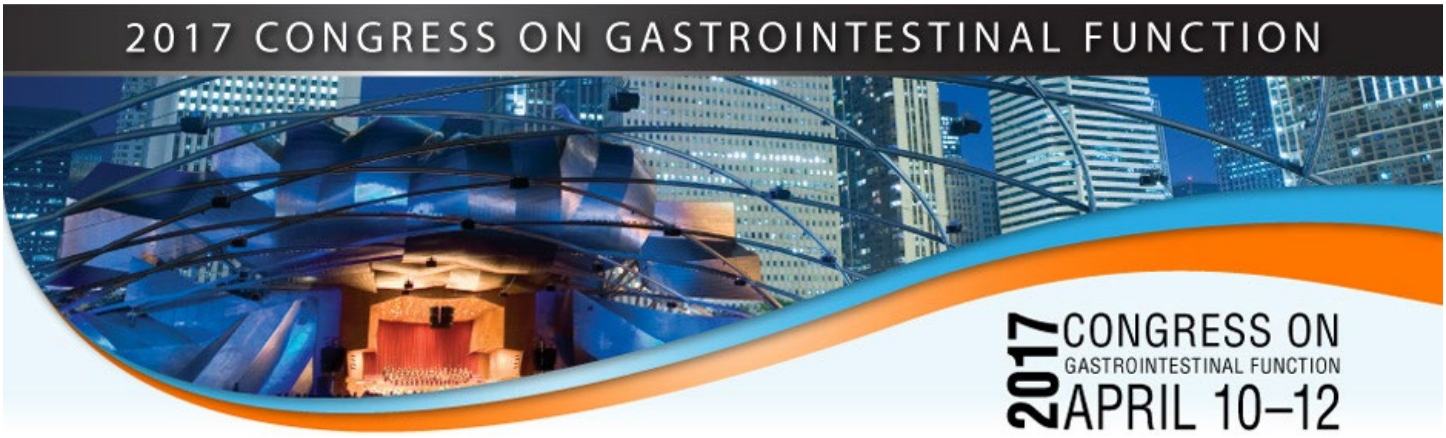

SCIENTIFIC PROGRAM AND ABSTRACTS

GLEACHER CENTER

UNIVERSITY OF CHICAGO

CHICAGO, ILLINOIS

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Monday, April 10

08:00–14:00 **Registration**
Gleacher Center, First Floor Foyer

Please pick up your registration materials, name tag, and mixer drink tickets at the registration desk before you enter the auditorium. Mount posters on boards provided (6th floor).

Special Session
Early Acquisition and Development of the Gut Microbiota: A Comparative Analysis

Chair: Rod Mackie, University of Illinois at Urbana-Champaign
Gleacher Center, First Floor, Tiered Classroom

08:30–08:40 **Introduction.**
Rod Mackie, Chair, *University of Illinois at Urbana-Champaign, USA*.

08:40–09:20 1 ***Invited talk:*** **Mopping up spilled milk: Restoring ecosystem function in the nursing infant gut microbiome.**
D. Mills*, *University of California*, *Davis, CA, USA*.

09:20–10:00 2 ***Invited talk:*** **The perinatal microbiome and finding unexpected answers.**
K. Aagaard*, *Obstetrics and Gynecology, Division of Maternal-Fetal Medicine and Departments of Molecular and Human Genetics, Molecular and Cell Biology, and Molecular Physiology and Biophysics at Baylor College of Medicine*, *Houston, TX, USA*.

10:00–10:30 **Tea break**

10:30–11:10 3 ***Invited talk:*** **Early life nutrition and gut microbiome development in the piglet.**
S. M. Donovan*, M. H. Monaco, and M. Wang, *University of Illinois*, *Urbana, IL, USA*.

11:10–11:50 4 ***Invited talk:*** **Assessment of neonatal gut microbiomes revealed microbial markers linked to calf gut health.**
N. Malmuthuge[1,2], G. Liang[1,3], and L. L. Guan*[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta*, *Edmonton, AB, Canada*, [2]*Vaccine and Infectious Disease Organization-International Vaccine Centre, University of Saskatchewan*, *Saskatoon, SK, Canada*, [3]*Department of Microbiology Perelman School of Medicine, University of Pennsylvania*, *Philadelphia, PA, USA*.

11:50–12:30 5 ***Invited talk:*** **The dynamics of microbiota development in chickens.**
R. J. Moore*[1], R. J. Hughes[2], and D. Stanley[3], [1]*RMIT University*, *Bundoora, Victoria, Australia*, [2]*South Australian Research and Development Institute*, *Roseworthy, South Australia, Australia*, [3]*Central Queensland University*, *Rockhampton, Queensland, Australia*.

12:30–13:00 **Chair, speakers, and panel discussion**

13:00–14:00 **Lunch (please make your own arrangements)**

2017 Opening Session
Bryant Memorial Lecture and Invited Presentations

Chair: Rod Mackie, Congress Chair, University of Illinois
Gleacher Center, First Floor, Tiered Classroom

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

14:10–15:00 6 ***Invited talk:*** **Bacterial energetics: The sixth antimicrobial target space for drug development.**
G. M. Cook*, *University of Otago, Department of Microbiology and Immunology*, *Dunedin, Otago, New Zealand*.

15:00–15:45 7 ***Invited talk:*** **Glycan uptake in the gut *Bacteroidetes*: The Sus paradigm.**
N. Koropatkin*, *University of Michigan*.

15:45–16:30 8 ***Invited talk:*** **Functional analysis of the gut microbiota using single-cell isotope probing.**
D. Berry*, *University of Vienna*, *Vienna, Austria*.

16:45–19:00 **Welcome Mixer**
Informal poster viewing session (please wear your name tag).
Refreshments: drink tickets, hors d'oeuvres, and cash bar.
Gleacher Center, First Floor, Tiered Classroom

Tuesday, April 11

08:30–09:00 **Continental breakfast**
Gleacher Center, First Floor, near Tiered Classroom

Podium presentations: Session 1

Chair: Isaac Cann, University of Illinois at Urbana-Champaign
Gleacher Center, First Floor, Tiered Classroom

09:00–09:45 9 ***Invited talk:*** **Modulation of the human gut microbiota—An ecological perspective.**
J. Walter*, *University of Alberta*, *Edmonton, Alberta, Canada*.

09:45–10:05 10 **High-resolution tracking of microbial colonization in fecal microbiota transplantation experiments via metagenome-assembled genomes.**
S. T. M. Lee*[1], S. Kahn[1], T. Delmont[1], N. Hubert[1], H. Morrison[2], D. Antonopoulos[1], D. Rubin[1], and A. Eren[1,2], [1]*University of Chicago Medicine*, *Chicago, IL, USA*, [2]*Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory*, *Woods Hole, MA, USA*.

10:05–10:25 11 **Using culturomics approaches to quantitate the diversity of bacteria that can be recovered from cultivation of infant feces.**
X. Pang[1], C. Méndez-García*[2], S. Donovan[2], M. Wang[2], M. Siegel[2], I. Cann[2], and R. Mackie[2], [1]*Shanghai Jiao Tong University*, *Shanghai, China*, [2]*University of Illinois*, *Urbana, IL, USA*.

10:25–11:00 **Coffee break**

11:00–11:20 12 **Large-scale network analysis of food, nutrients, and microbiome.**
P.-J. Kim*, *Korea Advanced Institute of Science and Technology*, *Daejeon, Republic of Korea*.

11:20–11:40 13 **Evaluation of organism identification for 16S rRNA sequencing of chicken cecal microbiome.**
J. C. Hsieh*[1], C. J. Schmidt[2], and S. J. Lamont[1], [1]*Iowa State University*, *Ames, IA, USA*, [2]*University of Delaware*, *Newark, DE, USA*.

11:40–12:00 14 **Stable isotope tracking of metabolically active rumen bacteria in an *in vitro* system under acidotic conditions.**
R. M. Petri* and Q. Zebeli, *University of Veterinary Medicine*, *Vienna, Austria*.

12:00–13:00 **Business Meeting (open to all attendees)**
Gleacher Center, First Floor, Tiered Classroom

12:00–13:30 **Lunch (please make your own arrangements)**

Podium presentations: Session 2

Chair: Isaac Cann, University of Illinois at Urbana-Champaign
Gleacher Center, First Floor, Tiered Classroom

13:30–14:10 15 ***Invited talk:* Microbial endocrinology—How does stress and tension influence the composition and functioning of gut bacteria?**
P. Freestone*, *University of Leicester*, *United Kingdom*.

14:10–14:30 16 **Environmental pollutant benzo[*a*]pyrene impacts the volatile metabolome and transcriptome of the human gut microbiota.**
C. Defois*[1], J. Ratel[2], S. Denis[1], F. Mercier[2], C. Gasc[1], E. Peyretaillade[1], E. Engel[2], and P. Peyret[1], [1]*UMR*, *Clermont-Ferrand, France*, [2]*Institut National de la Recherche Agronomique UR*, *Saint-Genes-Champanelle, France*.

14:30–14:50 17 **Chemerin is present in intestinal epithelia of calves with a potential role in barrier function.**
Y. Suzuki*[1], S. Roh[2], H. Hayashi[3], S. Koike[1], and Y. Kobayashi[1], [1]*Hokkaido University*, *Sapporo, Hokkaido, Japan*, [2]*Tohoku University*, *Sendai, Miyagi, Japan*, [3]*Rakuno Gakuen University*, *Ebetsu, Hokkaido, Japan*.

14:50–15:20 **Coffee break**

15:20–15:40 18 **The curious case of ruminal cellulose degradation: New players and old.**
A. E. Naas[1], L. M. Solden[2], I. M. Heggenes[1], N. M. Koropatkin[3], R. I. Mackie[4], V. G. H. Eijsink[1], K. C. Wrighton[2], M. Ø. Arntzen[1], and P. B. Pope*[1], [1]*Norwegian University of Life Sciences*, *Aas, Norway*, [2]*The Ohio State University*, *Columbus, OH, USA*, [3]*University of Michigan Medical School*, *MI, USA*, [4]*University of Illinois at Urbana-Champaign*, *IL, USA*.

15:40–16:00 19 **New roles in hemicellulosic sugar fermentation for the uncultivated *Bacteroidetes* family BS11.**
L. M. Solden*[1], D. W. Hoyt[2], W. B. Collins[3], J. E. Plank[1], R. A. Daly[1], E. Hildebrand[4], R. Wolfe[1], C. D. Nicora[2], S. O. Purvine[2], M. Carstensen[4], M. S. Lipton[2], D. E. Spalinger[5], J. L. Firkins[1], BA Wolfe[1], K. C. Wrighton[1], [1]*The Ohio State University*, *Columbus, OH, USA*, [2]*Pacific Northwest National Laboratory*, *Richland, WA, USA*, [3]*Alaska Department of Fish and Game*, *Palmer, AK, USA*, [4]*Minnesota Department of Natural Resources*, *Forest Lake, MN, USA*, [5]*University of Alaska Anchorage*, *Anchorage, AK, USA*.

16:00–16:30 20 **Analysis of the ammonium assimilation pathways of the human colonic bacterium *Bacteroides thetaiotaomicron*.**
M. Iakiviak*, I. Cann, and R. I. Mackie, *University of Illinois*, *Urbana-Champaign, IL, USA*.

16:30–18:30 **Poster Session and Mixer**
Gleacher Center, Sixth Floor (Room 621)

Wednesday, April 12

08:30–09:00 **Continental breakfast**
Gleacher Center, First Floor, near Tiered Classroom

Podium presentations: Session 3
Chair: Jeff Firkins, The Ohio State University
Gleacher Center, First Floor, Tiered Classroom

09:00–09:20 21 **PorkBoostEB: A new *Bacillus subtilis*-based probiotic that improves gut health in piglets.**
R. Cernat* and B. Nielsen, *Chr. Hansen A/S*, *Hørsholm, Denmark*.

09:20–09:40 22 **A formulated yeast blend and *Bacillus* probiotic fed to sows alters the fecal microbial ecology of their offspring.**
E. Davis*[1], J. Rehberger[1], J. Sawall[1], A. H. Smith[1], R. Song[2], and K. Friesen[2], [1]*Agro BioSciences Inc.*, *Wauwatosa, WI, USA*, [2]*NutriQuest*, *Mason City, IA, USA*.

09:40–10:00 23 **Modulation of yeast probiotic activities by manipulating cell wall mannan oligosaccharide.**
S. Kwak*, J.-J. Liu, and Y.-S. Jin, *The University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

10:00–10:30 **Coffee break**

10:30–11:10 24 ***Invited talk:* Microbial perspectives on host evolution.**
K. R. Amato*, *Department of Anthropology, Northwestern University*, *Evanston, IL, USA*.

11:10–11:30 25 **Interspecies hydrogen transfer and its effects on global transcript abundance in *Ruminococcus albus*, a predominant fiber-degrading species in the rumen, and *Wolinella succinogenes*, a syntrophic partner.**
R. R. Geier*[1,5], I. H. Kwon[3], I. Cann[3,4], and R. I. Mackie[2,3], [1]*Division of Nutritional Sciences, University of Illinois*, *Urbana, IL, USA*, [2]*Institute for Genomic Biology, University of Illinois*, *Urbana, IL, USA*, [3]*Department of Animal Sciences, University of Illinois*, *Urbana, IL, USA*, [4]*Department of Microbiology, University of Illinois*, *Urbana, IL, USA*, [5]*Agro BioSciences Inc.*, *Wauwatosa, WI, USA*.

11:30–11:50 26 **Structural biology of methanobacterial enzymes for fun and profit.**
V. Carbone[1], L. R. Schofield[1], Y. Zhang[1], I. M. Hannus[1], D. Schäfer[1], R. Atua[1], C. Sang[1], S. Molano[1], C. Nicol[1], B. P. Subedi[1], W. F. Martin[2], A. J. Sutherland-Smith[3], and R. S. Ronimus*[1], [1]*AgResearch*, *Palmerston North, New Zealand*, [2]*Heinrich-Heine Universität*, *Düsseldorf, Germany*, [3]*Massey University*, *Palmerston North, New Zealand*.

12:00–13:00 **Lunch (please make your own arrangements)**

Podium presentations: Session 4

Chair: Jeff Firkins, The Ohio State University
Gleacher Center, First Floor, Tiered Classroom

13:00–13:20 27 **Comparative microbiome analysis between sheep rumen and rabbits cecum provides new insight into their differential methane production.**
L. Mi*[1,2], B. Yang[1], Z. Yu[2], J. Liu[1], and J. Wang[1], [1]*Zhejiang University*, *Hangzhou, Zhejiang, China*, [2]*The Ohio State University*, *Columbus, OH, USA*.

13:20–13:40 28 **Winners and losers: Microorganisms impacted by inflammation in the gut.**
M. A. Borton*, A. Sabag-Daigle, J. Wu, L. M. Solden, B. S. O'Banion, R. A. Daly, R. A. Wolfe, J. F. Gonzalez, V. H. Wysocki, B. M. M. Ahmer, and K. C. Wrighton, *The Ohio State University*.

13:40–14:00 29 **Addition of arginine and citrate enhances bacterial growth and fluoroacetate degradation of *Cloacibacillus* sp. MFA1.**
S. Kang*, S. Denman, and C. McSweeney, *CSIRO*, *Brisbane, Queensland, Australia*.

14:00–14:30 **Coffee break**

14:30–14:50 59 **Biochanin A selectively inhibits carbohydrate-utilizing bacteria in the bovine rumen.**
B. E. Harlow*, G. E. Aiken, and M. D. Flythe, *USDA-ARS, Forage-Animal Production Research Unit*, *Lexington, KY, USA*.

14:50–15:10 31 **Modulation of sub-acute ruminal acidosis by active-dry yeast supplementation and its effect on rumen fungal and protozoal populations in liquid, solid, and epimural fractions.**
S. Ishaq*[1], O. AlZahal[2], N. Walker[2], and B. McBride[3], [1]*Ishaq Informatics*, *Bozeman, MT, USA*, [2]*AB Vista*, *Marlborough, United Kingdom*, [3]*University of Guelph*, *Guelph, Toronto, Canada*.

15:10–15:30	62	**Changes in rumen microbes' quantity and volatile fatty acid concentrations during adaptation period of dairy cows to changing diet.** L. Mamuad*[1,2], S. Kim[1,2], M. Lee[1], Y. Choi[1], C. Lee[2], Z. Yu[2], and S. Lee[1], [1]*Sunchon National University*, *Suncheon, Jeonnam, South Korea*, [2]*The Ohio State University*, *OH, USA*.
15:30–15:45		**Presentation of Russell Awards**
15:45		**Closing remarks and invitation to CGIF 2019**

Poster Presentations

Gleacher Center, 6th floor (Room 621)

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

32 **Effect of feeding increasing levels of *Aspergillus terreus* fermented palm kernel cake on methane emission in goats.**
J. B. Liang*[1], M. F. Mahadzir[1], S. Garba[1], M. F. Jahromi[2], S. C. L. Candyrine[1], R. Ronimus[3], S. Muetzel[3], and A. A. Samsudin[1], [1]*Universiti Putra Malaysia*, *Serdang, Selangor, Malaysia*, [2]*Agricultural Biotechnology Research Institute of Iran*, *Mashad, Iran*, [3]*AgResearch*, *New Zealand*.

33 **Effects of lysozyme on *in vitro* fermentation, methanogenesis, and microbial community structure of the rumen.**
Y. Chen[1], J. Shen*[1,2], W. Zhu[1], and Z. Yu[2], [1]*Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*The Ohio State University*, *Columbus, OH, USA*.

34 **Regulation of the small intestine development at preterm weaning.**
L. Kuchkarova*, G. Kudeshova, G. Dustmatova, and I. Karimova, *National University of Uzbekistan*, *Tashkent, Uzbekistan*.

