabolic signatures were obtained by HR-MAS NMR followed by multivariate pattern-recognition analysis to investigate differences between cohorts. Two dimensional correlation spectroscopy (COSY) was performed to identify metabolites. Principal component analysis (PCA) identified one outlier, which was excluded from further analysis. Orthogonal projection to latent structures analysis (OPLS) was applied to remaining cases and distinguished UC and control cohorts with significant predictive accuracy. The OPLS model supported good fit (R2Y 0.8362) and predictability (Q2Y 0.5026) parameters. The data analysis showed that colonic biopsies from UC patients contained relatively higher levels of antioxidants (ascorbate) and ethanolamine compared with those obtained from controls. A novel finding was that the model was capable of stratifying the samples based on severity (mild, moderate or severe), which has not been demonstrated in previous metabonomic studies on colonic samples of ulcerative colitis. HR-MAS NMR based metabolic profiling has identified distinct differences in UC patients and controls, in particular increased levels of antioxidants and amino acids, which may be required in greater quantities during catabolic conditions such as UC. This study shows the utility of HR-MAS NMR in exploring diagnostic possibilities, disease severity stratification and generating a better understanding of UC pathophysiology.

**Key Words:** metabonomics, magnetic resonance spectroscopy, inflammatory bowel disease

**Effects of the dose and viability of *Saccharomyces cerevisiae* on the ruminal bacterial population and fermentation and the performance of lactating dairy cattle**

Y. Jiang*[1], R. M. Martins[2], I. M. Ogunade[1], W. Rutherford[3], S. Qi[3], F. Owens[3], B. Smiley[3], K. Arriola[1], C. Staples[1], and A. T. Adesogan[1],
[1]Department of Animal Science, University of Florida, Gainesville, Florida, USA,
[2]Animal Science Department, Federal University of Vicosa, Vicosa, Minas Gerais, Brazil,
[3]DuPont Pioneer, Johnston, IA, USA.

The objective was to examine the effect of the dose and viability of supplemental *Saccharomyces cerevisiae* (SC) on ruminal fermentation, microbial diversity, and performance of lactating dairy cows. Four ruminally cannulated lactating Holstein cows in late lactation (284 ± 18 DIM) were assigned randomly to 4 treatments arranged in a 4 × 4 Latin square design with 4, 21-d periods. Cows were fed a total mixed ration supplemented with no yeast (control, CON) or with a low dose of live yeast ($5.7 \times 10^7$ cfu/d; LLY), a high dose of live yeast ($6.0 \times 10^8$ cfu/d; HLY), or a high dose of killed yeast ($6.0 \times 10^8$ cfu/d before boiling at 80°C; HDY). Ruminal pH and temperature were measured with indwelling probes. Ruminal fluid was collected 0, 2, 4, 6, 8 and 10 h after the morning feeding on d 21 for analysis of volatile fatty acids and ammonia-N. Microbial diversity was analyzed by high throughput sequencing of the V4 region of the 16S rRNA gene. In vivo digestibility was measured using chromic oxide as a marker. Supplemental LLY increased milk yield, milk fat and protein yields, feed efficiency, and in vivo apparent digestibility of dry matter and neutral and acid-detergent fiber. Feeding HLY instead of LLY decreased milk yield and feed efficiency. Treatments had no major effects on concentrations of ruminal ammonia-N, pH, or temperature, but killed yeast decreased the minimum ruminal pH. *Prevotella* were the most prevalent bacteria in the solid and liquid phases of the ruminal fluid followed by *Fibrobacter* and *Succinivibrionaceae*, respectively. Supplemental LLY increased the prevalence of both *Butyrivibrio* and *Ruminococcus* spp. in the liquid phase. Supplementing with live yeast instead of dead yeast increased DM digestibility, increased the prevalence of *Ruminococcus* in both liquid and solid phases and decreased proportions of *Succinivibrionaceae* and *Ruminobacter* in the liquid phase. Supplemental killed yeast decreased the prevalence of *Lachnospiraceae* and *Coprococcus* in the solid phase and increased those of *Ruminobacter* and *Porphyromonadaeae* in both solid and liquid phases. Overall, the treatments altered the ruminal microbial composition, but only the low dose of live yeast increased milk production and feed efficiency.

**Key Words:** yeast, dairy cow, ruminal bacteria

**Amylolytic bacteria in the equine hindgut: Effect of starch source and a case for antimicrobial-mediated competition**

A. E. Harlow*[1], L. M. Lawrence[1], and M. D. Flythe[2,1],
[1]Department of Animal and Food Sciences, University of Kentucky, Lexington, KY, USA,
[2]USDA, Agricultural Research Service, Forage-Animal Production Research Unit, Lexington, KY, USA.

Cereal grains are often included in equine diets. A high proportion of grain in the diet can allow starch to reach the hindgut where bacteria compete for the substrate, produce lactic acid, and decrease pH. The ecological theory of niche predicts that competition for a resource will negatively affect one or more of the competing organisms. The hypothesis of an earlier study was that starch introduction would cause a source-dependent press disturbance in the equine fecal microbial community, most notably in resident starch-utilizing (SU) bacteria. Feces were collected from horses, and cell suspensions were prepared by differential centrifugation and re-suspension in medium with ground corn or oats. SU, Lancefield

group D gram-positive cocci (GDGPC), and lactobacilli were enumerated after 24 h. Corn had >$10^4$-fold more SU, >10-fold more GDGPC, and >100-fold fewer lactobacilli than oats. Isolates from the highest dilutions of SU were identified by their 16S RNA gene sequence as *Enterococcus faecalis* and *Streptococcus bovis* in corn and oats, respectively. In an in vivo experiment, 10 horses were assigned to a corn (n = 5) or an oats diet (n = 5). Horses were gradually adapted to their final starch intake (3 g/kg BW/d) for 14 d. After 13 d, corn horses had $10^4$-fold more SU, >10-fold more GDGPC, and >10-fold fewer lactobacilli than oat horses. The predominant SU isolates from corn horses were again *E. faecalis*. Both experiments identified a negative correlation between lactobacilli and SU, indicating a potential competition between these bacteria for starch (r = −0.94, in vitro; r = −0.97, in vivo). The next experiment was conducted to determine if a *Lactobacillus* addition would mitigate SU proliferation, specifically GDGPC, with corn fermentation. The in vitro experiment was conducted as described above with ground corn ± live or dead (autoclaved) *L. reuteri*. The addition of live or dead *L. reuteri* decreased SU and GDGPC by >$10^4$- and 100-fold, respectively. To identify the mechanism of action, an energized *E. faecalis* isolate was co-incubated with dead *L. reuteri* cells or supernatant. The supernatant depleted the intracellular $K^+$ of *E. faecalis* in less than 10 min of exposure. This result demonstrates that one aspect of competition between lactobacilli and enterococci could be a membrane-active antimicrobial.