35 **Anti-*Salmonella* and uric acid-preserving effect of pine bark tannin in composted poultry litter.**
C. Arzola-Alvarez*[1], R. C. Anderson[2], M. E. Hume[2], O. Ruiz-Barrera[1], Y. Castillo-Castillo[3], B. R. Min[5], J. A. Byrd[2], D. J. Nisbet[2], J. Salinas-Chavira[4], M. Ontiveros-Magadan[1], and C. Rodriguez-Muela[1], [1]*Universidad Autonoma de Chihuahua*, *Chihuahua, Chihuahua, Mexico*, [2]*USDA, ARS*, *College Station, TX, USA*, [3]*Universidad Autonoma de Cd. Juarez*, *Cd. Juarez, Chihuahua, Mexico*, [4]*Universidad Autonoma de Tamaulipas*, *Cd. Victoria, Tamaulipas, Mexico*, [5]*Tuskagee University*, *Tuskagee, AL, USA*.

36 **Nitro-treatment of composted poultry litter, effects on *Salmonella*, *E. coli*, and nitrogen.**
O. Ruiz-Barrera*[1], C. Arzola-Alvarez[1], Y. Castillo-Castillo[2], A. Corral-Luna[1], R. C. Anderson[3], J. A. Byrd[3], M. E. Hume[3], D. J. Nisbet[3], and J. Salinas-Chavira[4], [1]*Universidad Autonoma de Chihuahua*, *Chihuahua, Chihuahua, Mexico*, [2]*Universidad Autonoma de Juarez*, *Ciudad Juarez, Chihuahua, Mexico*, [3]*ARS/USDA, Texas*, *College Station, TX, USA*, [4]*Universidad Autonoma de Tamaulipas*, *Ciudad Victoria, Tamaulipas, Mexico*.

Immunology (including host–microbe interactions)

30 **Starter feeding altered colonic mucosal bacterial communities and modulated mucosal immune homeostasis during milk-feeding period in lambs.**
J. Liu*[1], G. Bian[2], D. Sun[1], W. Zhu[1], and S. Mao[1], [1]*Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*Tianyi Health Science Research Institute*, *Zhenjiang, Jiangsu, China*.

37 ***Clostridium perfringens* infection of the chicken induces immunometabolic alterations in the duodenum that includes the glycolytic and insulin signaling and NLRP3 inflammasome-mediated inflammatory cell death.**
M. Kogut*[1] and R. Arsenault[2], [1]*USDA/ARS*, *College Station, TX, USA*, [2]*University of Delaware*, *Newark, DE, USA*.

38 **The effects of loxoribine, a toll-like receptor agonist, on *Escherichia coli* O157:H7 and *Salmonella* Typhimurium growth in rumen fluid.**
C. L. Swaggerty* and T. R. Callaway, *USDA/ARS*, *College Station, TX, USA*.

Microbiology (including ecology, (meta)genomics, physiology, and proteomics)

39 **Comparing the responses of rumen ciliate protozoa and bacteria to excess carbohydrate.**
C. Teixeira[1], R. de Paula Lana[1], J. Tao[2], and T. Hackmann*[2], [1]*Universidade Federal de Viçosa*, *Viçosa, Minas Gerais, Brazil*, [2]*University of Florida*, *Gainesville, FL, USA*.

40 **Census of the rumen microbiome using 16s rRNA gene-based pyrosequencing.**
E. A. Latham*[1,2] and W. E. Pinchak[2], [1]*Texas A&M University*, *College Station, TX, USA*, [2]*Texas A&M Agrilife Research*, *Vernon, TX, USA*.

41 **Resolution of the amino acid requirements for *Clostridium scindens* ATCC 35704, a major bile acid-dehydroxylating anaerobe in the human gut.**
R. Shrestha* and S. Daniel, *Eastern Illinois University*, *Charleston, IL, USA*.

42 **Effects of nitrogen source and monensin on *in vitro* ruminal ammonia production and proteolytic bacterial community structure.**
J. Shen*[1,2], Z. Yu[2], and W. Zhu[1], [1]*Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*The Ohio State University*, *Columbus, OH, USA*.

43 **Comparative analysis of bacterial microbial composition from the different ruminal ecological niche of Alxa Bactrian camel.**
J. Zhao*[1,2], Z. Yu[2], G. Wang[1], J. Li[1], and H. Wu[1], [1]*Inner Mongolia University for Nationalities*, *Tongliao, Inner Mongolia, China*, [2]*The Ohio State University*, *Columbus, OH, USA*.

44 **Determination of succession of rumen bacterial species in nursing beef calves.**
K. Smith*[1], A. Garza[1], K. Butterfield[1], A. Dickey[2], A. Lindholm-Perry[2], J. Wells[2], H. Freetly[2], and S. Ivey[1], [1]*New Mexico State University*, *Las Cruces, NM, USA*, [2]*U.S. Meat Animal Research Center*, *Clay Center, NE, USA*.

45 **Identification and biochemical characterization of three bile salt hydrolases in the human gut microbe *Bacteroides vulgatus* ATCC 8482.**
L. Ly*, S. Devendran, and J. Ridlon, *University of Illinois at Urbana-Champaign*, *Champaign, IL, USA*.

46 **Isolation and partial characterization of starch-degrading bacteria belonging to core phylotypes of rumen microbiota in Japanese Black cattle.**
S. Koike*[1], Y. Akiyama[1], T. Hashimoto[1], R. Inoue[2], T. Endo[3], Y. Suzuki[1], and Y. Kobayashi[1], [1]*Hokkaido University*, *Hokkaido, Japan*, [2]*Kyoto Prefectural University*, *Kyoto, Japan*, [3]*Hokkaido Research Organization*, *Hokkaido, Japan*.

47 **Prokaryotes inside and outside of ruminal ciliates isolated from rumen fluid and *in vitro* culture.**
T. Park* and Z. Yu, *The Ohio State University*, *Columbus, OH, USA*.

48 **Draft macronuclear genome of *Entodinium caudatum*.**
T. Park*, S. Wijeratne, T. Meulia, and Z. Yu, *The Ohio State University*, *Columbus, OH, USA*.

49 **Growth inhibitory effects of monensin on ruminal bacteria.**
N. Kim*, I. Cann, and R. I. Mackie, *University of Illinois Urbana-Champaign*, *Urbana, IL, USA*.

50 **A simplified bacterial community in gnotobiotic mice is insufficient to study community dynamics concerning resistant starch digestion.**
J. P. Sementkowski* and N. M. Koropatkin, *University of Michigan*, *Ann Arbor, MI, USA*.

51 **Bacteria and fungi in day-old turkeys vary between companies and flocks.**
A. H. Smith* and T. G. Rehberger, *Agro BioSciences*, *Milwaukee, WI, USA*.

52 **Pectin utilization by the human colonic bacterium *Bacteroides intestinalis* DSM17393.**
H. Müller Paul*, R. I. Mackie, and I. Cann, *Carl R. Woese Institute for Genomic Biology, University of Illinois*, *Urbana, IL, USA*.

53 **Feeding the natural plant extract β-resorcylic acid reduces enteric colonization and down-regulates attachment and gene expression of the foodborne pathogen *Campylobacter* in chickens.**
A. M. Donoghue*[1], B. R. Wagle[2], A. Upadhyay[2], K. Arsi[2], S. Shrestha[2], K. Venkitanarayanan[3], and D. J. Donoghue[2], [1]*Poultry Production and Product Safety Research Unit, ARS, USDA*, *Fayetteville, AR, USA*, [2]*Department of Poultry Science, University of Arkansas*, *Fayetteville, AR, USA*, [3]*Department of Animal Science, University of Connecticut*, *Storrs, CT, USA*.

54 **Elucidating the ecology of *Fibrobacter* spp. in the herbivore gut using comparative genomics.**
A. Neumann* and G. Suen, *University of Wisconsin-Madison*, *Madison, WI, USA*.

55 **Functional analyses of an esterase-enriched polysaccharide utilization locus conserved in diverse human colonic *Bacteroidetes*.**
G. V. Pereira*, D. Wefers, T. Natof, R. I. Mackie, and I. Cann, *University of Illinois at Urbana-Champaign*, *Urbana-Champaign, IL, USA*.

56 **Sequence-based analysis of the genus *Ruminococcus* resolves its phylogeny and reveals strong host association.**
A. J. La Reau*[1], J. P. Meier-Kolthoff[2], and G. Suen[1], [1]*University of Wisconsin-Madison*, *Madison, WI, USA*, [2]*Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures*, *Braunschweig, Germany*.

57 **The effects of nitrate and nitrite on rumen protozoal chemotaxis and yeast growth.**
C. M. Welty[1], S. Waits[1], A. M. Gehman[2], Y. Roman-Garcia*[1], J. Plank[1], R. Meller[1], and J. L. Firkins[1], [1]*The Ohio State University*, *Columbus, OH, USA*, [2]*Alltech Inc.*, *Nicholasville, KY, USA*.

58 **Conversion of 12-ketolithocholate to deoxycholate by *Clostridium scindens*, *Clostridium hylemonae*, and *Clostridium hiranonis*, major bile acid-metabolizing anaerobes in the human gut.**
L. Sallam*[1], J. Ridlon[2], G. Doden[2], H. Doden[2], and S. Daniel[1], [1]*Eastern Illinois University*, *Charleston, IL, USA*, [2]*University of Illinois Urbana-Champaign*, *Champaign, IL, USA*.

Nutrition and metabolism of livestock, humans, and companion animals

60 **Assessment of the effect of high quality hay on rumen health through epithelial gene expression and epimural microbiota.**
R. M. Petri*, M. T. Kleefisch, Q. Zebeli, and F. Kleverhusen, *University of Veterinary Medicine*, *Vienna, Austria*.

61 **Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small intestine-located microbiota.**
K. Perea, K. Perz, S. Olivo, A. Williams, M. Lachman, S. Ishaq*, J. Thomson, and C. Yeoman, *Montana State University*, *Bozeman, MT, USA*.

Prebiotics, probiotics, and DFM development

63 **Effect of antibiotics, Diamond V Original XPC, and *Lactobacillus plantarum* on broiler performance.**
S. Rasoulzadeh[1], S. Rahimi*[1], and K. Akbari[2], [1]*Tarbiat Modares University*, *Tehran, Tehran, Iran*, [2]*National Institute of Genetic Engineering and Biotechnology*, *Tehran, Tehran, Iran*.

64 **Comparison the effect of antibiotic, probiotic, prebiotic, phytobiotic, and *Bacillus subtilis* on broiler performance.**
M. Hamidi[1], S. Rahimi*[1], and N. Mojgani[2], [1]*Tarbiat Modares University*, *Tehran, Tehran, Iran*, [2]*Razi Vaccine and Serum Research Institute*, *Karaj, Alborz, Iran*.

65 **Functional properties of lablab bean husk and soybean husk in hindgut fermentation and microbiota of rats.**
H. Myint*, H.i Kishi, Y. Iwahashi, W. Saburi, S. Koike, and Y. Kobayashi, *Graduate School of Agriculture, Hokkaido University*, *Sapporo, Hokkaido, Japan*.

66 **Characterization of probiotic abilities of lactobacilli isolated from Iranian Koozeh traditional cheese.**
M. Tavakoli[1], Z. Hamidi-Esfahani*[1], M. A. Hejazi[2], M. H. Azizi[1], and S. Abbasi[1], [1]*Tarbiat Modares University*, *Tehran, Tehran, Iran*, [2]*Agricultural Biotechnology Research Institute of Iran*, *Tabriz, E. Azarbayjan, Iran*.

67 **Antagonistic effects of lipids against the bactericidal activity of thymol-β-d-glucopyranoside.**
R. C. Anderson*[1], G. Levent[1,2], B. Petrujkic[1,3], G. Ciftcioglu[2], R. B. Harvey[1], M. H. Hume[1], H. He[1], K. J. Genovese[1], R. C. Beier[1], C. L. Swaggerty[1], T. R. Callaway[1], and D. J. Nisbet[1], [1]*USDA/ARS*, *College Station, TX, USA*, [2]*Istanbul University*, *Istanbul, Turkey*, [3]*University of Belgrade*, *Belgrade, Serbia*.

68 **Identification and characterization of a 20β-hydroxysteroid dehydrogenase from the human gut microbe *Bifidobacterium adolescentis*.**
S. Mythen*, S. Devendran, and J. Ridlon, *University of Illinois at Urbana-Champaign*, *Urbana-Champaign, IL, USA*.

69 **Inhibitory effect of two indigenous *Bacillus* strains on growth of some plant pathogenic fungi and mycotoxins reduction.**
F. Siahmoshteh[1], Z. Hamidi-Esfahani*[1], and M. Razzaghi Abyaneh[2], [1]*Tarbiat Modares University*, *Tehran, Tehran, Iran*, [2]*Pasteur Institute*, *Tehran, Tehran, Iran*.

70 **The effect of antibiotic, probiotic and prebiotic (Diamond Original XPC) in reducing colonization of *Campylobacter jejuni* in intestine of broilers.**
N. Soltani[1], S. Rahimi*[1], and P. Khaki[2], [1]*Tarbiat Modares University*, *Tehran, Tehran, Iran*, [2]*Razi Vaccine and Serum Research Institute*, *Karaj, Alborz, Iran*.

71 **Improving gut microbiome function via high-throughput screening of biological and chemical compounds.**
L. Marsh and M. Hess*, *University of California*, *Davis, CA, USA*.

72 **Composting of laying hen litter with the addition of a yeast probiotic.**
Y. Castillo-Castillo*[1], J. D. Rivera[1], O. Ruiz-Barrera[2], M. Itza[1], C. Arzola[2], M. E. Hume[3], R. C. Anderson[3], J. Salinas[4], and A. Corral[2], [1]*Universidad Autonoma de Ciudad Juarez*, *Ciudad Juarez, Chihuahua, Mexico*, [2]*Universidad Autonoma de Chihuahua*, *Chihuahua, Chihuahua, Mexico*, [3]*USDA/ARS*, *College Station, TX, USA*, [4]*Universidad Autonoma de Tamaulipas*, *Ciudad Victoria, Tamaulipas, Mexico*.

73 **Influence of a direct-fed microbial on growth performance and digestibility of broiler chicks fed commercially available diets.**
J. Barnes*, J. Ison, and R. Carpenter, *BiOWiSH Technologies Inc.*, *Cincinnati, OH, USA*.

Invited Presentations

Early Acquisition and Development of the Gut Microbiota: A Comparative Analysis

1 Mopping up spilled milk: Restoring ecosystem function in the nursing infant gut microbiome.

D. Mills*,
University of California, Davis, CA USA.

Human milk contains numerous components that shape the microbial content of the developing infant gastrointestinal tract. A prominent feature of milk is an array of oligosaccharides and glycoconjugates that serve a passive immune function by sequestering and deflecting pathogens while simultaneously enriching a protective, “milk-oriented microbiota” often dominated by bifidobacteria. Recent research suggests the timing of establishment, and proper function of, the gut microbiota is critical for infant development. This community is initially established through environmental transfer to the gut and subsequently shaped by diet (milk) and host genetics. Once established, infant gut communities dominated by bifidobacteria exhibit low residual milk glycans and higher levels of short chain fatty acids in the feces, suggesting a strongly saccharolytic colonic microbiota. The mechanistic basis for milk glycan consumption by bifidobacteria has been the subject of active research. Different infant-borne bifidobacteria contain specific glycosidases and transport systems required to utilize milk oligosaccharides and glycoconjugates. In aggregate, these studies suggest a co-evolutionary relationship between mammalian milk glycans, infant-borne bifidobacteria and the infant host resulting in a programmed enrichment of a protective bifidobacterial-dominant community during a critical stage of infant development. Disruption of this programmed enrichment, by poor environmental transfer, antibiotic use, or infection, can lead to a “poorly functioning” milk-oriented microbiota that may pose a risk for negative health outcomes. Further analysis of this naturally evolved system will shed light on effective pre- and probiotic tools that support and ensure a protective gut microbiota for at-risk infants.

Key Words: milk, microbiota, infants, prebiotic, probiotic

2 The perinatal microbiome and finding unexpected answers.

K. Aagaard*,
Obstetrics & Gynecology, Division of Maternal-Fetal Medicine and Departments of Molecular & Human Genetics, Molecular & Cell Biology, and Molecular Physiology & Biophysics at Baylor College of Medicine, Houston, TX, USA.

Given the growing body of evidence that many (if not the vast majority) of chronic, non-communicable disease have their origins in fetal life, understanding the *in utero* factors that impact fetal metabolism and development are among the most important public health issues of our time. Based on the work of our lab and others, the past decade has seen the recognition that genomic, Genomic, epigenomic, and metagenomic mediators function in coordination to regulate the offspring developmental phenotype. Conversely, discoordination results in developmental variants or so-called ‘birth defects’. At a more detailed level, structural variants and nucleotide polymorphisms provide individual genomic variation, which can render varying susceptibility to early environmental exposures. Epigenomic variation is comprised of epigenetic modifications, including covalent histone modifications, DNA methylation, and non-coding RNAs. In recent years, we and others have brought forward the concept that another fundamental modifier of the developmental phenotype includes the metagenome, or the microbial community genetic repertoire. However, how and when the human microbiome takes up residence during development is of much debate. Human microbial communities are characterized by their metagenomic and

metabolic diversity, which varies by distinct body sites and influences human physiology. We are only beginning to characterize the complex set of interactions, which alters both community membership and function in early development. With respect to the potential source of microbiota at birth, it has generally assumed that the majority of seeding microbes originate in the lower genital tract, with microbiota ascending into the otherwise sterile intrauterine. However, we and others have recently demonstrated that (1) the vaginal and gut microbiome communities are distinctly structured in pregnancy, and (2) the placenta is in fact not sterile, but rather harbors a low-abundance microbiome, and (3) the maternal diet, and notably a high fat diet, has a particularly strong impact on the developing and early in life microbial community structure. Through ongoing longitudinal metagenomic studies characterizing the human and primate antenatal and perinatal microbiome, we are piecing together new understandings as to when, how and where it varies in the course of human gestation. We have taken 2 approaches to answering these questions in our studies. First, we use longitudinal cohorts of maternal-infant dyads collected across gestation and through 6 weeks post-delivery. Second, we utilize our well-established primate models of maternal high fat dietary exposure, both in the absence and presence of maternal obesity. Collectively, these recent experimental vignettes are examples of where understanding phenotype-genotype associations render insight to evolution and human reproductive traits. With mindful design of clinical translational studies accompanied by robust molecular and clinical data, we and others will continue to assemble the capacity to significantly discern causal inference of the role of the maternal and placental microbiome in the timing of parturition and establishment of the early microbiome. Key remaining questions include understanding what the true role of Cesarean delivery is in shaping the infant microbial community, or alternately understanding what maternal or pregnancy factors resulting in Cesarean delivery are actually the drivers of microbial variation. Finally, future efforts need to focus on ongoing studies relating perinatal exposures to both metabolic and behavioral health in the offspring.

3 Early life nutrition and gut microbiome development in the piglet.

S. M. Donovan*, M. H. Monaco, and M. Wang,
University of Illinois, Urbana, IL, USA.

The development of intestinal microbiota is a complex process influenced by host genetics and environmental factors such as gestational age, delivery mode, diet, pre- and probiotics, and antibiotics. The microbiota acquired in early life have short- and long-term implications for host metabolism and gastrointestinal, immune, and neurological function. This presentation will summarize our current understanding of the piglet microbiota using reports in the literature and our own findings. Over the past decade, we have investigated the development of gut microbiota composition and function in sow and artificially reared piglets. In addition, we have assessed how dietary milk oligosaccharides and prebiotics influence microbiota abundance, composition, and metabolic products. Last, we investigated the therapeutic potential of milk oligosaccharides and prebiotics to ameliorate the severity of infectious diseases in the piglet model. Our findings show that the sow-reared piglet microbiota develops over the first 14 d of life and is relatively constant until weaning at d 28, when a dramatic shift in composition is observed. Additionally, the microbiome of artificially reared piglets differs from that of sow-reared piglets, but is not significantly affected by route of delivery (Cesarean section vs vaginal). Prebiotics and milk oligosaccharides are supplemented to infant formula to act as prebiotics and provide fermentable substrates. We have shown that human milk oligosaccharides and oligosaccharides (FOS and GOS) significantly reduce the duration of rotavirus-induced diarrhea, affect microbiota composition and modulate the immune response to rotavirus infection. The piglet is an exceptional preclinical model for the human infant in terms of gastrointestinal, immune, and cognitive development. Therefore, investigations into the development and modulation of the piglet microbiome have dual purpose to improve both piglet and infant health.
(Funded by R01 HD061929.)

Key Words: *Sus scrofa*, microbiome, prebiotic, milk oligosaccharide

4 Assessment of neonatal gut microbiomes revealed microbial markers linked to calf gut health.

N. Malmuthuge[1,2], G. Liang[1,3], and L. L. Guan*[1],
[1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Vaccine and Infectious Disease Organization-International Vaccine Centre, University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.*

The understanding on pre-ruminant gut microbiome is very limited. Therefore, this study characterized the small intestinal (jejunum and ileum) microbiome of pre-weaned calves, aiming to understand the dynamics of postnatal microbial establishment within the first 6 weeks of life and how it potentially interacts with host mucosal immune system. Next-generation sequencing of digesta- and tissue-associated communities revealed remarkable variations in the microbial composition and the relative abundance of detected bacterial groups among individuals. Despite the highly individualized microbiome, we identified 2 taxonomic-based clusters of calves that were comprised of either high levels of *Lactobacillus* or *Bacteroides*. Moreover, *Bacteroides*, *Prevotella*, *Roseburia*, *Ruminococcus*, and *Veillonella* were low abundant or absent in the ileum of *Lactobacillus*-dominant calves. The analysis of metagenome profiles revealed that calves can be grouped to 2 function-based clusters with either high protein metabolism (cluster 1) or sulfur metabolism (cluster 2). When the small intestinal transcriptome was profiled, it indicated that the first week after birth is a very dynamic developmental period for the intestinal mucosal immune system. Similar changes were observed in the expression of miRNAs and microbiome during the first week of life, suggesting that the changes observed at transcriptome level may be regulated by both miRNAs and microbial colonization. Besides, the ileal transcriptome of the calves belonged to 2 taxonomic-based clusters revealed varied immune responses. The present study revealed that an establishment of small intestinal-specific microbiota from birth, and there are microbial markers (microbial functions and taxonomy) that can be used to broadly categorize calves, regardless of the highly individualized early microbiome. Findings from this study indicate that the colonizing microbiome is an essential factor regulating the rapid development of the mucosal immune system during the first week of life.

Key Words: neonatal gut microbiota, intestinal transcriptome, host-microbial interaction, immune function

5 The dynamics of microbiota development in chickens.

R. J. Moore*[1], R. J. Hughes[2], and D. Stanley[3],
[1]*RMIT University, Bundoora, Victoria, Australia,* [2]*South Australian Research and Development Institute, Roseworthy, South Australia, Australia,* [3]*Central Queensland University, Rockhampton, Queensland, Australia.*

Microbiota development in chickens is fundamentally different to that which occurs in other animals, including humans. In commercial chicken production, the birds are hatched in a clean environment and have no contact with adult birds. Therefore, chicks are not exposed to “chicken” microbiota but rather to microbes from diverse environmental origins. This random stochastic process of initial exposure has significant consequences for the subsequent development and stabilization of the gut microbiota. The most obvious consequence is the highly variable composition of gut microbiota found in commercial chickens. Different flocks of birds are often found to have very different microbiota compositions and even within a flock there can be very large bird-to-bird variations; even though chickens are coprophagic. The dramatic differences in microbiota composition in flocks that are derived from the same genetic line and have been exposed to the same environments, feed, and water indicate that the very earliest steps in microbiota formation must have profound and long-lasting influences on the establishment of adult microbiota. Experimental exposure of chicks to adult cecal microbiota derived from birds that were highly efficient at using energy did not improve the growth performance of the naïve chicks but did reduce

variation within a flock. Study of the dynamics of intestinal microbiota development over time showed that some genera, such as *Lactobacillus* varied little over time whereas the abundance of *Fecalibacterium* showed a slow and steady increase over time and *Enterobacter* was abundant only in the first few days post-hatch. It is important to understand how the gut microbiota becomes established and matures over time if we are to achieve our goal of manipulating the microbiota to maximize health and productivity outcomes in production animals.

Key Words: gut microbiota, chicken, dynamics, colonization

2017 Opening Session

6 Bacterial energetics: The sixth antimicrobial target space for drug development.

G. M. Cook*,
University of Otago, Department of Microbiology and Immunology, Dunedin, Otago, New Zealand.

The emergence and spread of drug resistant pathogens and our inability to develop new antimicrobials to combat resistance and persistence has motivated scientists to consider non-traditional targets where human homologs clearly exist. Bacterial energetics is an area showing promise for the development of new antimicrobials, but the success of this area will only grow further by understanding how these new drugs work against energetic targets in growing and non-replicating cells. The poorly defined molecular mechanisms of many antimicrobials are a hindrance to rational, mechanism-based drug discovery. Bedaquiline (BDQ), the first anti-tubercular to be approved by the FDA in 40 years, targets the mycobacterial F1Fo-ATP synthase, but not the mitochondrial enzyme. BDQ inhibits ATP synthesis leading to slow time-dependent cell killing. The mechanism of cell death was proposed to be due to ATP depletion. We hypothesized that mechanisms alternative to ATP depletion may be important in BDQ's bactericidal mode of action. Importantly, we proposed that the F1Fo-ATP synthase is critical for homeostatic control of the protonmotive force and inhibition of the complex may lead to uncoupling of the respiratory chain. We report that BDQ addition to resting mycobacterial cell suspensions activates oxygen consumption and dissipates the transmembrane pH gradient suggesting an uncoupler-like mode of action for BDQ. Activation of respiration was dependent on the terminal oxidase cytochrome bd (cydAB) and cydAB mutants were hypersusceptible to BDQ. Characterization of the BDQ mode of action in lipid-only (no ATP synthase target) vesicles demonstrated that bedaquiline could shuttle protons across lipid-bilayers with high affinity. Its relationship to the current modes of action in mycobacteria, or its effect on the mitochondrial inner membrane, will be discussed.

Key Words: bacterial energetics, mycobacteria, bedaquiline, uncouplers, energy generation

7 Glycan uptake in the gut *Bacteroidetes*: The Sus paradigm.

N. Koropatkin*,
University of Michigan.

The mammalian gut *Bacteroidetes* display a greatly expanded capacity for glycan degradation, with many having the ability to flexibly forage on at least a dozen complex polysaccharides. This glycolytic potential is packaged into discrete polysaccharide utilization loci (PUL) that encode the necessary machinery for the degradation and import of a distinct glycan structure. PUL-encoded protein complexes are referred to as starch utilization (Sus)-like systems after the first such system for starch import described in *Bacteroides thetaiotaomicron*. Sus-like protein complexes are present in nearly every gut *Bacteroidetes* yet restricted to this phylum, and their glycan specificity dictates the bacterial metabolic niche. The Sus of *B. thetaiotaomicron* is perhaps the simplest and best characterized of these glycan uptake systems, though more recently many other systems that target host mucins, hemicellulose, and even peptides

have been elucidated. All comprise a putative TonB-dependent transporter and 2 classes of carbohydrate-binding proteins: the SusD-like proteins and the surface glycan-binding lipoproteins (SGBPs). Within individual Sus-like systems, there is some redundant polysaccharide binding between the SusD-like proteins and the SGBPs, yet each protein plays a distinct role in carbohydrate import. Much of our work with the complexes for the acquisition of starch and xyloglucan has revealed that the presence of these proteins, and likely their interactions with the TonB-dependent transporter, are more important than their ability to bind glycan. Through a detailed understanding of how human gut bacteria acquire carbohydrate nutrition in the highly competitive gut ecosystem, we can develop prebiotic and probiotic strategies to manipulate the composition of this community toward improved human health.

Key Words: *Bacteroidetes*, carbohydrate, Sus, protein structure

8 Functional analysis of the gut microbiota using single-cell isotope probing.

D. Berry*,
University of Vienna, Vienna, Austria.

Microbial communities are essential for human nutrition and health as well as for the function of virtually all ecosystems. It remains challenging, however, to identify the activity and function of microbial cells under natural conditions. In recent years, sequencing-based approaches such as metagenomics and metatranscriptomics have greatly expanded our understanding of the composition and metabolic potential of the gut microbiota. Now, new tools are needed to determine the actual activities of the microbes in these communities. Stable isotope-based approaches are powerful tools to reveal microbial function in situ. This talk will focus on how microbes can be studied at the single-cell level using molecular methods combined with 2 powerful chemical imaging tools: nano-scale secondary ion mass spectrometry (NanoSIMS) and Raman microspectroscopy. We will discuss how isotope probing can be used to identify the microbes that are utilizers of specific compounds such as host- and diet-derived nutrients. We will also introduce a recently developed method to determine the general activity of cells using heavy water (D_2O). In this approach, active cells, irrespective of their physiology, incorporate D into their biomass, which can then be detected with Raman microspectroscopy or NanoSIMS. D-incorporation is a highly sensitive marker and activity can be detected before a single cell division. This can be combined with fluorescence in situ hybridization for the identification of active microbes. Additionally, labeled cells can subsequently be sorted using optical tweezers, allowing for targeted single cell genomics. Examples will be given of how these techniques can be used to study gut microbiota utilization of a range of compounds, including mucosal proteins, host-derived amino acids, and dietary and mucosal sugars, polysaccharides, and glycans. Future directions for these exciting new techniques will also be highlighted.

Key Words: mucus microbiota, stable isotope probing, single-cell genomics

Oral Abstracts
Podium presentations: Session 1

9 Modulation of the human gut microbiota—An ecological perspective.

J. Walter*,
University of Alberta, Edmonton, Alberta, Canada.

Diverse strategies have been used for several decades to improve human and animal health through the modulation of the gut microbiota, spanning from the administration of defined probiotic strains (or live biotherapeutics), whole microbial consortia (e.g., fecal bacteriotherapy), to the provision of bacterial growth substrates (prebiotics and dietary fiber). However, we still lack a conceptual understanding on how the gut microbiota can be modulated. In this presentation, I will summarize how ecological theory can provide a framework by which to understand characteristics of the human gut microbiota and the impact of microbiome-modulating strategies. I will present some of our own studies that investigated basic ecological questions regarding the temporal, spatial, and global patterns of the human microbiome, the factors that shape these patterns, and the ecological constrains within the human microbiome can be manipulated by diet and probiotics. The methodological toolset that is now available (e.g., through next-generation sequencing) provides an unprecedented opportunity to obtain phylogenetic, compositional, and functional information of microbial communities. When analyzed in the light of ecological theory, this has the potential to elucidate the factors and ecological processes that determine and potentially predict the response of the gut microbiota to therapeutic modulations.

Key Words: ecological theory, community ecology, microbiome modulation, probiotic

10 High-resolution tracking of microbial colonization in fecal microbiota transplantation experiments via metagenome-assembled genomes.

S. T. M. Lee*[1], S. Kahn[1], T. Delmont[1], N. Hubert[1], H. Morrison[2], D. Antonopoulos[1], D. Rubin[1], and A. Eren[1,2],
[1]*University of Chicago Medicine, Chicago, IL, USA,* [2]*Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA, USA.*

Fecal microbiota transplantation (FMT) is an effective treatment for recurrent *Clostridium difficile* infection and shows promise for treating other medical conditions associated with intestinal dysbioses. However, we lack a sufficient understanding of which microbial populations successfully colonize the recipient gut, and the widely used approaches to study the microbial ecology of FMT experiments fail to provide enough resolution to identify populations that are likely responsible for FMT-derived benefits. We used shotgun metagenomics together with assembly, and binning strategies to reconstruct metagenome-assembled genomes (MAGs) from fecal samples of a single FMT donor. We used mapping to follow the occurrence and distribution patterns of donor MAGs in 2 FMT recipients to investigate colonization properties of transferred microbial populations. Our analyses revealed that 22% of the 92 highly complete bacterial MAGs we identified from the donor successfully colonized and remained abundant in 2 recipient guts for at least 8 weeks. Most MAGs with high colonization rate belonged to the order *Bacteroidales*. The vast majority of those that lacked evidence of colonization belonged to the order *Clostridiales* and colonization success was negatively correlated with the number of genes related to sporulation. Although our data set showed a link between taxonomy and the colonization ability of a given MAG, we also identified MAGs that belong to the same taxon with different colonization properties, highlighting the importance of appropriate level of resolution

to explore the functional basis of colonization and to identify targets for cultivation, hypothesis generation, and testing in model systems. The analytical strategy adopted in our study can facilitate the identification of bacterial population genomes that may be critical to the success of FMT due to their colonization properties, and provide genomic insights to leverage our investigations beyond associations, and ultimately reveal the mechanistic underpinnings of this procedure.

Key Words: fecal microbiota transplantation, colonization, metagenomics, metagenome-assembled genomes

11 Using culturomics approaches to quantitate the diversity of bacteria that can be recovered from cultivation of infant feces.

X. Pang[1], C. Méndez-García*[2], S. Donovan[2], M. Wang[2], M. Siegel[2], I. Cann[2], and R. Mackie[2],
[1]*Shanghai Jiao Tong University, Shanghai, China,* [2]*University of Illinois, Urbana, IL, USA.*

There is a renewed interest in cultivation to isolate bacteria from the gut microbial ecosystem to better understand community composition at deeper taxonomic levels, as well as the ecological niche occupied by its microbial members. In this work, we lay the foundation for the quantification of culturability of bacteria present in the infant gut through culturomics. The bacterial composition of fecal samples from 3 breast-fed and 3 formula-fed vaginally delivered infants sampled at 3 and 12 mo was analyzed by 16S rRNA gene amplicon sequencing. Samples from breast-fed subjects were selected for culturomics using a Gut Microbiota Medium (GMM) and a habitat-simulating medium containing rumen fluid (RF). Further 16S rRNA amplicon sequencing was applied to plate washes of all colonies growing on plates at specific dilutions. The fecal communities of 3-mo-old breast-fed infants were dominated by *Bifidobacterium* (phylum *Actinobacteria*). The microbial diversity recovered at 12 mo was higher as compared with 3 mo, and *Bacteroides* (*Bacteroidetes*) was the most abundant genus detected at this age. In contrast, fecal samples from 3 mo old formula-fed babies showed increased proportions of *Enterobacteriaceae*, and the progression to a *Bacteroides*-enriched community was not observed. Isolates recovered included *Bifidobacterium*, *Bacteroides*, *Prevotella*, *Ruminococcus*, *Streptococcus*, *Lactobacillus*, *Clostridium*, *Veillonella*, *Enterococcus*, *Eubacterium*, *Staphylococcus*, *Dorea*, and *Collinsella* spp., as well as potentially new species (>20% of isolates for each condition). GMM better represented the composition of the original samples, with a maximum fraction of diversity recovered (measured as % of OTUs recuperated) of 44.5% of a dilution of 10^{-5}. Our culturomics endeavor resulted in 3 major findings: (1) extensive culturing to recover the highest bacterial diversity from the infant gut; (2) generated an index for the cultivability of infant gut bacteria; and (3) identified strong bacterial candidates for future design of a simplified infant gut microbial consortium to test in animal models.

Key Words: infant gut, bacterial diversity, 16S rRNA, culturomics

12 Large-scale network analysis of food, nutrients, and microbiome.

P.-J. Kim*,
Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea.