**Key Words:** acidosis, streptococci, carbohydrate

**Yeast with surface displayed xylanase as a new dual-purpose delivery vehicle of xylanase and yeast**

J. Wang*[1], B. He[1], W. Du[1], Y. Luo[1], Z. Yu[2], and J. Liu[1],
[1]Institute of Dairy Science, Zhejiang University, Hangzhou 310058, China,
[2]Department of Animal Sciences, The Ohio State University, Columbus, USA.

This study aimed to develop a yeast strain that surface displays a xylanase that was found from a rumen fosmid library and optimized by directed evolution (Orf6-$un_m$). Using *Saccharomyces cerevisiae* EBY100 as host, Orf6-$un_m$ was successfully displayed on its cell surface, yielding a specific xylanase activity of 136.8 U/g dry cells. The surface displayed Orf6-$un_m$ (referred to as YSD-Orf6-$un_m$) had optimal pH of 7 and optimal temperature of 50°C. Compared with the purified Orf6-$un_m$ from *E.coli*, YSD-Orf6-$un_m$ produced more xylose and had increased xylolytic activity. The yeast EBY100 with YSD-Orf6-$un_m$ (referred to as EBY100-pYD1-*orf6-un*$_m$) was evaluated for its effect on digestion and fermentation of corn stover using an in vitro experiment with 3 treatments: control without yeast addition, yeast EBY100, and EBY100-pYD1-*orf6-un*$_m$. Both EBY100 and EBY100-pYD1-*orf6-un*$_m$ increased concentration of total volatile fatty acids, acetate:propionate ratio, gas production, dry matter degradation, and total bacteria population, while shortening lag time of gas production. However, EBY100-pYD1-*orf6-un*$_m$ increased gas production and dry matter degradation, and shortened the lag time to greater magnitudes than EBY100. Supplementation of EBY100-pYD1-*orf6-un*$_m$ had little effect on the activities of cellulase or xylanase present in the liquid fraction of the in vitro fermentation cultures. EBY100-pYD1-*orf6-un*$_m$ may be used as a dual-purpose vehicle to deliver xylanase with live yeast to feed animals.

**Key Words:** ruminal fermentation, microbial population, xylanase, yeast surface display

**Early supplementation of alfalfa to starter diets altered electrophysiological properties and permeability of the gastrointestinal tracts in growing lambs**
B. Yang, S. Wang, B. He, J. Liu, and J. Wang*,
Institute of Dairy Science, Zhejiang University, Hangzhou, Zhejiang, China.

To investigate the effects of early supplementation of starter pellets with alfalfa in pre-weaning period on the permeability, expressions of tight junction proteins and cytokines of gastrointestinal tract during the pre- and post-weaning period, 6 of 66 male Hu lambs at the age of 10 d were slaughtered as control, and the other 60 lambs were randomly allocated to 2 dietary treatments: milk replacer and starter pellets (STA) without or with free-choice provision of chopped alfalfa (S-ALF). The animals were offered 300 g/d concentrate mixture and had free access to alfalfa after weaning at the end of wk 4 (age of 38 d). Data of tissue resistance and conductance showed that alfalfa supplementation increased or decreased permeability of ruminal epithelium and duodenal mucosa to some extents in pre- or post-weaning periods, respectively, whereas plasma concentrations of D-lactic acid, lipopolysaccharides, and immune globulin, and the morphological appearance of duodenum were not different between 2 treatments. Compared with the STA group, alfalfa supplementation increased the ruminal occludin expression, enhanced the duodenal expression of claudin-1 and occludin, and enhanced ileal expression of claudin-1 and claudin-4 during the pre-weaning period; alfalfa supplementation inhibited the weaning-caused compensatory increase in ruminal epithelial expression of claudin-1 and claudin-4. The change in ruminal expression of TNF-$\alpha$ with weaning was similar to claudin-1 and claudin-4. In summary, early supplementation of alfalfa to starter diet could maintain the normal function of the gastrointestinal barrier and help to relieve the stress from weaning and feed transition in growing lambs.

**Key Words:** young ruminant, alfalfa, weaning, permeability, gastrointestinal tract

**Early supplementation of alfalfa to starter diets altered electrophysiological properties and permeability of the gastrointestinal tracts in growing lambs**
A. Garcia*, C. Ariza, T. Rodriguez, and O. L. Mayorga,
Corporacion Colombiana de Investigacion Agropecuaria, Bogota, Cundinamarca, Colombia.

Microbial protein synthesis is limited by the amount of energy available in the rumen. Growth and maintenance of microbial cells and end products of fermentation are a source of nutrients to the animal but also place a limit on productive efficiency. The purpose of this work was to assess the pattern of ATP availability produced by temporal colonization of rumen microbes on *Penisetum clandestinum* and its relationship with end products of fermentation in batch cultures. In vitro incubations were set up in Van Soest medium with 10% sieved rumen fluid and 5% substrate, with time intervals of 0, 2, 4, 8, 12, 18, and 24 h. Temporal microbial colonization was assessed using quantitative PCR (qPCR) with universal bacterial primers.