Recent progress in data-driven analysis methods, including network-based approaches, is revolutionizing many classical disciplines. These techniques can also be applied to food and nutrition, which must be studied to design healthy diets. Using nutritional information from over 1,000 foods, we systematically evaluated the nutrient composition of each food in regards to satisfying daily nutritional requirements. The nutrient balance of a food was quantified and termed “nutritional fitness.” Nutritional fitness offers a way to prioritize recommendable foods within a global network of foods, in which foods are connected based on the similarities of their nutrient compositions. We identified several key nutrients whose levels in foods can critically affect the nutritional fitness of the foods. Analogously, pairs of nutrients can have the same effect. This result, involving the tendency among nutrients to exhibit correlations in their abundances across foods, implies a hidden layer of complexity when exploring for foods whose balance of nutrients within pairs holistically helps meet

nutritional requirements (published, PLoS ONE 10 e0118697). However, considering only the direct effect of nutrients on our body may not be enough to understand the full picture of dietary effects. For example, our resident gut microbial community, or gut microbiome, is largely affected by our diets, and linked not only to our health, but also to various disorders such as obesity, cancer, and diabetes. We constructed the comprehensive literature-curated global interaction network of the human gut microbiome mediated by various chemicals. Using our network, we investigated the disease-related infrastructure of the gut microbial ecosystem in type 2 diabetes (T2D) patients. Our network analysis reveals core microbial groups with distinctively large metabolic influence, and their metabolic products that may contribute toward disease through maintaining the structural integrity of T2D-specific microbial communities. Our network framework shows promise for investigating complex microbe-microbe and host-microbe chemical cross-talk implicated in disease.

Key Words: big data analysis, network science, diet, human nutrition, gut microbiome

13 Evaluation of organism identification for 16S rRNA sequencing of chicken cecal microbiome.

J. C. Hsieh*[1], C. J. Schmidt[2], and S. J. Lamont[1],
[1]*Iowa State University, Ames, IA, USA,* [2]*University of Delaware, Newark, DE, USA.*

Profiling of the microbiome with 16S rRNA sequencing is the most common approach used to analyze microbial samples collected from human and animals. Despite the wide-spread use of 16S analysis, high resolution identification of organisms present in microbial samples is a difficult task for bioinformatics tools such as QIIME, Mothur, and MG-RAST. The Affymetrix Axiom Microbiome Array provides a novel solution to high-resolution organismal identification through targeted sequencing. In this study, we evaluated organisms identified by MG-RAST from digesta sampled from the chicken ceca using the results from targeted sequencing. Chicken cecum digesta samples (n = 80) were flash-frozen in liquid nitrogen after collection. DNA was isolated with MO-BIO PowerFecal DNA Isolation Kit. 16S amplicons were generated with a V1-V3 primer set and sequenced with Illumina MiSeq 300x2. Sequencing outputs were submitted to MG-RAST and profiling was performed against the M5nr database. A threshold of >1% of total reads was applied to remove organisms identified from spurious mapping. DNA was also submitted to Affymetrix for profiling by the Axiom Microbiome Array. Positive predictive value (PPV) and sensitivity was calculated for MG-RAST identifications assuming the identification by targeted sequencing as standard. MG-RAST had an average PPV of 0.38 (SD = 0.06) at the genus level and 0.54 (SD = 0.10) at the family level. For sensitivity, the average was 0.44 (SD = 0.07) at the genus level and 0.57 (SD = 0.08) at the family level. MG-RAST was consistently not able to identify the *Bifidobacterium* genus due to the variable region chosen for 16S sequencing. There was also no correlation ($R^2 = 0.04$) between the number of organisms identified by targeted sequencing and the α-diversity predicted by MG-RAST. We demonstrated the limitations of high-resolution organism identification with 16S rRNA sequencing in this data set. The availability of a new independent data source from targeted sequencing opens the possibility for the evaluation of performance of specific bioinformatics tools for organism identification on experimental data.

Key Words: chicken, bioinformatics, 16S rRNA, MG-RAST, targeted sequencing

14 Stable isotope tracking of metabolically active rumen bacteria in an *in vitro* system under acidotic conditions.

R. M. Petri* and Q. Zebeli,
University of Veterinary Medicine, Vienna, Austria.

Rumen microbes elicit crucial metabolic functions and are important for cattle health. High production feeding practices induce sub-acute ruminal acidosis (SARA) and ecological imbalances in the rumen. In this study, we simulated SARA conditions in an *in vitro* Rusitec model to evaluate rumen microbiota, their metabolic viability and function. Four treatment groups using 12 fermenters were established with either the addition of C^{12}-lactate or C^{13}-

lactate to both the control and SARA diets (n = 3/treatment). The addition of C^{13} was used to allow for high-density centrifugation to determine the metabolically active bacteria groups. Rumen microbial samples were taken daily over 10 d with SARA being induced on d 6–10. Replicates were pooled for each day. DNA was extracted and underwent 16S rRNA MiSeq paired-end sequencing. Analysis for microbial diversity showed significant effects ($P < 0.05$) of the SARA conditions on bacterial populations. This corresponds to the significant effect of the SARA model on the mean pH ($P = 0.03$) and trend on redox values ($P = 0.06$). Statistical analysis showed 50 OTUs were significantly affected by SARA ($P < 0.05$) and/or the addition of lactate to the fermenter. Of the 11 OTUs that were impacted by the addition of lactate, the 4 were identified as belonging to the *Bacteroidales* family and 5 were identified as *Clostridiales* family. Predicted metabolic pathway analysis (PICRUSt) showed an effect of SARA on membrane transport and carbohydrate metabolism pathways. Samples of microbial DNA from control and SARA treatments were processed using high density gradient centrifugation and the resulting heavy DNA was used for targeted qPCR based on *Clostridiales* targets identified from sequencing analysis. Total gene copies for Bacteria, *Clostridium* Cluster XIVa and *Prevotella* genus were higher in the control treatment compared with the SARA diet. However, total gene copies for *Ruminococcus albus* were increased in the heavy DNA fraction of the SARA diet. This research validates the use of stable isotope tracking in rumen *in vitro* analysis for identification of metabolically active microbial groups.

Key Words: C^{13}-lactate, subacute acidosis, MiSeq, PICRUSt

Podium presentations: Session 2

15 Microbial endocrinology—How does stress and tension influence the composition and functioning of gut bacteria?

P. Freestone*,
University of Leicester, United Kingdom

Animals share a close and life-long relationship with a huge variety of resident microbial species. The microbiota, particularly within the gut, have been shown to aid nutrition, regulate metabolism and shape development and function of the nervous and immune systems. More recently, studies have suggested that the gut microbiota can even directly modulate the mood of their host. Evidence is considerable that a bidirectional communication takes place between animals and their microbiome co-habitants, and that this inter-kingdom dialog is capable of directly influencing health. This talk explores how host hormonal signals are sensed by the gut microbiota, and what in return the microbiome residents are signaling to their hosts that can affect physical and even mental wellbeing.

Key Words: gut microbiota, stress, interkingdom dialogues

16 Environmental pollutant benzo[a]pyrene impacts the volatile metabolome and transcriptome of the human gut microbiota.

C. Defois*[1], J. Ratel[2], S. Denis[1], F. Mercier[2], C. Gasc[1], E. Peyretaillade[1], E. Engel[2], and P. Peyret[1],
[1]*UMR* 454 *Microbiologie, Environnement Digestif et Santé, Clermont-Ferrand, France,* [2]*Institut National de la Recherche Agronomique UR*370 *Qualité des Produits Animaux, Saint-Genes-Champanelle, France.*

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous persistent pollutants arising from oil and derivatives along with incomplete organic matter combustion. The latter including both anthropogenic (engine exhausts, domestic heating, tobacco smoke, some cooking practices) and natural sources (forest fires, volcanism). Population exposure occurs mainly with contaminated food products, which introduces the pollutant to the digestive tract. Benzo[a]pyrene (B[a]P), being the most studied PAH due to its high mutagenic and carcinogenic properties, has been chosen as

marker of PAH occurrence and toxicity. While its metabolism by host cells is well known, the impact on the gut microbiota, which plays a key role in human health remains unexplored. By means of 16S barcoding, metatranscriptomics and volatolomics, we studied the impact of B[a]P on human gut microbiota. B[a]P exposure did not induce a significant change in the microbial structure; however, some slight modifications appeared as the increase of the genera *Sutterella*, *Sporanaerobacter* and *Oscillospira* and the reduction of *Rikenellaceae* and *Blautia*. B[a]P exposure alters the microbial volatolome dose-dependently. While the studied microbiota structures were clearly different, their metabolic response was rather similar. Metatranscriptomes revealed that the transcript levels related to several metabolic pathways were upregulated such as vitamin and cofactor metabolism, cell wall compound metabolism, DNA repair and replication systems, and aromatic compound metabolism, whereas the transcript levels related to the glycolysis-gluconeogenesis pathway and bacterial chemotaxis toward simple carbohydrates were downregulated. These primary findings show that food pollutants, such as B[a]P, alter human gut microbiota activity. The observed shift in the volatolome demonstrates that B[a]P induces a specific deviation in the microbial metabolism. Major adaptive mechanisms of the fecal microbiota to counteract the toxic properties of the pollutant have been revealed by metatranscriptomics.

Key Words: human gut microbiota, polycyclic aromatic hydrocarbons, 16S amplicon sequencing, volatolomics, metatranscriptomics

17 Chemerin is present in intestinal epithelia of calves with a potential role in barrier function.

Y. Suzuki*[1], S. Roh[2], H. Hayashi[3], S. Koike[1], and Y. Kobayashi[1],
[1]*Hokkaido University, Sapporo, Hokkaido, Japan,* [2]*Tohoku University, Sendai, Miyagi, Japan,* [3]*Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.*

Maintenance of the environment in gastrointestinal tract (GIT) is essential for health and prevention of diseases in calves. Intestinal epithelial barrier consists of highly integrated mechanisms including tight junction, mucin layer, immune cells, cytokines and antimicrobial peptides. Chemerin is a secreted protein with antibacterial property and chemoattractant ability to antigen-presenting cells. Our previous data demonstrated that chemerin was expressed within mammary epithelial cells and secreted into milk of dairy cow, suggesting this protein as a novel regulator of nasal immunity. This study aimed to investigate the presence and expressional change of chemerin in GIT of calves, and to understand its physiological role in the intestinal epithelial barrier. Gene and protein expression of chemerin in the epithelia of gastrointestinal tract (forestomach, small intestine and large intestine) of 3-week-old Holstein calves (n = 4) were investigated by RT-PCR and immunoblotting. Chemerin-expressing cells in GIT were visualized by immunohistochemistry. The levels of mRNA and protein of chemerin in duodenum, jejunum and ileum were measured by RT-qPCR and immunoblotting, and compared between different ages (3-week-old, 13-week-old, and 10-mo-old; n = 3 to 7 at each age). Differences in expressional levels of chemerin were considered significant when $P < 0.05$ by Tukey's HSD test. As a result, chemerin mRNA was highly expressed in duodenum, jejunum, and ileum compared with the other parts of GIT. Accordingly, chemerin protein was detected in these tissues. Most of chemerin-positive cells were present in basal portion of small intestinal villi. Expression level of chemerin in ileum was significantly lower in 10-mo-old calves, whereas those in duodenum and jejunum were not different between ages. In summary, chemerin is present in intestinal epithelia of calves, whose level changes as calves grow. This study indicated the potential role of chemerin in immune and barrier capacity of intestinal epithelia due to its antibacterial and chemotactic ability.

Key Words: calf, small intestine, epithelial barrier, chemerin

18 The curious case of ruminal cellulose degradation: New players and old.

A. E. Naas[1], L. M. Solden[2], I. M. Heggenes[1], N. M. Koropatkin[3], R. I. Mackie[4], V. G. H. Eijsink[1], K. C. Wrighton[2], M. Ø. Arntzen[1], and P. B. Pope*[1],

[1]*Norwegian University of Life Sciences, Aas, Norway,* [2]*The Ohio State University, Columbus, OH, USA,* [3]*University of Michigan Medical School, MI, USA,* [4]*University of Illinois at Urbana-Champaign, IL, USA.*

In nature, obligate herbivorous ruminants have a close symbiotic relationship with their gastrointestinal microbiome, which proficiently deconstructs plant biomass. Despite decades of research, cellulose degradation in the rumen has thus far mostly been attributed to a limited number of culturable representatives, including *Firmicutes* and *Fibrobacteres* species. The infamous *Fibrobacter succinogenes* uses an enigmatic mechanism that does not comprise cellulosomes nor the well-known cellobiohydrolase genes that are considered essential in secreted cellulase systems. Moreover, the *Bacteroidetes* are known to exert large influence within the rumen, however evidence for association of this phylum with cellulose degradation is limited. We have used meta-omics, bioinformatics and biochemistry to obtain new information pertaining to polysaccharide hydrolysis by *F. succinogenes* and to discover and characterize a novel cellulolytic *Bacteroidetes* family ('Candidatus MH11') that is not closely related to any previously described taxa. We demonstrate the hydrolytic capacity of outer membrane vesicles (OMVs) that are released by *F. succinogenes* when grown on cellulose. In particular, OMVs were found to contain membrane-bound multiprotein complexes and are able to depolymerize cellulose and a broad range of linear and branched hemicelluloses and pectin. With respect to the *Bacteroidetes*, metabolic reconstruction of the first Ca. MH11-affiliated genome bin ('Candidatus *Polyenzymogenes ruminantium*') revealed a comprehensive inventory of carbohydrate active enzymes, including predicted cellulases that are putatively secreted via the *Bacteroidetes*-specific Type 9 secretion system. Selected genes have been expressed and their products biochemically characterized *in vitro*, including multi-modular cellulases with 2 or more catalytic domains, which are modular arrangements that are unique to rumen *Bacteroidetes*. We will discuss these results in the greater context of the varying mechanisms that ruminal microorganisms employ to degrade cellulose.

Key Words: rumen microbiology, metagenomics, metaproteomics, biochemistry, cellulose

19 New roles in hemicellulosic sugar fermentation for the uncultivated *Bacteroidetes* family BS11.

L. M. Solden*[1], D. W. Hoyt[2], W. B. Collins[3], J. E. Plank[1], R. A. Daly[1], E. Hildebrand[4], R. Wolfe[1], C. D. Nicora[2], S. O. Purvine[2], M. Carstensen[4], M. S. Lipton[2], D. E. Spalinger[5], J. L. Firkins[1], B. A. Wolfe[1], K. C. Wrighton[1],

[1]*The Ohio State University, Columbus, OH, USA,* [2]*Pacific Northwest National Laboratory, Richland, WA, USA,* [3]*Alaska Department of Fish and Game, Palmer, AK, USA,* [4]*Minnesota Department of Natural Resources, Forest Lake, MN, USA,* [5]*University of Alaska Anchorage, Anchorage, AK, USA.*

Ruminants have co-evolved with their gastrointestinal microbial communities that digest plant materials to provide energy for the host. Some arctic and boreal ruminants have already shown to be vulnerable to dietary shifts caused by changing climate, yet we know little about the metabolic capacity of the ruminant microbiome in these animals. Here we use meta-omics approaches to sample rumen fluid microbial communities from Alaskan moose foraging along a seasonal lignocellulose gradient. Winter diets with increased hemicellulose and lignin strongly enriched for BS11, a *Bacteroidetes* family lacking cultivated or genomically sampled representatives. We show that BS11 are cosmopolitan host-associated bacteria prevalent in gastrointestinal tracts of ruminants and other mammals. Metagenomic reconstruction yielded the first 4 BS11 genomes; phylogenetically resolving 2 genera within this previously taxonomically undefined family. Genome-enabled metabolic analyses uncovered multiple pathways for fermenting hemicellulose monomeric sugars to short-chain fatty acids (SCFA), metabolites vital for ruminant energy. Active hemicellulosic sugar fermentation and SCFA production was validated by shotgun proteomics and rumen metabolites, illuminating the role BS11 play in carbon transformations

within the rumen. Our results also highlight the currently unknown metabolic potential residing in the rumen that may be vital for sustaining host energy in response to a changing vegetative environment.

Key Words: ruminant, *Bacteroidetes*, metagenomics, nutrition, arctic

20 Analysis of the ammonium assimilation pathways of the human colonic bacterium *Bacteroides thetaiotaomicron*.

M. Iakiviak*, I. K. O. Cann, and R. I. Mackie,
University of Illinois, Urbana-Champaign, IL, USA.

In ruminants, efficient rumen function and proper host metabolism is dependent on the nitrogen supply in animal feeds. Assimilated ammonium accounts for up to 70% of the microbial protein production, which satisfies up to 85% of the host protein requirements. Similar numbers for the human colon have not been determined. However, colonic bacteria are responsible for the production of ammonium, derived from host-secreted urea and endogenous and dietary proteins, that provides the preferred nitrogen source for microbial growth. *Bacteroides thetaiotaomicron*, a model organism for human gut *Bacteroidetes*, encodes genes for the capture of ammonium through the 2 primary pathways, the glutamate dehydrogenase (GDH) pathway and the glutamine synthetase/glutamate synthase (GS/GOGAT) pathway. To gain insight into the genomic features underlying ammonium uptake and assimilation in this bacterium, comparative transcriptomic analysis using RNA-Seq was employed on cultures growing under excess or limiting ammonium concentrations. A single genomic locus was identified with highly increased transcription when the organism grows under limiting ammonium concentration, encoding for the GS/GOGAT pathway. The relative contribution of each gene to ammonium assimilation was assessed through construction of genomic deletion strains for each of the 3 GS, 1 GOGAT, and 2 GDH genes. The deletion of one of the glutamate dehydrogenase (gdhA) significantly impeded growth of the organisms under both nitrogen conditions. Taken together, the results demonstrate the importance of the GDH pathway for constitutive ammonium assimilation. However, when the organism grows under nitrogen limitation, the GS/GOGAT pathway is induced. To extrapolate the significance of the findings, a comparative bioinformatic analysis, using all of the available sequenced *Bacteroides* genomes, revealed high conservation of the critical genomic loci in gut species. Understanding of nitrogen metabolism in gut microbes is essential for a complete depiction of their ecological implications on the host's metabolism in health and disease.