Forage degradation was measured by the in vitro dry matter digestibility (IVDMD) technique and the production of gas total. The components of colonization were assessed following extraction in glutaraldehyde 3% in PBS. ATP was analyzed by the luciferin-luciferase bioluminescent reaction. The methane production was measured by methane detector and volatile fatty acids (VFA) by gas chromatography. The colonized bacteria concentration changed over time ($P < 0.01$) from 5.97 to 15.8 $Log_{10}$ bacterial DNA [ng/mg of DM]. ATP released by rumen microbes was correlated with bacteria content during the log phase of growth (r = 0.69; $P < 0.01$). Differences in ATP concentration according to colonization components were observed in range from 9.6 to 410 ng/mL, showed negative correlation with total gas production (r = −0.43, $P = 0.06$ and methane (r = −0.48, $P = 0.04$). The lost energy during the colonization indicated a dynamic availability of ATP for growth and maintenance of rumen microbes, where the material was degraded up to 69%. The bacterial colonization pattern revealed positive correlations with acetic acid (r = 0.73, $P < 0.01$), propionic acid (r = 0.71, $P < 0.01$), IVDMD (r = 0.71, $P < 0.001$), available ATP (r = 0.69, $P < 0.01$), total gas (r = 0.90, $P < 0.01$), and methane (r = 0.93, $P < 0.01$) production. The rumen fluid ATP concentration was greater in colonized plant material than in the planktonic phase. In conclusion, ATP was dynamically associated with colonization and fermentation patterns by rumen microbes to degrade forage under conditions mimicking the rumen.

**Key Words:** rumen, ATP, methane, q-PCR, colonization

**Evaluation of different essential oils in modulating methane production, rumen fermentations, and microbial population in vitro**

G. Cobellis*[1,2], M. Trabalza-Marinucci[1], and Z. Yu[2],
[1]Department of Veterinary Medicine, University of Perugia, Perugia, Italy,
[2]Department of Animal Sciences, The Ohio State University, Columbus, Ohio, USA.

Essential oils (EO) can decrease $CH_4$ production by rumen microbiome, but they often decrease feed digestion at effective doses. The aim of this study was to evaluate commonly used EO with respect to efficacy in decreasing $CH_4$ production and affecting rumen function. EO from oregano, rosemary, ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, anethole, and eucalyptus were evaluated in vitro individually at 1.125 mL/L or in 3-way combinations (equal ratio) at 0.8 mL/L. The in vitro fermentation (48 h, 39°C) was done in triplicate using artificial saliva as medium, fresh rumen fluid as inoculum, and ground alfalfa hay plus a dairy concentrate mixture (50:50) as feed substrate. The results showed that all the EO, except anethole and eucalyptus, decreased ($P < 0.001$) total gas and $CH_4$ production, but they also decreased ($P < 0.001$) dry matter (DM) digestibility and fermentation. All the 3-way EO combinations reduced ($P < 0.001$) total gas (12–19.3%) and $CH_4$ (37.7–78.5%) production and $NH_3$ concentration (42.1–59.2%). The greatest $CH_4$ inhibition resulted from the EO combination of oregano, rosemary, and cinnamon leaves (−78.5%) and of ceylon cinnamon, dill seeds, and eucalyptus (−52.3%). DM degradability was decreased ($P < 0.001$) by 16.2–26.5% by all the EO combinations except that of ceylon cinnamon, dill seeds, and eucalyptus. The VFA profiles were affected by nearly all the EO combinations, but the concentrations of total VFA were not, including the EO combinations of ceylon cinnamon, dill seeds,

and eucalyptus. Results of qPCR assays evidenced no effects of the EO combinations on the abundance of total bacteria, *Prevotella ruminicola*, or *Streptococcus bovis* but a decrease ($P < 0.001$) in the abundances of *Archaea* (65.6–85.5%), *Fibrobacter succinogenes* (96.1–99.8%), *Ruminococcus amylophilus* (83.7–96.2%), *P. bryantii* (54.7–97.4%), *Selenomonas ruminantium* (45.5–71.2%) and *Megasphaera elsdenii* (57.3–98.7%). The abundances of *R. albus* and *R. flavefaciens* was increased ($P < 0.001$) by several of the EO combinations, including that of ceylon cinnamon, dill seeds, and eucalyptus. The results of this study suggest that low concentrations of EO in combination may be a practical approach to mitigate $CH_4$ emission from cattle without adverse effect on feed digestion or fermentation.

**Key Words:** essential oil, methane, rumen fermentation, rumen bacteria

**Induction of subacute ruminal acidosis affects the ruminal microbiome**

J. C. McCann*, S. Alqarni, S. Luan, F. C. Cardoso, and J. J. Loor,
University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Subacute ruminal acidosis (SARA) negatively affects the dairy industry by decreasing dry matter intake, milk production, profitability, and increasing culling rate and death loss. Six lactating Holstein cows were used in a replicated 2 × 2 Latin square design to determine the effects of SARA induction on the ruminal microbiome. Experimental periods were 10 d with d 1 to 3 for ad libitum intake of control diet, followed by 50% feed restriction on d 4, and ad libitum access on d 5 of the control diet (control) or control diet + 4.6 kg of a 50:50 wheat/barley pellet (challenge). Ruminal samples were collected on d 1 and 6 of each period before morning feeding and separated into liquid and solid fractions. Bacterial DNA was extracted, the V4 region of the 16S rRNA gene was amplified, and paired-end sequencing was conducted on the MiSeq Illumina platform. Output paired-end reads were merged using PANDASeq and analyzed using QIIME pipeline. Bacteroidetes and Firmicutes were the predominant phyla in both fractions, collectively accounting for more than 90% of sequences. Within the solid fraction, the relative abundance of *Streptococcus*, *Ruminococcus*, and *Succiniclasticum* was increased after SARA induction on d 6 ($P = 0.04$). In contrast, relative abundance of *Moryella* and *Blautia* was decreased on d 6 in the solid fraction ($P = 0.04$). Within the liquid fraction, relative abundance of *Prevotella* and *Lactobacillus* was increased on d 6 ($P = 0.03$). Although the relative abundance of *Streptococcus* increased after SARA induction on d 6 ($P = 0.01$), there was a tendency for a greater increase for the challenge treatment in the liquid fraction ($P = 0.07$). *Ruminococcus* relative abundance increased from d 1 to d 6 for the challenge treatment ($P = 0.01$), but no effects were observed for the control treatment in the liquid fraction ($P = 0.77$). Christensenellaceae and Erysipelotrichaceae families decreased in the liquid fraction from d 1 to d 6. Overall, results indicate feed restriction and subsequent refeeding caused a greater effect on the ruminal microbiome than the additional starch in the challenge treatment. However, the results may still be indicative of the rumen bacterial community response to SARA.

**Key Words:** rumen, acidosis, nutrition, cattle

**Effect of probiotics on the activity of intestinal carbohydrases in rats with lead intoxication**

L. S. Kuchkarova*, G. T. Kudeshova, I. I. Karimova, N.Sh. Nadzhmutdinova, and R. O. Atoeva
National University of Uzbekistan, Tashkent, Tashkent Uzbekistan.

It is known that lead ions from food and water lead to several disturbances in the body, including to dysbacteriosis. We suggested that the intake of lead ions in the digestive tract triggers other disorders of intestinal digestion associated with dysbiosis. The purpose of current work is to study the effect of probiotic Bifidumbacterin on the carbohydrate digestion in growing rats with lead intoxication. Growing Wistar rats were divided into 4 groups: 3 experimental and 1 control groups. In the first experimental group 10-, 11- and 12-d old rats were administered with lead acetate (5 mg/kg per day); then, from the 13th day of life for 5 d, rats were treated with saline. In the second group, 10- to 12-d-old rats were treated with saline, and 13- to 17-d rats were administered probiotic Bifidumbacterin (Russia) in a dose of $2 \times 10^4$ cfu/kg per day. In the third experimental group, rats in the same time and same dose were treated first 3 d with lead acetate and then 5 d with probiotics. Rats in the control group were treated with an equivalent volume of saline in the same period. All drugs were administered orally. Rats were killed on the 18th day of postnatal life. Lead intoxication decreased the body weight ($P < 0.01$) and the mass of the small intestine ($P < 0.01$) in growing rats. Introduction of Bifidumbacterin led to a slight increase in body weight gain and the small intestine weight in rats treated with lead ions ($P < 0.05$), but not in the rats treated with saline. Maltase activity was 4.9 ± 0.3 mmol/min/g tissue, and lactase activity was 6.9 ± 0.4 mmol/min/g tissue in control rats. Lead intoxication resulted in a decrease of the lactase and maltase activity of 22.9 and 32.4%, respectively. Differences were observed in intestinal enzyme activity in rats treated with the probiotic and the control groups. The administration of Bifidumbacterin to rats after lead intoxication led to some recovery in activities of maltase and lactase in small intestine. Our data suggest that Bifidumbacterin helps recover activity of intestinal maltase and lactase in growing rats after lead intoxication, but it does not exert any influence on the activity of disaccharidases in rats treated with saline.

**Key Words:** lead intoxication, intestinal disaccharidases, probiotic, growing rats

**Bacteria and bile: It takes a lot of gall**

J. Ridlon*[1,2],
[1]Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA, USA,
[2]McGuire Veterans Affairs, Richmond, VA, USA.

Bile salts are cholesterol derivatives released from the gallbladder into the small bowel where they function to solubilize lipids and lipid-soluble vitamins. When bile salts reach the terminal ileum, they are actively transported into the portal circulation making their way back to the liver in what is termed the enterohepatic circulation. Bile salts are also hormones regulating their own synthesis, as well as glucose and

lipid homeostasis. The enterohepatic circulation links host and microbial steroid-metabolizing genes, allowing for a cocktail of steroids to be produced, some of whose physiological and pathophysiological effects are now beginning to be explored. A host-bile acid-microbiome axis is emerging in which the bile acid pool shapes the structure and function of the gut microbiome, and the gut microbiome shapes the size and composition of the biliary pool. Gut bacteria can produce 32 metabolites from cholic acid alone. Bacteria inhabiting the large intestine are the sole producers of toxic secondary bile acids, namely deoxycholic acid and lithocholic acid. These bacteria are represented by a few species in the genus *Clostridium* and constitute less than one percent of the gut microbiome. Organs throughout the body accumulate distinct patterns of bile acids and bile salts, and these patterns differ markedly between germ-free and conventional animals. Bile acids activate several host nuclear receptors (NR), G-coupled protein receptors (GCPR), and cell-signaling pathways that regulate glucose and lipid metabolism and energy homeostasis. Bile acids vary in their ability to activate different NR and GCPR. In some instances, secondary bile acids are better ligands for host receptors than the natural ligands; for instance, lithocholic is the highest affinity ligand to the vitamin D receptor. Therefore, the gut microbiota should be thought of as an 'endocrine organ'. Unraveling this complex host-bile acid-microbiome axis points to strategies for treating diseases of the gastrointestinal tract and beyond.

**Key Words:** bile acid, cell-signaling, nuclear receptor, microbiome, deoxycholic acid

**Effect of varying levels of urea-molasses fermented wheat straw on ruminal characteristics, nutrient digestibility, blood urea nitrogen, and nitrogen balance**

M. Nisa*[1], M. Ali[2], and M. Sarwar[2],
[1]Department of Food Science, Nutrition and Home Economics, Govt. College University,, Faisalabad, Pakistan,
[2]Institute of Animal Science, University of Agriculture, Faisalabad, Pakistan.