Key Words: *Bacteroides*, nitrogen, glutamine synthetase, RNA-Seq, glutamate synthase

Podium presentations: Session 3

21 PorkBoostEB: A new *Bacillus subtilis*-based probiotic that improves gut health in piglets.

R. Cernat* and B. Nielsen,
Chr. Hansen A/S, Hørsholm, Denmark.

The scheduled phase-out of specific antibiotic growth promoters in the EU requires management changes and a need for new probiotics among which *Bacillus* spp.-based feed additives are highly relevant for pig industry. This work was aimed at screening a pool of 260 new spore formers and selecting the best 2 candidates for *in vivo* trials. PorkBoostEB was identified as *Bacillus subtilis* ssp. *subtilis*. *In vitro* screening included antibiotic susceptibility testing, tolerance to bile and acid pH, sporulation and antimicrobial activity against *Cl. perfringens* Type A and Type C, *S.* typhimurium, ETEC O149:K91:F4ac and O147:K89:F4, and *E. coli* O101:K-:F5 strain. The adhesion of PorkBoostEB spores and vegetative cells to porcine jejunal IPEC-J2 cells was also investigated. *E. coli* strains were further used as *in vitro* challenge models. For the *in vivo* trial, 216

newly weaned piglets were randomly allocated to control or treatment group, and fed equal standard diets without or with PorkBoostEB. PorkBoostEB has a considerably higher ability to attach *in vitro* to small intestinal epithelium when present as spores (71.2% ± 0.8) rather than vegetative cells (15.0% ± 1.5) previously found to also be able to attach to the mucus-secreting HT-29 MTX cells (12.7% ± 0.9) and to Caco-2 cells (10.7% ± 1.5). Our data are in agreement with the very few *in vitro* studies conducted so far on other *Bacillus* spp.-based products.The *in vitro* challenge data showed a significant reduction ($P < 0.005$) in the adhesion of ETEC O149:K91:F4ac and *E. coli* O101:K-:F5 strain to IPEC-J2 cells in the presence of PorkBoostEB. Although an F4 strain as well, the adhesion of *E. coli* O147:K89:F4 was not affected. This suggests that PorkBoostEB's protective effect is more likely to be pathogen-specific. Results showed that feed supplementation with PorkBoostEB had a numeric or significant effect on daily gain (235 g/d vs. 218 g/d) and feed conversion (1.15 kg/kg vs. 1.21 kg/kg; $P < 0.05$) as well as improved fecal scoring ($P < 0.01$).

Key Words: probiotic bacilli, PorkBoostEB, antimicrobial activity, protective effect, intestinal epithelium

22 A formulated yeast blend and *Bacillus* probiotic fed to sows alters the fecal microbial ecology of their offspring.

E. Davis*[1], J. Rehberger[1], J. Sawall[1], A. H. Smith[1], R. Song[2], and K. Friesen[2],
[1]*Agro BioSciences Inc., Wauwatosa, WI, USA,* [2]*NutriQuest, Mason City, IA, USA.*

Yeast and *Bacillus* feed additives improve health and productivity when added to livestock diets. To determine the effect of a yeast blend and *Bacillus* probiotic on the fecal microbial ecology of sows and their offspring, 500 sows were fed one of 4 dietary treatments through gestation and lactation (1) a control, basal diet; (2) basal diet with *Bacillus subtilis* 2-strain probiotic; (3) basal diet with a formulated yeast blend containing yeast extract, hydrolyzed yeast, and yeast culture; or (4) basal diet with *Bacillus* probiotic+yeast blend. Fecal samples were collected from 25 sows/treatment at the end of gestation and lactation, from pigs in the sows' litters at 5 and 18 d of age, and from post-weaning pigs at 24 and 35 d of age. Fecal samples were plated for *E. coli* counts; microbial DNA was isolated for terminal restriction fragment length polymorphism (TRFLP) analysis and statistically analyzed using Canoco 5. Presumptive identification of TRFLP peaks was done with 2 restriction enzymes using MICA. Fecal counts of *E. coli* were lower in gestating sows fed the *Bacillus* probiotic compared with sows fed diets devoid of *Bacillus*, but the overall fecal microbial ecology of gestation and lactation sows did not differ. Fecal microbial ecology of 5-d-old pigs from sows fed the Yeast treatment differed from pigs born to sows fed the other 3 treatments, with peaks putatively identified as clostridia being proportionally higher. Five-day-old pigs from sows fed the *Bacillus*+Yeast treatment tended to differ from pigs born to sows fed the other 3 treatments, such that *Enterobacteriaceae* and lactobacilli peaks were present at greater proportions. Fecal microbial ecology of 24-d-old weaned pigs born to sows fed the *Bacillus* treatment tended to differ from the other treatments. Fecal microbial ecology of 35-d-old weaned pigs born to sows fed the Yeast treatment differed from the other 3 treatments, with peaks representing lactobacilli proportionally higher in the Yeast treatment. These data indicate that yeast and *Bacillus* feed additives fed to sows both singly and in combination shift the fecal microbial ecology of their offspring.

23 Modulation of yeast probiotic activities by manipulating cell wall mannan oligosaccharide.

S. Kwak*, J.-J. Liu, and Y.-S. Jin,
The University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Saccharomyces boulardii is a commercialized probiotic yeast strain for GI disorders. As compared with *S. cerevisiae*, *S. boulardii* shows higher cell wall mannan oligosaccharide (MOS) levels and stronger probiotic activities including the adhesion activity against pathogenic bacteria such as *Salmonella enterica*. In this study, we aimed to investigate the effect of cell wall MOS on the adhesion activity of yeast. We constructed

engineered yeast strains containing various cell wall MOS contents through CRISPR-Cas9 based genome editing and compared their interactivities with *S. enterica*. To enhance the cell wall MOS levels, biosynthesis of GDP-mannose, the only substrate for MOS biosynthesis in yeast, was manipulated. GDP-mannose pathway coding genes, such as PMI40 and PSA1, were overexpressed under a strong constitutive promoter to reinforce GDP-mannose synthesis. PFK26 and PFK27 (6-phosphofructo-2-kinase) were deleted to slow down upper-glycolysis and shunt metabolic fluxes toward GDP-mannose pathway. There was a synergistic effect on the intracellular GDP-mannose level when the GDP-mannose pathway was overexpressed, and PFK26-PFK27 was deleted simultaneously. PFK26-PFK27 double deletion allowed PMI40-PSA1 overexpressing *S. boulardii* to accumulate 2.15 mg of GDP-mannose per 1 g dry cell mass, which is 10 times higher than the GDP-mannose level of wildtype (0.208 mg/g cell). However, enhanced GDP-mannose level did not significantly affect cell wall MOS content. Therefore, we additionally overexpressed SED1 and DPM1, a highly mannosylated cell wall protein and an ER membrane protein for mannosyl group transmission. Additional co-overexpression of SED1 and DPM1 led 19% increased MOS content of high GDP-mannose accumulating *S. boulardii* strain. We measured adhesion activities of the engineered *S. boulardii* strain, wildtype *S. boulardii* and brewing yeast *S. cerevisiae* against *S. enterica* serovar Typhimurium. The engineered *S. boulardii* showed 69% higher and 30% higher adhesion activities as compared with *S. cerevisiae* and wildtype *S. boulardii*, respectively.

Key Words: *Saccharomyces boulardii*, GDP-mannose, mannan oligosaccharide, *Salmonella enterica*

24 Microbial perspectives on host evolution.

K. R. Amato*,
Department of Anthropology, Northwestern University, Evanston, IL, USA.

The mammalian gut is home to a diverse community of microbes whose function is generally assumed to impact host fitness. However, while widespread use of terms such as 'co-evolution' and 'holobiont' imply these impacts, a relatively small body of literature directly addresses the potential roles of gut microbes in contributing to mammalian host evolutionary trajectories. Here I outline a general framework for exploring the relationship between gut microbes and host ecology and evolution. Using this framework, I briefly review the current state of knowledge, employing specific examples from non-human primates to highlight advances and remaining gaps in our knowledge. Specifically, I utilize research focused on host diet and energetics to examine the evolution of host behavior and physiology in the context of gut microbial function. While gut microbiota research is proliferating rapidly, there remain important gaps in our understanding of host-gut microbe interactions that will require an evolutionary perspective to fill. Likewise, gut microbiota research will be an important tool for filling remaining gaps in evolutionary biology.

25 Interspecies hydrogen transfer and its effects on global transcript abundance in *Ruminococcus albus*, a predominant fiber-degrading species in the rumen, and *Wolinella succinogenes*, a syntrophic partner.

R. R. Geier*[1,5], I. H. Kwon[3], I. K. Cann[3,4], and R. I. Mackie[2,3],
[1]*Division of Nutritional Sciences, University of Illinois, Urbana, IL, USA,* [2]*Institute for Genomic Biology, University of Illinois, Urbana, IL, USA,* [3]*Department of Animal Sciences, University of Illinois, Urbana, IL, USA,* [4]*Department of Microbiology, University of Illinois, Urbana, IL, USA,* [5]*Agro BioSciences Inc., Wauwatosa, WI, USA.*

Interspecies hydrogen transfer (IHT) is a central process in anaerobic environments linking the transfer of reducing power from fermentation of organic molecules to inorganic electron acceptors via hydrogen. IHT plays a significant role in enteric fermentation as well as the global methane cycle. *Ruminococcus albus* 7 is a hydrogen-producing, fermentative bacterium which encodes HydABC and HydA2, as well as a putative hydrogen-sensing protein, HydS. HydABC is an electron-bifurcating ferredoxin- and NAD-dependent [FeFe]-hydrogenase, HydA2 is

a ferredoxin-dependent [FeFe]-hydrogenase. We hypothesized that HydS transcriptionally regulates HydA2 in a manner dependent on the presence of a hydrogen-utilizing syntroph. To test this hypothesis, *R. albus* 7 and a hydrogen-utilizing bacterium, *Wolinella succinogenes* DSM 1740, were grown in pure culture and in coculture. Hydrogen accumulated in the *R. albus* pure culture only. Production of acetate increased and ethanol decreased when *R. albus* was grown in coculture with *W. succinogenes*. RNA was extracted at mid-log phase for sequencing to compare transcriptomic profiles. Transcript abundance of HydA2 was 90-fold lower in coculture. The electron-bifurcating hydrogenase, HydABC, had a small change in transcript abundance in coculture (1.3-fold increase). This suggests HydS might be sensing hydrogen levels and regulating the transcription of HydA2. These results also suggest that the electron-bifurcating hydrogenase (HydABC) functions in central metabolism regardless of external hydrogen concentration. *W. succinogenes* reduced all the fumarate to succinate in both cultures. The [NiFe]-hydrogenase in *W. succinogenes* had an increase in transcript abundance of nearly 3-fold. The transcripts for fumarate reductase had an increase in abundance in coculture of 1.2-fold. This is the first study to demonstrate at the genome and metabolite levels that both *R. albus* and *W. succinogenes* benefit from symbiotic IHT.

Key Words: RNA-seq, *Ruminococcus albus*, *Wolinella succinogenes*, interspecies hydrogen transfer

26 Structural biology of methanobacterial enzymes for fun and profit.

V. Carbone[1], L. R. Schofield[1], Y. Zhang[1], I. M. Hannus[1], D. Schäfer[1], R. Atua[1], C. Sang[1], S. Molano[1], C. Nicol[1], B. P. Subedi[1], W. F. Martin[2], A. J. Sutherland-Smith[3], and R. S. Ronimus*[1],
[1]*AgResearch, Palmerston North, New Zealand,* [2]*Heinrich-Heine Universität, Düsseldorf, Germany,* [3]*Massey University, Palmerston North, New Zealand.*

The rumen archaeal community is typically dominated by hydrogenotrophic methanogens from the family *Methanobacteriales*. Rumen methanogenic archaea possess several unusual traits such as isoprenoid-based lipids linked to sn-glycerol-1-phosphate via ether bonds, a unique energy metabolism (methanogenesis) that incorporates 6 specialized cofactors, and cell walls comprised of pseudomurein. Most of the enzymes responsible for these methanogen-specific traits are not found in rumen bacteria, fungi and protozoa, or the host animal. Thus, one viable approach for controlling methane emissions from rumen methanogens is to develop small molecule inhibitory compounds that specifically target the unique enzymes of the methanogens. To accelerate inhibitor discovery, x-ray crystallographic techniques were used to determine the structures of enzymes from rumen methanogens or from related thermophilic methanobacteria. Structures from thermophilic methanogens offer some advantages as they are more stable, can often be more-easily purified and are less flexible at crystal-forming temperatures. Over 300 enzymes were targeted for structure determination, which produced in excess of one hundred soluble enzymes for crystallographic screening. Over 30 different enzymes have produced crystals and 17 structures have been solved thus far. We exemplify the structural data with examples from the methanogenesis pathway (5,10-methenyltetrahydromethanopterin cyclohydrolase; MCH), lipid biosynthesis (sn-glycerol-1-phosphate dehydrogenase, G1PDH), cofactor biosynthesis (2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5′-phosphate reductase; MthRED) and cell wall biosynthesis (an UDP-aminosugar 4-epimerase). A subset of the available structures has been the focus of in silico screening of large compound libraries to identify possible inhibitors for use in ruminants. Several hit classes have been identified and tested in both in *in vitro* tests (enzyme assays, pure cultures of methanogens and/or rumen fluid-based assays) or *in vivo* experiments.

Key Words: *Methanobacteriales*, pseudomurein, cofactors, methanogenesis, rumen

Podium presentations: Session 4

27 Comparative microbiome analysis between sheep rumen and rabbit cecum provides new insight into their differential methane production.

L. Mi*[1,2], B. Yang[1], Z. Yu[2], J. Liu[1], and J. Wang[1],
[1]*Zhejiang University, Hangzhou, Zhejiang, China,* [2]*The Ohio State University, Columbus, OH, USA.*

The rumen and the hindgut represent 2 main evolutionary fermentation sites in herbivorous mammals, where feeds are converted to VFA, microbial protein, ammonia, and gases. In the rumen, the gaseous fermentation products, mainly CO_2 and H_2, are primarily used by methanogens to produce CH_4, whereas in the hindgut, much of the CO_2 and H_2 are used in the production of acetate and other VFA. Therefore, a better understanding of the microbiome of these 2 digestive sites can provide insight into the microbial underpinning of the differential CH_4 output from ruminant and non-ruminant herbivores, which may help develop new strategies to achieve effective methane mitigation. To this end, we compared the CH_4 from 5 adult sheep and 15 adult rabbits, all of which were fed alfalfa hay, and comparatively analyzed the ruminal microbiome of the sheep and cecal microbiome of the rabbits. As determined by open-circuit respiratory chambers, the sheep produced more CH_4 than the rabbits per unit of BW, digestible NDF, and ADF intake. 16S rRNA gene amplicon sequencing analysis revealed differences in communities of both archaea and bacteria. Of the 3 genera of methanogens detected, *Methanobrevibacter*, *Methanosphaera* and vadinCA11, both species *Methanobrevibacter millerae* and *Methanobrevibacter woesei* were predominant in rabbit cecum, but only *Methanobrevibacter millerae* was predominant in sheep rumen. *Firmicutes* and *Bacteroidetes* had similar predominance in sheep rumen and rabbit cecum, but a higher relative abundance of the genera *Prevotella*, *Butyrivibrio*, *Succiniclasticum*, and *Mogibacterium* was observed in sheep rumen. More interestingly, the families *Ruminococcaceae* and *Clostridiaceae*, and the genus Blautia, all of which contain known acetogens, were more predominant in rabbit cecum than in sheep rumen. Future research on the physiochemical factors in the rabbit cecum that shape the microbiome therein may lead to new strategies to effectively lower CH_4 emission from ruminants. The hydrogen-consuming bacteria present in the rabbit cecum may also be used to lower CH_4 emission from ruminants.

Key Words: archaea, bacteria, cecum, methane, rumen

28 Winners and losers: Microorganisms impacted by inflammation in the gut.

M. A. Borton*, A. Sabag-Daigle, J. Wu, L. M. Solden, B. S. O'Banion, R. A. Daly, R. A. Wolfe, J. F. Gonzalez, V. H. Wysocki, B. M. M. Ahmer, and K. C. Wrighton,
The Ohio State University.

Salmonella is a leading cause of foodborne illness in the US, affecting 1.2 million Americans annually. While this pathogen is known to induce host inflammation, the impacts of *Salmonella* presence and interrelated inflammation on the gut microbiome are largely unknown. We used a CBA/J mouse model to evaluate the intestinal responses to *Salmonella* induced inflammation. In parallel, we evaluated chemically induced inflammation by dextran sodium sulfate (DSS) and a non-inflammation control. Fecal samples were collected before and after treatment, while cecal samples were collected at d 16 post-treatment. We performed single gene taxonomic as well as metagenomic analyses, and paired this data to metabolite and inflammation quantification (lipocalin-2). We demonstrated that inflammation, chemically- or pathogen-induced, restructures the gut environment. Despite the same pathogen inoculum, *Salmonella* relative abundance ranged from 46% in 3 high responder mice to no more than 7% in the remaining 7 low responder mice. These high and low responders, along with the chemical DSS group, established a significant inflammation gradient with DSS and low responders having at

least a 2 log-fold lower lipocalin-2 concentration than high responders. Inflammation amount correlated to microbial community membership and to decreased butyrate and overall short chain fatty acid concentrations. Low-level inflammation, chemical or pathogen induced, enriched for *Akkermansia* spp. and members of *Bacteroidetes* family S24–7. High inflammation drastically reduced microbial diversity relative to all other treatment groups. This reduction was specifically driven by decreases in *Alistipes* and *Lachnospiraceae* members. Conversely, members of the *Enterobacteriaceae* and *Lactobacillus* were enriched concomitant with high levels of *Salmonella* induced inflammation. Reconstructed genomes from these inflammation sensitive and resistant organisms are providing insight into the metabolic functions and adaptations used to withstand the inflamed gut environment and will reveal new therapeutic strategies for restoring the microbiota after inflammation.

Key Words: *Salmonella*, 16S rRNA gene, metagenomics, *Alistipes*

29 Addition of arginine and citrate enhances bacterial growth and fluoroacetate degradation of *Cloacibacillus* sp. MFA1.