The experiment was conducted to determine the influence of varying levels of urea-molasses fermented wheat straw (FWS) ensiled with rumen digesta for 21 d on ruminal characteristics (pH, $NH_3$-N), nutrient digestibility, blood urea nitrogen and nitrogen balance in Nili Ravi buffalo bulls. Four isocaloric and isonitrogenous diets were formulated. Control diet (C) contained 50% of un-treated wheat straw and 50% concentrate on a dry matter (DM) basis, whereas the FWS 60, FWS 70, and FWS 80 diets contained 60, 70, and 80% of FWS, respectively. Diets C, FWS 60, FWS 70, and FWS 80 were offered to bulls for 15 d; the first 10 d were the adaptation period, and the next 5 d were the collection period. After 15, 30, and 45 d, re-randomization was done, and the same procedure was replicated on the same animals. Nutrient digestibilities were determined by total fecal collection. Fecal and urinary samples were collected, composited and stored. Nutrients intake were similar in all animal fed diets having varying levels of FWS. The DM, crude protein, neutral detergent fiber and acid detergent fiber digestibilities increased in bulls fed FWS 60 and FWS 70 diets compared with those fed FWS 80 and the C diet. A similar trend was noticed in N balance. Higher ruminal $NH_3$-N and pH was observed in bulls fed FWS 60 compared with FWS 70, FWS 80, and C diets. Bulls fed on the diet of FWS 60 had

higher blood urea nitrogen than other diets. The results showed that urea molasses-treated wheat straw fermented with rumen digesta could replace 60% concentrate without any adverse effect on ruminal characteristics, nutrient intake, and nutrient digestibility in bulls.

**Key Words:** urea-treated wheat straw, ruminal characteristics, nutrient digestibility, nitrogen balance

**Fermentation of spent craft brewer's yeast by caprine rumen bacteria**

B. E. Harlow*[1], R. Bryant[2], and M. D. Flythe[3,1],
[1]University of Kentucky, Department of Animal and Food Sciences, Lexington, KY, USA,
[2]Asheville Flavor Innovations LLC, Asheville, NC, USA,
[3]USDA, ARS, Forage-Animal Production Research Unit, Lexington, KY, USA.

Protein in ruminant diets can be lost due to proteolysis and deamination in the rumen. Ruminants utilize rumen microbial protein, but the value of rumen bypass protein is recognized. Protein supplements include legumes and a myriad of byproducts, including spent brewer's yeast long included in feeds. However, recently introduced American craft beers often include more hops (*Humulus lupulus* L.) than previous recipes, and hops compounds accumulate in the residual yeast. These compounds include α- and β-acids, which are antimicrobial to the rumen hyper ammonia-producing bacteria (HAB), major contributors to amino acid degradation. The goal was to determine fermentation characteristics of a craft brewer's yeast (CY; ~3.5 mg/g combined hops acids) or a baker's yeast (BY; no hops acids) by caprine rumen microorganisms and HAB. Data were analyzed by ANOVA with Tukey's test post hoc. Mixed rumen microorganism cell suspensions were made by harvesting rumen fluid from fistulated goats (n = 3 wethers, 2 y), by differential centrifugation. Cells were re-suspended in medium with 40, 20, 10, or 5 mg/mL BY or CY as the nitrogen and energy source. After 24 h (39 °C), HAB were enumerated and ammonia ($NH_4$) was measured. At least 10-fold fewer HAB were enumerated from CY than from BY in each concentration ($P < 0.05$). Similarly, $NH_4$ production from CY was reduced by approximately 60% compared with BY (40, 20 or 10 mg/mL; $P < 0.05$). The most HAB ($10^9$ cells/mL) and greatest $NH_4$ concentration (53 mM) were measured in 40 mg/mL BY. In comparison, $10^7$ HAB cells/mL and 25 mM $NH_4$ were observed in 40 mg/mL CY. Addition of hops β-acids (45 ppm) to BY fermentations (40 mg/mL) produced a similar inhibitory response to CY alone. Pure culture experiments (n = 3) were conducted with *Peptostreptococcus anaerobius* BG1, a previously isolated caprine HAB. Ammonia production by BG1 from BY was 5.5, 3.8, 2.5 and 1.8 times greater than from CY at 40, 20, 10 and 5 mg/mL concentration, respectively ($P < 0.05$). Ammonia production was greater when Trypticase (15 mg/mL) was included as an additional substrate, but similar inhibition was observed in CY treatments ($P < 0.05$). These results indicate that rumen microorganisms deaminated the amino acids in CY to a lesser degree than in BY, and inhibition of HAB by residual hops compounds is the likely mechanism of action.

**Key Words:** microflora, in vitro, livestock

**Session: Prebiotics, probiotics, and DFM development**

**Utilization of prebiotic carbohydrates by the human gut acetogen *Blautia producta*: You are what your acetogens eat!**

K. Ohseki and S. L. Daniel*,
Eastern Illinois University, Charleston, IL, USA.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating bacterial activities in the colon, thus improving host health. To date, most studies have focused on the effects of prebiotics on bifidobacteria and lactobacilli in the gut. However, evidence now suggests that prebiotics may also stimulate other gut populations, especially bacteria in the phylum *Firmicutes*. In *Firmicutes*, the family *Lachnospiraceae* is active in the colonic fermentation of complex polysaccharides. Whether *Blautia* spp., organisms in *Lachnospiraceae*, utilize prebiotic carbohydrates is presently unknown. *Blautia producta* U-1, a human gut acetogen, was grown at 37°C in an anaerobic undefined medium (UM; minerals, metals, 0.1% yeast extract, bicarbonate/$CO_2$ buffer system, resazurin, and cysteine). Stock solutions of disaccharides (lactose, lactulose), tetrasaccharide (stachyose), sugar alcohols (adonitol, dulcitol, erythritol, lactitol, sorbitol, xylitol) and complex carbohydrates [inulin, Litesse (polydextrose), Nutraflora, pectin, Orafti (raftilose), starch] were added to UM to the following initial concentrations: di- and tetrasaccharides, 10 m*M*; sugar alcohols, 10 m*M*; and complex carbohydrates, 0.25%. Lactitol, lactulose, stachyose were growth supportive; in contrast, most sugar alcohols were not growth supportive. *B*. *producta* U-1 grew at the expense of Litesse, Nutraflora, and Orafti. The natural prebiotic inulin was not growth supportive. Growth by *B*. *producta* U-1 at the expense of prebiotics carbohydrates was rapid (completed within 12–18 h), and cell yields with different prebiotics were essentially the same except for Litesse which approximated half that of other cultures. Acetate was a major end product detected, and small amounts (0–3.7 m*M*) of lactate were also observed. Acetate yields in lactose, stachyose, Nutraflora, and Orafti cultures ranged from 25 to 30 m*M*, whereas acetate levels in lactitol and lactulose cultures were higher (40–43 m*M*). Prebiotics may have broad stimulatory effects on many gut populations, including intestinal acetogens. Prebiotics that stimulate gut acetogens and acetogenesis would potentially benefit host health by reducing colonic gas volume and producing acetate, a usable metabolite for the animal host.

**Key Words:** gut, acetogens, prebiotic carbohydrates, *Blautia product*

**Efficacy of butyric acid and monolaurate to combat bacterial enteritis problems in broilers**

F. Dias*[1], A. Schwarz[1], T. Rogge[2], J. de Gussem[3], and H. van Meirhaeghe[1],
[1]Vetworks, Poultry Vet Consultant, Poeke, Flanders, Belgium,
[2]Proviron, Chemistry Company, Oostende, Flanders, Belgium,
[3]Poulpharm, Poultry Vet Lab., Izegem, Flanders, Belgium.

Several trials have shown that butyric acid plays an important role in the development of the intestinal wall. It serves as an important energy source for enterocyte proliferation, speeds up gut repair, strengthens gut barrier functions, has

antiinflammatory and antioxidant activity and regulates intestinal water intake. This study reports on a series comprising 2 floor pen trials and a field trial involving multiple commercial houses. The aim of the study was to evaluate the efficacy of a specific formulation of butyric acid and monolaurate for the control of bacterial enteritis (BE) in broilers. Floor pen trials BE induction: To provoke bacterial enteritis (BE) in experimental conditions, a feed rich in nonstarch polysaccharides (NSP), wheat/rye and highly methylated citrus pectin (HMC) was given. In trial 1, a high performance feed with high energy and protein levels was used to compare BE reduction strategies to a positive control. In trial 2, a lower performance feed, with lower energy and protein content, was used to compare BE reduction strategies to a negative control. Field trial: From the results of the floor pen trials, wwe selected a specific blend of butyric acid and monolaurate, Provifeed Optigut, because of its beneficial effect on average end weight (AEW) and feed conversion ratio (FCR) from field trials. The trial group included 176,500 birds in 5 houses: Provifeed Optigut (4 kg/t; metric ton = 1,000 kg) d 0–10, (2 kg/t) d 11–31, and (0 kg/t) d 32–42. The control group included 207,700 birds in 6 houses: commercial feed, incl. traditional organic acids. Results: (1) floor pen trials Optigut groups / average: improvement AEW 8.5% (197 g), FCR 6 points; (2) field trials Optigut groups / average: improvement AEW 4.5% (113 g), FCR 5 points. Our trials demonstrated that Provifeed Optigut can be considered as potential alternative to antibiotic treatment of BE in broilers.

**Key Words:** bacterial enteritis, organic acids, butyric acid, monolaurate

**Anti-*Salmonella* effect of thymol-β-D-glucopyranoside in porcine jejunal, cecal, and rectal gut contents**

G. Levent*[1,2], G. Ciftcioglu[2], R. C. Anderson[1], R. C. Beier[1], and D. J. Nisbet[1],
[1]United States Department of Agriculture, Agricultural Research Service, Food and Feed Safety Research Unit, College Station, TX, USA,
[2]Istanbul University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, Istanbul, Turkey.

Thymol is a natural antimicrobial with activity against zoonotic pathogens, but its rapid absorption in the proximal gut prevents its passage to the lower gut where these pathogens primarily reside. Thymol-β-D-glucopyranoside (β-thymol) passes more effectively than free thymol to the lower gut but needs to be hydrolyzed by β-glycoside-hydrolyzing bacteria to liberate free thymol. β-Thymol has been shown to be bactericidal against *Salmonella* when cultured in fecal environment with β-glycoside-hydrolyzing gut microbes, but its activity in different gut compartments has not been studied. This study aimed to analyze the presence of β-thymol hydrolyzing-activity in digesta collected from various gut compartments of a weaned pig and as evidenced by the anti-*Salmonella* effect of free thymol potentially liberated by these populations. Jejunal, cecal and rectal contents were added (6, 15, and 15 g, respectively) to separate 100-mL volumes of anaerobic Mueller-Hinton broth inoculated with 5.6 ± 0.12 $\log_{10}$ cfu/mL *Salmonella* Typhimurium DT104 (*ST*). The suspensions were distributed to crimp top tubes (10 mL/tube) supplied with or without β-thymol to achieve 0 or 1.5 m*M* and incubated anaerobically under 100% $N_2$ at 39°C. Survivability of *ST* was determined by plating serial dilutions on Brilliant

Green Agar supplemented with novobiocin and chloramphenicol (25 µg/mL each), and viable colonies counted after 24 h incubation (37°C) were analyzed by an ANOVA. After 6 h incubation, viable *ST* in cecal and rectal suspensions were decreased ($P < 0.05$) from those in controls (5.78 ± 0.18 and 6.15 ± 0.07 $\log_{10}$ cfu/mL, respectively) by 1.5 and 1.6 $\log_{10}$ cfu/mL by β-thymol treatment. Similarly, *ST* counts in β-thymol-treated cecal and rectal suspensions were decreased 1.93 and 1.12 $\log_{10}$ cfu/mL from control counts (4.9 ± 0.37 and 5.97 ± 0.23 $\log_{10}$ cfu/mL, respectively) after 24 h incubation. In jejunal suspensions, control and treated *ST* counts differed by only 0.3 $\log_{10}$ cfu/mL after 6 h (approximately 9.0 $\log_{10}$ cfu/mL) but were decreased ($P < 0.05$) from controls (7.15 ± 0.07 $\log_{10}$ cfu/mL) by 3.6 $\log_{10}$ by β-thymol after 24 h. Results reveal an anti-*Salmonella* effect of β-thymol in microbial populations from various porcine gut compartments; however, more studies are needed to understand the kinetics of β-thymol activity in swine gut.