S. Kang*, S. Denman, and C. McSweeney,
CSIRO, Brisbane, Queensland, Australia.

Cloacibacillus sp. MFA1 (MFA1) is an asaccharolytic ruminal bacterium belonging to the *Synergistetes* phylum. It degrades the plant toxin fluoroacetate (FA) by anaerobic dehalorespiration, which generates energy that stimulates growth. The general nutritional requirements, cell growth, FA degradation, and amino acid/peptide utilization of the bacterium were investigated under different culturing conditions. The growth of MFA1 and its FA degradation rate were enhanced by peptide-rich protein hydrolysates (tryptone and yeast extract) compared with an amino acid rich protein hydrolysate (casamino acid). To test induction of the putative FA degrading gene expression by non-toxic compounds, the pGL4-promo vector containing a luciferase (*luc2*) gene was constructed as a reporter assay. The *luc2* gene expression in the vector was controlled by the cloned promoter of the FA degrading gene, sourced from MFA1 genomic DNA. The promoter was highly induced in response to addition of arginine and citrate in the reporter assay. As expected, *luc2* gene transcription levels were also increased by these 2 supplements when assayed by quantitative reverse transcription PCR (qRT-PCR). In its natural cellular setting, when MFA1 was cultured with arginine, and citrate, FA degradation related genes [Fe-S oxidoreductase and glycine reductase complex B (GrdB)] were highly expressed and these supplements promoted both cell growth and FA degradation. We conclude that peptide-rich protein hydrolysates aregeneral nutritional stimulants for MFA1, and that arginine and citrate may specifically enhance cell growth and FA degradation. Sources of such nutritional supplements may be candidates as animal feed supplements to induce FA detoxification in ruminants exposed to FA containing plants. Furthermore, the constructed reporter assay system could be used to screen for nutrients or other non-toxic compounds from various plant extracts as stimulants for cell growth and FA degradation of MFA1.

Key Words: *Cloacibacillus* sp. MFA1, fluoroacetate and luciferase reporter assay

30 See Poster Abstracts (page 36)

59 Biochanin A selectively inhibits carbohydrate-utilizing bacteria in the bovine rumen.

B. E. Harlow*, G. E. Aiken, and M. D. Flythe,
USDA-ARS, Forage-Animal Production Research Unit, Lexington, KY, USA.

Rumen carbohydrate-utilizing (C-U) bacteria play an important role in both feed conversion (e.g., cellulosis) and disease pathogenesis (e.g., sub-acute ruminal acidosis, SARA). Historically, antibiotics (e.g., monensin, MON) have been used to enhance health and productivity by selectively inhibiting C-U. Biochanin A (BCA), an isoflavone produced by red clover (*Trifolium pratense*), also selectively inhibits C-U *ex vivo*. The objective was to determine the effect of BCA on C-U in the rumen, including amylolytic (AMY) and cellulolytic (CELL) bacteria. Rumen

fistulated Holstein steers (n = 12) were assigned to 1 of 4 treatments (randomized complete block, 2 × 2 factorial): HF CON (high fiber control), SARA CON, MON (200 mg d^{-1}), or BCA (6 g d^{-1}). Diets included corn silage and dried distillers grain ± cracked corn ad libitum. The study consisted of a 2-wk adaptation, a 1-wk HF period, and an 8-d SARA step-up challenge (pH: 5.5–5.0; 40% corn, 4 d; 70% corn, 4 d). Samples for pH and enumeration were taken on the last day of each period (4 h). Total AMY, Lancefield group D gram-positive cocci (GPC), lactobacilli (LAC), total CELL, and 2-deoxy-D-glucose (2-DG) resistant CELL were enumerated. In situ dry matter digestibility (DMD) was evaluated for the last 72 h of each period. Data were analyzed by repeated measures ANOVA using the MIXED procedure of SAS. During the HF period, pH, AMY, and CELL were unaffected by treatment. However, BCA increased 2-DG CELL and DMD 4% (HF CON: 62%). SARA challenge decreased pH (HF CON: 6.1; SARA CON 40%: 5.5, 70%: 5.3). However, BCA and MON partially mitigated pH decline (BCA 40%: 5.9, 70%: 5.6; MON 40%: 5.8, 70%: 5.4). SARA challenge increased AMY, GPC, and LAC. Although, BCA had minimal effects on AMY during the 40% SARA challenge, BCA decreased AMY, LAC, and GPC when steers were fed 70% corn. Similar results were observed with MON (excluding LAC). SARA CON decreased CELL, 2-DG CELL, and DMD (40%: 50%, 70%: 32%). BCA mitigated CELL inhibition and increased DMD (40%: 55%, 70%: 49%). These results indicate that BCA may be an effective antibiotic alternative for improving feed conversion and mitigating SARA in beef cattle.

Key Words: rumen, biochanin A, bovine

31 Modulation of sub-acute ruminal acidosis by active-dry yeast supplementation and its effect on rumen fungal and protozoal populations in liquid, solid, and epimural fractions.

S. Ishaq*[1], O. AlZahal[2], N. Walker[2], and B. McBride[3],
[1]*Ishaq Informatics, Bozeman, MT, USA,* [2]*AB Vista, Marlborough, United Kingdom,* [3]*University of Guelph, Guelph, Toronto, Canada.*

Sub-acute ruminal acidosis (SARA) is a gastrointestinal functional disorder in livestock characterized by low rumen pH, which alters rumen function, microbial diversity, and host performance or immune function. Information is lacking on the effect of SARA or yeast supplementation on ruminal protozoa and fungi, despite their roles in fiber fermentation. Sixteen multiparous, lactating Holstein cows were fed a high-fiber (HF, 77% forage:23% concentrate) for 6 wk, then abruptly transitioned to a high-grain (HG, 49% forage:51% concentrate) diet for an additional 4 wk to induce SARA, with and without *Saccharomyces cerevisiae* (ADY) supplementation. Rumen fluid, solids, and epimural areas were sampled to determine diversity using the protozoal 18S rRNA and the fungal ITS genes via Illumina MiSeq sequencing. Diet-induced SARA dramatically increased rare fungal taxa; yet total diversity (Inverse Simpson and Shannon Indices) was decreased in epimural fractions, and was very low in fluid for fungi and protozoa. ADY significantly affected fungal taxa for both diets, but did not affect overall diversity. Diet-induced SARA increased the protozoa *Entodinium furca monolobum*, *E. caudatum*, and *Polyplastron multivesiculatum*. Yeast increased *Isotricha intestinalis* but decreased *Entodinium furca* spp. in HF, and in HG increased *P. multivesiculatum* and *Entodinium* spp., while decreasing species of *Eudiplodinium*, *Eremoplastron*, and *Ostracodinium*. Multivariate analyses showed diet type was most significant in driving diversity, followed by ADY (AMOVA, ANOSIM, weighted UniFrac). Interactions between treatment and diet were location specific, in epimural fractions and occasionally in solid fractions. Only sample location was a significant factor for protozoa (PERMANOVA, P = 0.001, Monte Carlo). SARA induced by a HG diet reduced diversity of rumen fungi and protozoa and selected against fiber-degrading species. Supplementation with ADY mitigated this reduction, presumptively by triggering diversity shifts (as seen even in HF diet) that resulted in pH stabilization.

Key Words: Illumina, rumen microbiome, *Saccharomyces cerevisiae*

62 Changes in rumen microbes' quantity and volatile fatty acid concentrations during adaptation period of dairy cows to changing diet.

L. Mamuad*[1,2], S. Kim[1,2], M. Lee[1], Y. Choi[1], C. Lee[2], Z. Yu[2], and S. Lee[1],
[1]*Sunchon National University, Suncheon, Jeonnam, South Korea,* [2]*The Ohio State University, OH, USA.*

In *in vivo* nutritional studies such as evaluation of different feeds, feeding ratios, and probiotics, an adaptation period is included. During the adaptation period, animals become acclimated to the treatments before data are collected. During this period, the rumen microbiota also undergoes populational and metabolic changes. Hence, this study was conducted to evaluate the effects of changing diet during adaptation on the rumen microbiota and its fermentation characteristics. Three Holstein-Friesian cows were used in this study and assigned as replicates. They were initially fed with Italian ryegrass ad libitum and 2 kg concentrate diet per day and for 30 d. Then, the concentrate diet fed to the animals was increased to 8 kg per day for 14 d and then decreased to 2 kg concentrate diet per day for another 14 d. Rumen fluids were collected 2 h after morning feeding through stomach tubing at the start of the experiment, and at d 7 and 14 of the feeding trials. pH, volatile fatty acids (VFA), and abundance of select groups of rumen microbes were determined. The linear, quadratic, cubic, and quartic effects of treatments were analyzed using orthogonal polynomial coefficients to describe the functional relationships among treatment levels. Highest pH of 7.02 was observed at the start of the experiment, while the lowest pH of 6.80 was observed at d 14 of consuming 8 kg concentrate daily. Concentrations of acetic, propionic, and butyric acids also peaked ($P < 0.05$) at d 14 d of consuming 8 kg concentrate: at 56.76 m*M*, 13.28 m*M*, and 6.60 m*M*, respectively. Changing diet had linear, quadratic, cubic, and quartic effects on acetic acid concentration, quadratic, cubic, and quartic effects on butyric acid concentration, and both quadratic and quartic effects on propionic acid concentration. The total bacteria quantity was comparable throughout the feeding trials while the lowest methanogen quantity was observed at the start of the experiment while the highest was observed at d 14 of consuming 8 kg concentrate daily.

Key Words: adaptation, concentrate, microbiota, rumen, volatile fatty acids

NOTES

Poster Abstracts

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

32 Effect of feeding increasing levels of *Aspergillus terreus* fermented palm kernel cake on methane emission in goats.

J. B. Liang*[1], M. F. Mahadzir[1], S. Garba[1], M. F. Jahromi[2], S. C. L. Candyrine[1], R. Ronimus[3], S. Muetzel[3], and A. A. Samsudin[1],
[1]*Universiti Putra Malaysia, Serdang, Selangor, Malaysia,* [2]*Agricultural Biotechnology Research Institute of Iran, Mashad, Iran,* [3]*AgResearch, New Zealand.*

Lovastatin, produced during growth of certain fungi, is a HMG-CoA enzyme inhibitor, and has been reported to reduce methanogenesis in ruminants. Studies have suggested that the effective dosage of lovastatin to mitigate enteric methane (CH_4) is 2.26 mg/kg LW for sheep, but dosage at 2.6 mg/kg LW had resulted in adverse effect in cattle. Studies on the use of lovastatin to mitigate enteric CH_4 emission have been short-term and there is little data that examines the potential of the rumen microbiota to adapt to the effect of lovastatin over time, as well as effect of lovastatin to the health of the host animals. This study examines the responses to dietary supplementation of different doses of naturally produced lovastatin, produced using palm kernel cake (PKC) and *Aspergillus terreus*, over a period of 12 wk in goats. Twenty male Saanen goats (4–5 mo old, average LW of 26 kg) were randomly assigned in equal number to 4 treatments. Animals were fed a total mixed ration (700 g daily in 2 equal portions 08:00 and 20:00) containing 50% rice straw, 22.8% concentrate and 27.2% of various proportions of PKC that contained lovastatin or not to achieve the target feeding levels of 0, 2, 4, and 6 mg lovastatin/kg LW/day. Methane emissions were measured using open circuit respiration chambers at 4-, 8-, and 12-wk feeding periods. At each period, intake and microbial community composition of the rumen fluid were determined. Daily CH_4 emission from goats fed the control diet (no lovastatin) averaged 17.40 L/goat. Lovastatin supplementation reduced CH_4 production by 5.1, 20.5 and 38.4% (averaged of 3 periods), respectively, for the 2, 4 and 6 mg/kg LW groups. When the data were adjusted to CH_4/kg DM intake, CH_4 reductions were 7.2, 15.2 and 16.7%, respectively. No significant differences were detected for total methanogens or *Methanobacteriales* (log_{10} copy number/mL), or their ratios, between the control and treatments. No overt adverse physiological effects were observed in the goats except that some animals in the 2 higher dosage treatments showed reduced DMI.

Key Words: lovastatin, methane production, goats, microbial community

33 Effects of lysozyme on *in vitro* fermentation, methanogenesis, and microbial community structure of the rumen.

Y. Chen[1], J. Shen*[1,2], W. Zhu[1], and Z. Yu[2],
[1]*Nanjing Agricultural University, Nanjing, Jiangsu, China,* [2]*The Ohio State University, Columbus, OH, USA.*

Lysozyme hydrolyzes peptidoglycan of gram-positive bacteria, many of which are hydrogen producers in the rumen. Thus, lysozyme can potentially affect fermentation and methane production by ruminal microbiome. This study aimed to investigate the effects of lysozyme on *in vitro* rumen fermentation, methanogenesis and microbial community structure. Lysozyme was added to *in vitro* ruminal cultures at 5 doses (per liter): 0 (L-0, Control), 1 (L-1), 10 (L-10), 100 (L-100), and 1000 mg (L-1000). Methane production were measured at different time of incubation. Culture samples collected at 24h were analyzed for fermentation parameters and microbial populations. In addition, samples of L-0, L-10, and L-1000 were also used subjected to metagenomics analysis of bacterial community using Illumina sequencing of 16S rRNA gene amplicons. Compared with control, methane production, DMD, NH_3-N and TVFA were not influenced by L-1 ($P > 0.05$). Methane production

and NH3-N concentration were reduced, and propionate concentration was increased by L-10 ($P < 0.05$), while DMD or TVFA were not affected ($P > 0.05$). Methane production was reduced, and propionate concentration was increased by L-100 and L-1000 ($P < 0.05$), but DMD were also decreased significantly ($P < 0.05$). Total bacteria, fungi and methanogens were significantly reduced by L-1000 ($P < 0.05$), but were not influenced ($P > 0.05$) by L-1, L-10, or L-100. Principal component analysis of the sequencing data showed clear differences in the composition of the ruminal bacterial community among treatments. The abundance of propionate-producing bacteria (e.g., *Selenomonas* and *Succinivibrio*) was increased by lysozyme, resulting in more hydrogen being directed to production of propionate instead of methane. Moreover, the reduced NH_3-N concentration in L-10 was probably due to the lower abundance of proteolytic bacteria (e.g., *Prevotella* and *Bacteroides*) inhibited by lysozyme. Appropriate lysozyme addition (10 mg/L) may be used to modulate ruminal microbial ecology and reduce methanogenesis and ammoniagenesis by rumen microbiome without adversely affecting feed digestion or fermentation.

Key Words: bacterial community, *in vitro*, lysozyme, methane

34 Regulation of the small intestine development at preterm weaning.

L. Kuchkarova*, G. Kudeshova, G. Dustmatova, and I. Karimova,
National University of Uzbekistan, Tashkent, Uzbekistan.

In experiments on white rats it has been shown that preterm weaning leads to delay and/or disruption of age-depended rearrangements of the small intestine mucous, reflected in a lower density of enterocytes, lower villi height, and more expressed cell desquamation from the villi top in the early weaned rats compared with intact ones. However, in that case, some increase of intestinal definitive disaccharidases (maltase, γ-amylase, and sucrase) activity took place on the background of constant lactase activity. Intestinal mass and body weight in preterm weaned rats were reduced. The oral administration of some phytoecdisteroids (ecdysterone, turkesteron) for 5 d (1 mg/kg) before weaning declined and/or prevented structural damage and reducing intestine mucous of growing rats. In addition, well-marked increase of enteral "definitive" disaccharidases activity took place after the administration of these drugs. This suggests that treatment of growing animals with phytoecdysteroids has pronounced maturation effect, which "prepares" the suckling intestine to digest adult food. Intestinal microstructure abnormalities in early weaned rats contribute to high permeability of the epithelium to the foreign protein. This permeability is often a cause of diarrhea and other related diseases in the growing organism during weaning. In the majority of farm animals weaning period comes increase milk production. This leads to diarrhea and other illnesses, which affect both welfare and productivity of livestock. These data suggest the ability using of phytoecdisteroids for intestinal maturation in premature weaned farm animals.

Key Words: small intestine, maturation, phytoecdisteroids, growing rats

35 Anti-*Salmonella* and uric acid-preserving effect of pine bark tannin in composted poultry litter.

C. Arzola-Alvarez*[1], R. C. Anderson[2], M. E. Hume[2], O. Ruiz-Barrera[1], Y. Castillo-Castillo[3], B. R. Min[5], J. A. Byrd[2], D. J. Nisbet[2], J. Salinas-Chavira[4], M. Ontiveros-Magadan[1], and C. Rodriguez-Muela[1],
[1]*Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico,* [2]*USDA,ARS, College Station, TX, USA,* [3]*Universidad Autonoma de Cd. Juarez, Cd. Juarez, Chihuahua, Mexico,* [4]*Universidad Autonoma de Tamaulipas, Cd. Victoria, Tamaulipas, Mexico,* [5]*Tuskagee University, Tuskagee, AL, USA.*

Poultry litter contains appreciable amounts of uric acid which makes it a good crude protein supplement for ruminants, but the litter must be treated to kill bacterial pathogens. Presently, we examined the antimicrobial and uric acid-

preserving activity of pine bark tannin during the early stage of poultry litter composting. One-year-old wood chip-based poultry litter exposed to 2 to 3 flocks with no use of antibiotics was used in this study. Condensed tannins from pine bark (*Pinus palustris*) were extracted using a Sephadex LH-20. The litter was distributed (11 g) to 50-mL conical centrifuge tubes (n = 3 tubes/treatment), amended with 1.3 mL of pine bark tannin (9% tannin wt/vol) or 0.4 *M* sodium phosphate buffer (control) and then inoculated with a novobiocin and naladixic acid-resistant *Salmonella* Typhimurium (STNN) to achieve 3.0 $\log_{10}$ cfu/g. The tubes were closed with caps, sealed with parafilm and incubated at 37°C for 3 d after which time the tube contents were serially diluted (10-fold) and plated on 3M *E. coli*/coliform Petri-film and Brilliant Green Agar supplemented with 25 and 20 µg/mL novobiocin and naladixic acid, respectively, for enumeration of wildtype *E. coli* and the challenge STNN strain. Fluids were analyzed colorimetrically for determination of ammonia, uric acid and urea concentrations. ANOVA revealed that pine bark tannin treatment decreased ($P < 0.05$) STNN populations in the litter by 0.6 log units compared with controls (6.0 ± 0.2 $\log_{10}$ cfu/g). Wildtype *E. coli* populations were unaffected by tannin treatment (6.4 ± 0.1 $\log_{10}$ cfu/g). Ammonia accumulation was decreased ($P < 0.05$) 23% in tannin-treated litter compared with the control (2.8 ± 0.1 µmol/g). Conversely, the residual uric acid concentration was 1.6-fold higher ($P < 0.05$) in litter treated with the pine bark tannin than in the control litter (15.5 ± 1.3 µmol/g). Urea concentrations (10.2 ± 1.6 µmol/g) were unaffected by tannin treatment. Results suggest that pine bark tannin treatment may help preserve crude protein contained as uric acid in composted litter while aiding *Salmonella* control.