**Key Words:** thymol-β-D-glucopyranoside, *Salmonella*, pigs, gut

**Effect of *in ovo* and hatcher spray of probiotics on microbial properties of gastrointestinal tract and hatcher cabinets**

B. M. Hargis*[1], L. E. Graham[1], K. D. Teague[1], J. C. Bielke[1], R. E. Wolfenden[2], G. Tellez[1], and L. R. Bielke[1],
[1]Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA,
[2]Pacific Vet Group-USA, Inc., Fayetteville, AR, USA.

Early colonization of beneficial bacteria in chicks may improve gastrointestinal tract (GIT) development and performance, but appropriate application methods need investigation. *In ovo* and hatchery application of probiotics were investigated. Effects of 2 co-administered egg shell-origin MRS-recoverable bacteria (MRSR) on hatchability and GIT recovery of selected bacterial groups on day of hatch were evaluated. Embryos (d18) were *in ovo* injected with saline (CON) or cfu MRSR. After hatch, GIT samples were collected for microbial recovery on MRS and tryptic soy agar (TSA). Hatchability was not affected by *in ovo* treatment. Foregut MRS recovery was 6.05 ± 0.32 $\text{Log}_{10}$ cfu, vs. 3.25 ± 0.63 in CON ($P < 0.05$). In the hindgut, MRSR treatment resulted in 8.71 ± 0.16 $\text{Log}_{10}$ cfu vs. 5.57 ± 1.01 $\text{Log}_{10}$ MRS-recoverable in CON ($P < 0.05$). Recovery on TSA was similar for both samples. Analogous results may reflect ability of administered bacteria to grow MRS and TSA. For hatchery application, combinations of either dry (D) or aqueous suspension (W) of *Bacillus* spores (BS) plus lactic acid bacteria (W; LAB) were sprayed at transfer only, or 3 time points (transfer, 20% and 50% hatch). Combinations included single spray of DBS + LAB (W; Trt1), single spray of WBS + LAB (Trt2), or 3× spray WBS + LAB (Trt3). Six (each) TSA, Rogosa (RA), and MacConkey's (MCA) agar plates were distributed in cabinets at transfer, ~50% pip, ~20%, ~50%, and ~75% hatch. At ~20% pip and ~20% hatch recovery on MCA was suppressed ($P < 0.05$) in cabinets in all treatments. At ~50% hatch, Trt1 had 1.0 $\log_{10}$ lower cfu on MCA compared with controls, and Trt3 resulted in increased LAB cfu on RA and TSA ($P < 0.05$). By ~75% hatch, MCA cfu were the same for all treatments, but Trt1 increased *Bacillus* recovery and Trt3 increased LAB cfu on RA. Trt2 did not result in differences at any time points, suggesting that multiple applications are best for survival of LAB, and spray of DBS is optimal for persistence. These studies suggest that *in ovo* and

hatcher application of probiotics may be appropriate methods for early distribution to chicks to promote beneficial microflora development.

**Key Words:** probiotics, *in ovo*, hatcher cabinets, *Bacillus*, lactic acid bacteria

**A novel yeast strain *Meyerozyma guilliermondii* isolated from native fruits from Colombian ecosystems as a prospective probiotic to be used in dairy systems**

T. Rodriguez*, M. Chaparro, M. Gomez, C. Castillo, A. Garcia, C. Ariza, and O. Mayorga,
Corporación Colombiana de Investigación Agropecuaria - Corpoica, Mosquera, Cundinarmarca, Colombia.

Yeast products are expected to improve milk yield and weight gain in cattle by stimulating microbial ruminal activity. However, their responses on animal productivity and doses are strain-dependent, opening a new field of research for new strains more specialized. This work aimed to identify and assess the inclusion of 3 levels of 2 types of vehiculized yeast cells with promising characteristics as a dairy probiotic. Molecular identification was carried out by 454 pyrosequencing of ITS1 and ITS4 regions and matched using NCBI database and BLASTn algorithm. A 21-d oral toxicity test of the native yeast culture was conducted in male and female Wistar rats by gavage at 3 doses of 0, 1250, 2500, and 5000 mg/kg/day. The vehiculization of native yeast cells was developed in amylaceous polysaccharide (FAP) and non-amylaceous polysaccharide (FNP) compounds. The inclusion doses of this vehiculized probiotic were $10^6$, $10^7$, and $10^8$ cfu/mL compared with yeast free system (YCF) and commercial yeast (CY) as controls. Forage degradation was measured by dry matter disappearance (IVDMD) and production of total gas in vitro. Anaerobic incubations were carried out with Van Soest medium, 10% sieved rumen fluid, 5% substrate and sampling in times 0, 2, 4, 8, 12, 18 and 24 h. The data were parametrized using nonlinear models and compared by orthogonal contrasts. Methane production was measured by a laser sensor. The yeast strain was classified as *Meyerozyma guilliermondii*, with a coverage of 99% and identity of 99%. The vehiculized probiotic is not pathogenic, based on our finding that the LD50 was highest at the 5000 mg/kg/day doses. The vehiculized probiotic correlated with inclusion levels (r = 0.72, *P* = 0.075). FAP produced the highest total gas values compared with the rest of the treatments: for FAP, 120; FNP, 112; YCF, 103; and CY, 100 mL total gas/200 mg DM. Methane production decreased at the $10^8$ cfu/mL dose of vehiculized probiotic with FAP (33.4 ppm) and FNP (29.7 ppm) compared with YCF (37.4 ppm), consequently improving IVDMD by 10%. The *M. guilliermondii* is a promising strain with wide biotechnological applications.