Key Words: *Salmonella*, *E. coli*, nitrogen metabolism, pine bark tannin, poultry litter

36 Nitro-treatment of composted poultry litter, effects on *Salmonella*, *E. coli*, and nitrogen.

O. Ruiz-Barrera*[1], C. Arzola-Alvarez[1], Y. Castillo-Castillo[2], A. Corral-Luna[1], R. C. Anderson[3], J. A. Byrd[3], M. E. Hume[3], D. J. Nisbet[3], and J. Salinas-Chavira[4],

[1]*Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico,* [2]*Universidad Autonoma de Juarez, Ciudad Juarez, Chihuahua, Mexico,* [3]*ARS/USDA, Texas, College Station, TX, USA,* [4]*Universidad Autonoma de Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico.*

Poultry litter is a potentially valuable crude protein feed for ruminants whose gut microbes transform the nitrogen in uric acid into microbial protein. However, poultry litter must be treated to kill pathogens before feeding, but risks volatilization losses of nitrogen as ammonia. Presently, 200-g of poultry litter were treated with ethyl nitroacetate (ENA) or 3-nitropropionate (3NPA) in 100 mL of 0.4 *M* phosphate buffer to achieve 25 µmol/g litter. The litter was distributed (11 g) to 50-mL tubes and inoculated with a novobiocin and naladixic acid-resistant *Salmonella* Typhimurium (STNN) to achieve 3.0 $\log_{10}$ cfu/g. Tubes were incubated 6 d at 37°C and then 3 d at 50°C. Upon sampling (d 3, 6 and 9), 3 tubes of each treatment were diluted and plated on 3M *E. coli*/coliform Petri-film and BGA supplemented with 25 and 20 µg/mL novobiocin and naladixic acid, respectively, for enumeration of *E. coli* and STNN strain. Repeated measures ANOVA revealed a main effect ($P < 0.05$) of treatment on STNN but not *E. coli* populations, with STNN being decreased >1.0 $\log_{10}$ by nitro-treatment compared with controls (4.2 ± 0.2 $\log_{10}$ cfu/g). A main effect due to days of incubation, reflecting an effect of composting, was observed on both STNN and *E. coli*, with populations decreasing to non-detectable levels by d 9. Interactions between treatment and day of incubation were not observed for STNN or *E. coli*. Main effects of treatment were observed on ammonia accumulations and uric acid degradation ($P < 0.05$), with 17 to 24% less ammonia accumulating in nitro-treated litter than controls (3.4 ± 1.4 µmol/g) and 18% more uric acid remaining in 3NPA-treated litter than in controls or ENA-treated litter (18.1 ± 3.8 µmol/g and 18.5 ± 5.0 µmol/g, respectively). Accumulations of ammonia and urea increased ($P < 0.05$) due to day of incubation, the latter more rapidly ($P < 0.05$) during the early days of incubation in control litter than in 3-NPA- or ENA-

treated litter (2.3 ± 0.6, 1.5 ± 0.4 and 0.5 ± 0.6 μmol/g per h, respectively). Results suggest that nitro-treatment may help preserve uric acid in composted litter while aiding *Salmonella* control.

Key Words: nitrocompound, nitrogen, poultry litter

Immunology (including host–microbe interactions)

30 Starter feeding altered colonic mucosal bacterial communities and modulated mucosal immune homeostasis during milk-feeding period in lambs.

J. Liu*[1], G. Bian[2], D. Sun[1], W. Zhu[1], and S. Mao[1], [1]*Nanjing Agricultural University, Nanjing, Jiangsu, China,* [2]*Tianyi Health Science Research Institute, Zhenjiang, Jiangsu, China.*

This study aims to investigate the effect of starter feeding on the colonic mucosal bacterial community and on mucosal immune homeostasis in pre-weaned lambs. We selected 8 pairs of 10-d-old lamb twins. One twin was fed breast milk (M, n = 8) while the other was fed breast milk+starter (M+S, n = 8). The lambs were slaughtered at 56 d old. The colonic content was collected to determine the pH and the concentration of volatile fatty acids (VFA) and lactate. The colonic mucosa was collected to characterize the bacterial community using Illumina MiSeq sequencing and to determine mRNA expression of cytokines and toll-like receptors (TLR) using quantitative real-time PCR. Statistical analyses were performed using the SPSS software. The data found to have a normal distribution were analyzed by the Independent Samples *t*-test procedure. The Kruskal-Wallis test was used to analyze variables found to have a non-normal distribution. Correlation analysis was assessed by Spearman's correlation test using GraphPad Prism v.5. The results show that starter feeding decreased luminal pH and increased the concentration of acetate, propionate, butyrate, total VFA, and lactate in the colon. The principal coordinate analysis and analysis of molecular variance show that starter feeding significantly affected the colonic mucosal bacterial communities with a higher relative abundance of the dominant taxa unclassified S24–7, *Oscillibacter*, *Prevotella*, *Parabacteroides*, *Bifidobacterium*, *Ruminobacter*, and *Succinivibrio*, and a lower proportion of unclassified *Ruminococcaceae*, RC9_gut_group, Blautia, *Phocaeicola*, *Phascolarctobacterium*, unclassified BS11_gut_group, and *Campylobacter* in lambs. Meanwhile, starter feeding decreased mRNA expression of TLR4 and cytokines TNF-α and IFN-γ in colonic tissue. Furthermore, the changes in the colonic mucosal mRNA expression of TLR and cytokines were associated with variations in mucosal bacterial composition. These findings may provide new insights into colonic mucosal bacteria and immune homeostasis in developing lambs.

Key Words: starter feeding, colonic mucosa, bacterial community, immune homeostasis, lamb

37 *Clostridium perfringens* infection of the chicken induces immunometabolic alterations in the duodenum that includes the glycolytic and insulin signaling and NLRP3 inflammasome-mediated inflammatory cell death.

M. Kogut*[1] and R. Arsenault[2], [1]*USDA-ARS, College Station, TX, USA,* [2]*University of Delaware, Newark, DE, USA.*

Clostridium perfringens (CP) infection of the gut is a central requirement for the establishment of necrotic enteritis (NE), despite CP very often being a member of the commensal microbiota in broiler chickens. Little is known about the immune mechanisms directed against CP during necrotic enteritis infections. An integral component of immune regulation is through the metabolic pathways necessary to support energetically demanding protective or pathogenic responses. Understanding these links in immunometabolism is critical to understanding basic immune responses to CP. Using a species-specific kinome immunometabolism peptide

array, we investigated changes in signaling pathways in the duodenum of broilers given a live-attenuated vaccine against IBD followed by a CP challenge. In these experiments, all birds received a commercial infectious bursa disease vaccine on d 10 of age followed by an orally administered CP challenge on d 15, 16, and 17. The studies were terminated at d 21 when birds were sacrificed and a 40-mg sample of duodenal tissue was collected from each bird, flash frozen, homogenized, and applied to the peptide array protocol. We observed metabolic changes that affected glucose metabolism through the glycolytic and the insulin signaling pathways. Within 4 d of challenge infection, we observed changes in the duodenal phosphorylation state of the enzymes up and down the glycolytic pathway. In addition, changes to a large subset of the protein intermediates of the insulin pathway were altered by infection. Immunologically, infection induces pyroptosis by increased phosphorylation of several peptides in both the TLR1/NFAT and NLRP3 (Caspase 1, CARD9, PRTPIP1) signaling pathways. This is the first report of significant regulatory metabolic and inflammatory signaling pathways induced by CP infection and provides new insights in the mechanisms essential for the establishment of NE in chickens.

Key Words: *Clostridium*, chicken, immunometabolism, glycolysis

38 The effects of loxoribine, a toll-like receptor agonist, on *Escherichia coli* O157:H7 and *Salmonella* Typhimurium growth in rumen fluid.

C. L. Swaggerty* and T. R. Callaway,
USDA/ARS, College Station, TX, USA.

Escherichia coli O157:H7 and *Salmonella* Typhimurium are 2 of the leading causes of bacterial foodborne illness in the United States. Human illness resulting from either of these bacteria have been traced to cattle either by handling/consuming raw or undercooked beef products, direct contact with an infected animal, or indirectly through water sources contaminated by cattle feces. To reduce the on farm incidence of foodborne pathogenic bacteria, pre-harvest intervention strategies aimed at reducing populations of *E. coli* O157:H7 and *S.* Typhimurium in cattle should be developed and implemented. Toll-like receptors (TLR) are a group of pattern recognition receptors that are differentially expressed on leukocytes and in non-immune cells, and detect and initiate the first response against invading bacteria and viruses by recognizing specific pathogen-associated molecular patterns. Loxoribine is a guanosine analog that is a potent stimulator of the innate immune system and acts through TLR7. The objectives of the present study were to determine the effects of loxoribine on 1) maximum specific growth rate of *E. coli* O157:H7 and *S.* Typhimurium and 2) final pH and growth of *E. coli* O157:H7 and *S.* Typhimurium in mixed rumen microorganism fermentation. *E. coli* O157:H7 and *S.* Typhimurium growth curves (n = 3) were conducted in LB broth containing concentrations of loxoribine (25–200 µg) which did not affect the growth rate of either bacterium at any concentration tested. In mixed rumen microorganisms (n = 2) loxoribine concentrations from 25 to 200 µg did not alter the pH of rumen fluid fermentations. When ruminal fluid fermentations were inoculated with 10^6 colony-forming units (cfu) *E. coli* O157:H7 or 10^6 cfu *S.* Typhimurium there was no effect of loxoribine treatments on populations of *E. coli* O157:H7 or *S.* Typhimurium. In conclusion, loxoribine did not have an effect on the growth of *E. coli* O157:H7 or *S.* Typhimurium, nor did it alter the pH or growth of *E. coli* O157:H7 and *S.* Typhimurium in rumen fluid; however, additional studies using *in vitro* cell culture should be considered.

Key Words: cattle, *E. coli*, loxoribine, *Salmonella*, toll-like receptor

Microbiology (including ecology, (meta)genomics, physiology, and proteomics)

39 Comparing the responses of rumen ciliate protozoa and bacteria to excess carbohydrate.

C. Teixeira[1], R. de Paula Lana[1], J. Tao[2], and T. Hackmann*[2],
[1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil,* [2]*University of Florida, Gainesville, FL, USA.*

When given excess carbohydrate, certain microbial species respond by storing energy (synthesizing reserve carbohydrate), but other species respond by dissipating the energy as heat (spilling energy). To determine the importance of these responses in the rumen microbial community, this study quantified how mixed ciliate protozoa responded to glucose, and compared their response to that of mixed bacteria. We hypothesized that ciliates would synthesize more reserve carbohydrate and spill less energy than would bacteria. Ciliates and bacteria were isolated from rumen fluid using filtration and centrifugation, resuspended in nitrogen-free buffer to limit growth, and dosed with 5 m*M* glucose. Compared with bacteria, ciliates consumed glucose >3-fold faster and synthesized reserve carbohydrate 4-fold faster. They incorporated 53% of glucose carbon into reserve carbohydrate—double the value for bacteria. Energy spilling was not detected for ciliates, as all heat production (104%) was accounted by synthesis of reserve carbohydrate and endogenous metabolism. For bacteria, reserve carbohydrate and endogenous metabolism accounted for only 68% of heat production, and spilling was detected within 11 min of dosing glucose. These results suggest that ciliates alter the course of carbohydrate metabolism in the rumen by outcompetingbacteria for excess carbohydrate, maximizing reserve carbohydrate synthesis, and minimizing energy spilling.

Key Words: rumen, ciliate protozoa, bacteria, energy spilling, reserve carbohydrate

40 Census of the rumen microbiome using 16s rRNA gene-based pyrosequencing.

E. A. Latham*[1,2] and W. E. Pinchak[2],
[1]*Texas A&M University, College Station, TX, USA,* [2]*Texas A&M Agrilife Research, Vernon, TX, USA.*

In this study, the collective prokaryotic diversity in the rumen was examined by performing a meta-analysis of the 16s rRNA gene sequences available. A search string and exclusion criteria were developed and standardized. Raw reads were processed using the default settings in the QIIME pipeline. This included 34 studies as of June 2016, with 22,003 OTUs, representing 68 phyla and 788 genera. Across all samples, *Firmicutes* represented 35.5% of relative abundance and *Bacteroidetes* 26.7%. The core and pan-microbiome were determined. We found a strong clustering of the rumen microbiota by study suggesting that technical differences between laboratories cause significant differences in the observed diversity. However, some factors produced sufficient changes in the gut microbiota to influence clustering patterns including genus of animal, fraction of the rumen, sample collection method, primer type, and diet. For example, wild ruminants had the most spatially heterogeneous microbiota, while diary were the most conserved. Regardless of experimental factor, the samples representing the solid or fiber fraction of the rumen separated from the liquid fraction. Other parameters examined included age, DNA extraction method, gender, sampling time, sequencing method, and stage of production. Together, this study shows that cross-study comparisons of the rumen microbiota are valuable, but challenging due to the large and varied effect of many of the parameters. Future studies may want to include more subtle difference in methodology with controlled populations of animals and standardized protocols to determine what are the primary influencers of the rumen microbial ecosystem across ruminant species and diets. It

also underscores the importance of open access data with detailed metadata.

Key Words: microbiome, rumen, census, meta-analysis

41 Resolution of the amino acid requirements for *Clostridium scindens* ATCC 35704, a major bile acid-dehydroxylating anaerobe in the human gut.

R. Shrestha* and S. Daniel,
Eastern Illinois University, Charleston, IL, USA.

Cholate and chenodeoxycholate are primary bile acids that play vital roles in food digestion. However, primary bile acids are converted in the gut to toxic secondary bile acids, which contribute to colon cancer and gallstone disease. Secondary bile acids are the result of 7α-dehydroxylating anaerobes, and one of the key players in this conversion is *Clostridium scindens*. Interestingly, apart from its 7α-dehydroxylating and desmolase activities, little is known about the physiology of *C. scindens*. Thus, the focus of this study was to determine the amino acid requirements of *C. scindens*. *C. scindens* ATCC 35704 was grown in anaerobic defined medium (DM) containing glucose, minerals, trace metals, amino acids, vitamins, bicarbonate, CO_2 gas phase, and sodium sulfide. Amino acids were divided into 6 groups: Glutamate group (glutamine, glutamate, proline and arginine), serine group (serine, glycine and cysteine), aspartate group (aspartate, asparagine, methionine, lysine, threonine and isoleucine), pyruvate group (alanine, valine and leucine), aromatic group (tryptophan, tyrosine and phenylalanine) and histidine group (histidine), and the "leave one [group] out" technique was used to eliminate non-essential amino acids. Growth was observed without the glutamate and aspartate groups, suggesting that one or more of the 10 amino acids in the remaining 4 groups were required. Once cells were adapted to these 10 amino acids, good growth occurred without the serine, pyruvate, and histidine groups; growth did not occur in the absence of the aromatic group. When DM was supplemented with tryptophan, tyrosine or phenylalanine, growth was only observed with tryptophan, indicating that tryptophan was a required amino acid. This, together with our findings that riboflavin, pantothenate, and pyridoxal are required vitamins for *C. scindens* ATCC 35704, means that the nutritional requirements have now been resolved for this important member of the human gut microbiome.

Key Words: bile acids, 7α-dehydroxylation, nutritional requirements, amino acids, vitamins

42 Effects of nitrogen source and monensin on *in vitro* ruminal ammonia production and proteolytic bacterial community structure.

J. Shen*[1,2], Z. Yu[2], and W. Zhu[1],
[1]*Nanjing Agricultural University, Nanjing, Jiangsu, China,* [2]*The Ohio State University, Columbus, OH, USA.*

Low nitrogen utilization efficiency in dairy cattle challenges the sustainable production of dairy products. This study evaluated the effects of monensin on ruminal ammonia production and proteolytic bacterial community structure when different nitrogen sources were added. The experiment was designed with a 2 × 2 factorial arrangement: 2 nitrogen sources, Casein (Cas) and Tryptone (Try), and 2 levels of monensin, 0 (C) and 5 μ*M* (M), resulting in 4 treatments: Cas-C, Cas-M, Try-C, and Try-M. Ruminal fluid collected from 3 cannulated Holstein dairy cows was used as the inoculum. Each treatment culture was consecutively transferred 6 times after 24 h incubation. The qPCR and Illumina sequencing of 16S rRNA gene amplicons were utilized to examine and compare the proteolytic bacteria communities among the treatments. The results showed significant differences in ammonia production among treatments from the 2nd to 6th transfer, with Try-C having the highest ammonia (70.2 m*M*), followed by Cas-C (36.3 m*M*) and Try-M (25.4 m*M*), and Cas-M (3.97 m*M*). In the 6th transfer enrichment cultures, the population of total bacteria was reduced by monensin but was unaffected by nitrogen sources. Low abundance of *Clostridium sticklandii* and *C. aminophilum*, 2 known hyper-ammonia producing bacteria, were detected in the ruminal fluid inoculum. However, *C. aminophilum* was enriched in Cas-C and Ty-C, while *C. sticklandii* could not be detected in any enrichment culture. Principal component analysis (PCA) showed that the bacterial communities differed among

the treatments. Proteolytic bacterial populations also differed among the treatments. It was noted that the genus *Peptostreptococcus* accounted for as much as 41% of total bacteria in Try-C, but it was less than 0.02% in the other 3 treatments. Nitrogen source composition and monensin can affect ruminal ammonia production through modulating ruminal proteolytic bacterial community structure, and some hyper ammonia-producing bacteria of *Peptostreptococcus* may be one of the main culprits contributing to high ammonia concentration in the rumen.