**Key Words:** *Meyerozyma guilliermondii*, yeast, probiotic, livestock

**Reduction of pathogenic organisms through daily feeding of probiotics in multiple species of production livestock and companion animals**

S. Lerner*, W. Kreikemeier, C. Jamison, A. McDonald, and K. Nestor,
Nutrition Physiology Company, LLC, Overland Park, Kansas, USA.

Effective probiotics have been demonstrated to competitively inhibit the colonization of the lower gastrointestinal (GI) tract by pathogenic microorganisms; throughout the literature, this appears to be a concentration-dependent phenomenon. Reduction in the presence of pathogenic bacteria in the lower GI tracts of production animals is associated with improved digestive and immune function and a reduced shedding of potentially foodborne-illness-causing pathogens. Likewise, a reduction in the presence of pathogenic bacteria in the lower GI tracts of companion animals is associated with reduced risk of zoonotic illnesses. In this work, daily feeding of $1 \times 10^7$ cfu of *Lactobacillus* (NP-51) to chickens (*Gallus gallus domesticus*) and turkeys (*Meleagris gallopavo*), $1 \times 10^9$ cfu of *Lactobacillus* (NP-51) to dogs (*Canis familiaris*), and $1 \times 10^9$ cfu of *Lactobacillus* in combination with $1 \times 10^9$ cfu of *Propionibacterium* in dairy and beef cattle (*Bos taurus*) results in a significant improvement in performance (growth in the production species and maintenance of weight in the companion species) and a significant reduction in a variety of pathogenic organisms, including *E. coli* O157:H7, the other "big 6" shiga toxin-producing *E. coli* species (STEC), *Salmonella enterica*, and *Clostridium perfringens*. In addition, in the presence of additional challenges with organisms that cause secondary infections and necrotic enteritis, daily feeding of the probiotic *Lactobacillus* strain significantly ameliorated their physiologic effect and the resulting mortality and morbidity. In conclusion, daily feeding of an effective number of a single strain of *Lactobacillus* can have a demonstrable benefit in a variety of monogastric animals and birds and, in combination with *Propionibacterium*, can have a similar effect in ruminants.

**Key Words:** *Lactobacillus*, pathogens, competitive exclusion, production animals, direct-fed microbial

### Isolation and identification of probiotic bacteria from the gut of yellow perch, *Perca flavescens*, and evaluation of their probiotic potential against *Vibrio anguillarum*

J. Stiverson*, Z. Yu, and K. Dabrowski,
The Ohio State University.

This study aimed to isolate potential probiotic strains from the intestinal tract of healthy yellow perch, *Perca flavescens.* Contents from the intestinal tract were collected and plated on TSA containing 5% sheep blood. Cultures were grown 24 h before challenging with a sprayed layer of *Vibrio anguillarum* (the cause of vibriosis in aquaculture). Colonies that exhibited inhibition to *Vibrio anguillarum* were selected. These isolate were further evaluated for their inhibition to *Vibrio anguillarum* and *Aeromonas salmonicida* using cross streaking. Seven isolates were found be inhibitory to these pathogens. Based on 16S rRNA sequencing, 6 of the isolates belong to *Lactococcus lactis* and one belongs to *Pseudomonas*. The culture supernatant of the isolates did not inhibit the growth of *V. anguillarum.* However, in co-culture with one isolate (referred to as *L. lactis* V9), the growth of *V. anguillarum* was dramatically decreased while that of *L. lactis* V9 was not affected. These results suggest that *L. lactis* V9 does not produce metabolites that are inhibitory to *V. anguillarum*, but it out-competes the latter for nutrients. Because these isolates are native to the perch gut, they can thrive within the host and provide a longer lasting probiotic effect. Future in vivo studies are warranted to confirm this theory.

**Key Words:** probiotics, *Vibrio anguillarum*, yellow perch, aquaculture

**Specific bacterial strains associated with high milk production and favorable milk profiles in lactating dairy cows: A case study using a novel platform for DFM product discovery**

C. Belnap* and M. Ashby,
Taxon Biosciences Inc., Tiburon, CA, USA.

Modification of the rumen microbiome using direct-fed microbial (DFM) products may enhance milk production and mitigate systemic health problems, such as subacute ruminal acidosis (SARA). SARA occurs when microbial production of organic acids exceeds rumen absorption capacity, thereby lowering rumen pH and disrupting milk production. Taxon Biosciences utilizes a novel 2-D clustering software platform to link specific microbes, represented by unique small subunit 16S rRNA gene sequence tags, to environmental metadata. Taxon's platform can be utilized to identify beneficial rumen microorganisms and subsequently evaluate these microbes as new DFM products. This study is in contrast to traditional analyses of microbial communities that examine abundance of large groups of microbes (e.g., Firmicutes), or target clades of microbes using qPCR approaches. To track the rumen microbiome response to SARA, 5 cannulated lactating dairy cows were transitioned to a high concentrate diet resulting in low rumen pH and decreased milk production. Shifts in the rumen microbiome were tracked during onset of SARA followed by a recovery period. A total of 30 rumen samples were sequenced to generate a database of 2,850,684 microbial 16S rRNA gene sequence tags. Correlation of microbial abundances to 62 metadata parameters including feed, rumen chemistry, milk production, and milk characteristics were examined. Large shifts in microbial abundance were strongly associated with low rumen pH during onset of SARA, and microbial distributions at the end of the 7-d recovery period were distinct from the initial communities. Several rumen genera were examined using operational taxonomic units (OTUs) at 97% or greater DNA sequence identity, and as unique individual sequences. Distinct sequences within OTU groups showed vastly different distributions indicating OTUs do not provide sufficient resolution to adequately characterize changes to the rumen microbiome. These data revealed that specific strains, represented by a single unique sequence, might be beneficial to cows subjected to high concentrate diets. The ability to distinguish closely related (>97% similar), yet functionally different microorganisms is critical to designing more effective DFM products.

**Key Words:** rumen, microbiome, acidosis, direct-fed microbial, 16S rRNA