Key Words: ammonia, *in vitro*, proteolytic bacterial community, rumen

43 Comparative analysis of bacterial microbial composition from the different ruminal ecological niche of Alxa Bactrian camel.

J. Zhao*[1,2], Z. Yu[2], G. Wang[1], J. Li[1], and H. Wu[1],
[1]*Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia, China,* [2]*The Ohio State University, Columbus, OH, USA.*

Similar to ruminants, pseudoruminants such as camels depend on the microbiota in their pseudorumen (a 3-chambered forestomach) to digest fibrous feed. Compared with the ruminal microbiome of cattle and sheep, the microbiome of the pseudorumen of camels are poorly understood. The objective of this research was to characterize the bacteria partitioned into different niches: liquid phase (LP), solid phase (SP), and epimural phase (EP), of the pseudorumen of Alxa Bactrian camel. Samples of the 3 phases were collected from 6 slaughtered Alxa Bactrian camels. Community compositions of bacteria were determined through sequencing 16S rRNA genes amplicons of the V3-V5 hypervariable regions on a MiSeq platform. UniFrac analysis revealed that the bacterial community of LP was clearly different from that of SP and EP. From 619,517 quality-checked sequences, 774 operational taxonomic units (OTUs) were identified at a 97% sequence identity. As in the rumen, *Bacteroidetes* (46.6% of the total sequences) and *Firmicutes* (32.8%) were the 2 most predominant, with other minor phyla also being represented: *Verrucomicrobia* (4.4%), *Spirochaetes* (3.3%), *Proteobacteria* (2.8%), *Fibrobacteres* (2.6%), *Tenericutes* (2.4%), and *Lentisphaerae* (1.2%). *Bacteroidetes* was more predominant in LP than in SP and EP, while *Fibrobacteres* was more predominant in SP than in LP. At genus level, a total of 117 taxa were observed across all the samples, but 48 of them represented new genera. Only 0.1% of the total sequences were assigned to archaea. The results showed that the camel pseudoruminal microbiome was structurally similar but compositionally distinct from that of true rumen, reflecting the different dietary and genetic impact of camels on their pseudoruminal microbiome, an untapped microbial resource. These findings also clearly demonstrated the compositional differences among LP, SP, and EP, indicating that those bacterial communities are specific and adapted to their niches.

Key Words: Alxa Bactrian camel, bacteria, microbiome, pseudorumen

44 Determination of succession of rumen bacterial species in nursing beef calves.

K. Smith*[1], A. Garza[1], K. Butterfield[1], A. Dickey[2], A. Lindholm-Perry[2], J. Wells[2], H. Freetly[2], and S. Ivey[1],
[1]*New Mexico State University, Las Cruces, NM, USA,* [2]*U.S. Meat Animal Research Center, Clay Center, NE, USA.*

Ruminants are typically born with a rumen devoid of microorganisms. The succession of the microbial population in the rumen from birth to animal maturity is of interest due to the key role that the rumen microbial population plays in the overall health and productivity of the host animal. There is limited data available regarding the development of bacterial populations in beef calves managed under traditional systems. We hypothesize that calves raised in differing environments will alter rumen bacterial population development. The objective of this study was to investigate the effects of environment on the succession of the bacterial population in the rumen of nursing beef calves over time. Ruminal samples were collected on d 7, 35, 63, 91, 119, 147, and 175 of age via oral lavage from Angus calves- in New Mexico (NMANG, n = 10) and Nebraska (NEANG, n = 10). The pastures at each location differed by plant species and diet quality. Samples were used for metagenome

analysis of the V1-V3 region of the 16S rRNA gene using the Illumina MiSeq. A total of 122 samples produced 333,068 operational taxonomic units. Metagenomic analysis revealed a day by environment interaction for phylum ($P < 0.05$). *Bacteroidetes* and *Firmicutes* were the predominant phyla regardless of environment. On d 7, *Bacteroidetes* was greater ($P < 0.01$) in NEANG. However, by d 175 NMANG had a greater percentage of *Bacteroidetes* ($P < 0.01$). *Firmicutes* was the predominant phylum for NMANG on d 7 and remained greater ($P < 0.01$) than NEANG throughout the sampling days. The predominant genera in NEANG on d 7 were *Prevotella*, CF321, *Fibrobacter*, and *Campylobacter* ($P < 0.05$); however, these populations did not differ from NMANG on d 175 ($P < 0.05$). Butyrivibrio was greater ($P < 0.01$) in NMANG on d 7, but showed no differences by d 175. Genera richness increased from d 7 to d 175 with NMANG having greater ($P < 0.01$) richness throughout sampling days. Results show environmental effects that may be driven by diet quality and composition on the succession of the bacterial population in nursing beef calves.

Key Words: Angus, calf, metagenomics, rumen bacteria

45 Identification and biochemical characterization of three bile salt hydrolases in the human gut microbe *Bacteroides vulgatus* ATCC 8482.

L. Ly*, S. Devendran, and J. Ridlon,
University of Illinois at Urbana-Champaign, Champaign, IL, USA.

During digestion, bile salts are secreted from the gallbladder and become susceptible to microbial biotransformation in the colon. The resulting bile acids act as hormones and regulate host-microbe interactions, ultimately causing major changes to host physiology. Bile salt hydrolases (BSHs) catalyze the gateway reaction to all downstream bile acid metabolism by deconjugating glycine and taurine from the bile acid. Whole-genome sequencing has revealed multiple copies of BSHs found in the genomes of bacteroides species, the predominant genus in the gut microbiota. Here, 3 putative BSHs (WP_012055977, WP_032944961, WP_005850163) have been identified in *Bacteroides vulgatus* ATCC 8482 based on sequence homology to a previously identified and characterized BSH from *Bacteroides fragilis*. The molecular mass of the 3 BSHs have been determined to be 39.4, 39.7 and 63.0 kDa, respectively. Using the ninhydrin colorimetric method to detect deconjugated amino acids, each purified BSH was screened for substrate specificity against 10 bile salts: taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, taurolithocholic acid, tauroursodeoxycholic acid, tauro-α-muricholic acid, tauro-β-muricholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid, and glycoursodeoxycholic acid. Based on the substrate specificities, Michaelis-Menten constants for each BSH were determined from Lineweaver-Burk plots. Additionally, a phylogenetic tree of other *bacteroides* species was constructed based on homologous BSH sequences. The goal of this research is to understand the physiological role of BSH for intestinal *Bacteroides* spp.

Key Words: bile acid, *Bacteroides*, bile salt hydrolase

46 Isolation and partial characterization of starch degrading bacteria belonging to core phylotypes of rumen microbiota in Japanese Black cattle.

S. Koike*[1], Y. Akiyama[1], T. Hashimoto[1], R. Inoue[2], T. Endo[3], Y. Suzuki[1], and Y. Kobayashi[1],
[1]*Hokkaido University, Hokkaido, Japan,* [2]*Kyoto Prefectural University, Kyoto, Japan,* [3]*Hokkaido Research Organization, Hokkaido, Japan.*

Japanese Black (JB) is the most popular beef breed and accounts for 60% of beef cattle in Japan. To produce well-marbled meat, JB cattle are fed concentrate-based diet for over one year. Therefore, starch-degrading bacteria play an important role in the stable ruminal fermentation in JB cattle. In this study, we aimed to characterize starch-degrading bacteria belonging to core phylotypes of rumen microbiota in JB cattle. Ruminal content was collected from 74 fattening JB cattle using a stomach tube and was used for bacterial community analysis (MiSeq) and isolation of starch degrading bacteria (roll tube method). MiSeq analysis of the microbiota

revealed that *Lachnospiraceae*, *Prevotellaceae*, *Ruminococcaceae*, unclassified *Bacteroidales* and unclassified *Clostridiales* occupied more than 50% of rumen microbiota in JB cattle. Therefore, these 5 phylotypes were considered as core members of the microbiota, and we attempted to isolate starch-degrading bacteria belonging to these phylotypes. Among 178 newly isolated strains, 12 strains were classified into either of target phylotypes; 5 strains for *Lachnospiraceae*, 5 strains for *Ruminococcaceae* and 2 strains for *Prevotellaceae*. Based on the phylogenetic positions, representative strains were selected and used for characterization. Under different pH conditions, *Lachnospiraceae* strain grew at pH 5.5; meanwhile, strains of *Prevotellaceae* and *Ruminococcaceae* did not. Strains of *Lachnospiraceae* and *Ruminococcaceae* adhere to feed particles within 30 min of incubation, and the extent of adhesion was higher in concentrate particles than in hay particles. Although *Prevotellaceae* strain did not adhere to feed particles within 30 min of incubation, amount of particle-adherent cells seemed to gradually increase during 24 h of incubation. Therefore, the strains belonging to core phylotypes of rumen microbiota in JB cattle might be involved in feed digestion in different manners.

Key Words: rumen microbiota, isolation, starch degrader

47 Prokaryotes inside and outside of ruminal ciliates isolated from rumen fluid and *in vitro* culture.

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

Ruminal protozoa are ciliates that primarily rely on predation of prokaryotes for their survival. It remains to be determined if they select their preys or symbionts. This study examined the bacteria and archaea that reside inside and outside of single protozoal cells. Single cells of *Entodinium caudatum* and *Epidinium caudatum* were isolated from a monoculture of each species after 0, 2, 4, and 8 h after feeding and washed. The free-living prokaryote (FLP) fraction of each culture was also collected. The free-living bacteria and archaea and those associated with the single protozoal cells were identified using Illumina sequencing of 16S rRNA gene amplicons of V4-V5 hypervariable regions. *Proteobacteria* dominated the prokaryotes associated with the isolated single protozoal cells (PAP) of both protozoal species. The FLP of both monocultures have well separated bacterial communities, but the cells of both *Ent. caudatum* and *Epi. caudatum* shared some associated bacteria mostly belonging to *Proteobacteria*. Acquisition of FLP into PAP was prominent in *Epi. caudatum* whereas both protozoal species contained *Shewanella* as dominant genus in PAP which have not considered in rumen microbiome. No significant shift in major bacterial groups was found in both FLP and PAP over time of feeding. Hydrogenosome-containing *Epidinium* had more archaean than mitosome-containing *Entodinium*. Single cells of 8 different genera of ruminal ciliates were also isolated from the rumen fluid of 5 Jersey dairy cows after 2 h of morning feeding. Prokaryotes were also harvested from rumen fluid after removal of protozoa. *Proteobacteria* was dominant in PAP, and *Shewanella* is the largest genus. *Sediminibacterium*, *Limnobacter* and *Wolinella* were only found in PAP among the major genera. There was no significant difference in archaeal abundance, but *Methanimicrococcus* was only detected in *Diploplastron*. The results suggest that ruminal protozoa have some selectivity over the prokaryotes they associate with. However, further research is needed to distinguish true symbionts from engulfed prokaryotes as feed.

Key Words: amplicon sequencing, protozoa-associated prokaryote, *Proteobacteria*, ruminal ciliate, *Shewanella*

48 Draft macronuclear genome of *Entodinium caudatum*.

T. Park*, S. Wijeratne, T. Meulia, and Z. Yu,
The Ohio State University, Columbus, OH, USA.

Entodinium caudatum is a representative species of the genus *Entodinium* that is the most dominant protozoa in the rumen. *E. caudatum* is also the most researched ruminal protozoa, but lack of and inability to establish an axenic culture have hindered understanding its actual role in the rumen. The objective of this study was to sequence the macronuclear (MAC)

genome of *E. caudatum* to aid understanding of its metabolism, physiology, and ecology to achieve the information about their genetic functions. Cells of *E. caudatum* were isolated from a laboratory monoculture and washed. Macronuclei were isolated and lysed using physical and chemical methods. Following purification of macronuclei using Percoll gradient centrifugation and confirmation by PCR amplification of the actin gene, MAC DNA was extracted and RNA was removed. The MAC DNA was sequenced using an Illumina MiSeq platform. Approximately 40 million paired-end (2 × 300 bp) reads were generated. Illumina adapters removing and quality trimming were performed using Trimmomatic (version 3.2.2) with the quality cut off set to Phred quality 20 for a window size of 4 (SLIDINGWINDOW:4:20). Any reads shorter than 75 bp were removed. Then, the quality-checked reads (70,540,444 in total) were de novo assembled using Spades. The coverage reached 98 × from a total 66,294 contigs with N50 being 2,606 (the longest contig is 147,262 bp). The telomere sequences of *E. caudatum* were unknown, but 4 of the scaffolds were capped at both ends with the telomere sequences of *Tetrahymena thermophila*, the most research model ciliate species. Some of the scaffolds were only capped at one end with the *T. thermophila* telomere sequences. Annotation of draft MAC genome and comparison with that of other ciliates will help better understand the metabolism, physiology, and ecology of *E. caudatum* and its importance in rumen function and interaction with other members of the rumen microbiome.

Key Words: *Entodinium caudatum*, genome assembly, macronucleus, ruminal ciliate

49 Growth inhibitory effects of monensin on ruminal bacteria.

N. Kim*, I. K. O. Cann, and R. I. Mackie,
University of Illinois Urbana-Champaign, Urbana, IL, USA.

Monensin, an ionophore antibiotic, is widely used in ruminant animal diets to improve the production efficiency and to prevent coccidial infections in poultry. Current knowledge concerning the mechanism increasing feed efficiency comes from manipulation of ruminal fermentation through alteration of microbial populations and their activity by monensin that selectively suppresses growth of rumen bacteria with a G+ type of cell wall. However, little is known about its cellular mode of action in ruminal bacteria with the different cell wall types. The growth inhibitory effects of monensin were investigated by growing representative rumen strains of G- (*Prevotella bryantii* B14), G+ (*Ruminococcus albus* 7 and *Streptococcus bovis* JB1), and G-intermediate (*Selenomonas ruminantium* HD4, *Megasphaera elsdenii* T81 and *Butyrivibrio fibrisolvens* D1) in batch culture over a range of monensin concentrations. *P. bryantii* B14, which is known for its intrinsic resistance to monensin, showed inhibited growth at 7.0 µg/mL. In the G+ strains, *R. albus* 7 was sensitive to 0.2 µg/mL while growth of S. bovis JB1 was resistant to 4.0 µg/mL and showed delayed growth at a concentration of 7.0–20.0 µg/mL with prolonged incubation of up to 18 h. Populations of *S. ruminantium* HD4 and *M. elsdenii* T81 were insensitive up to 50.0 µg/mL and 25.0 µg/mL, respectively, whereas growth of *B. fibrisolvens* D1, which has a G+ cell wall type, was inhibited by 0.2 µg/mL. Among the strains that exhibited resistance to monensin, *P. bryantii* B14, *S. ruminantium* HD4, and *M. elsdenii* T81 grew to a similar MaxOD at the late-exponential phase of growth. In contrast, the maximal growth of S. bovis JB1 decreased with respect to the increasing monensin concentrations. The results suggest that most of the strains showed susceptibility to monensin that was related to their cell wall type. Furthermore, the delayed growth of *S. bovis* JB1 indicates the possibility of adaptation by selection of monensin-resistant populations even within bacteria that have a G+ cell wall type. Further studies on cellular and molecular mechanisms will expand our understanding of adaptation of this phenotype in ruminal bacteria.

Key Words: monensin, ruminal bacteria, growth inhibitory

50 A simplified bacterial community in gnotobiotic mice is insufficient to study community dynamics concerning resistant starch digestion.

J. P. Sementkowski* and N. M. Koropatkin,
University of Michigan, Ann Arbor, MI, USA.

Dietary resistant starch is a propitious prebiotic therapy for the treatment of inflammatory bowel disease and diabetes. Resistant starch is not processed in the small intestines and thus, becomes nutrition for gut bacteria. Degradation of resistant starch is a specialized function of species such as *Bifidobacterium adolescentis* and *Ruminococcus bromii*, but how they compete and influence starch digestion by the rest of the gut community is unknown. Using a gnotobiotic mouse model, we conducted 3 trial experiments in which mice were colonized with intentional communities of both resistant starch degraders and non-resistant starch degraders: *Bifidobacterium adolescentis*, *Ruminococcus bromii*, *Bacteroides thetaiotaomicron*, and *Eubacterium rectale*. *Lactobacillus reuteri* was included in the second trial. *Bacteroides ovatus* replaced *Bacteroides thetaiotaomicron* in the third trial. Mice were initially fed a control diet and switched to a diet high in resistant starch. Fecal samples were collected throughout the experiments and qPCR was performed on DNA isolated from fecal samples to determine the relative abundance of each species in the mouse gut. In 3 trial experiments, we were unable to effectively colonize mice with all intentional species. *Ruminococcus bromii*, one of the resistant starch degraders, did not colonize any mice in amounts significant enough for analysis. In all trials, *Bacteroides* spp. overwhelmingly dominated, making up approximately 85 to 99% of the gut bacterial communities in which they were included, as measured via the last fecal sample collected before experiment conclusion. Our findings indicate that a more complete community may be necessary to study how resistant starch affects the composition and metabolic output of the gut microbiota. While the strains used in this study were known to colonize gnotobiotic mice, co-colonization information was limited or unavailable. Metabolic differences and spatial distribution within the gut between the species used in this study may explain our results.

Key Words: resistant starch, prebiotic, microbiota, gnotobiotic

51 Bacteria and fungi in day-old turkeys vary between companies and flocks.

A. H. Smith* and T. G. Rehberger,
Agro BioSciences, Milwaukee, WI, USA.

In commercial turkey production, eggs are washed and sanitized, and poults may be injected with antibiotics before placing in a brooder barn. Poults are then raised without exposure to adults. Our hypothesis is that most of the microbiota is obtained through horizontal transmission and very little by maternal transmission of bacteria resulting in highly variable patterns of colonization. Illumina sequencing of marker genes for bacteria, lactic acid bacteria (LAB) and fungi was performed to determine the variety of microbes present in day-old poults. DNA was extracted using a Qiagen PowerFecal DNA Kit from gastrointestinal contents of 118 d-old turkey poults from several flocks across 2 companies. PCR was performed with 16S V4 rRNA primers for total bacteria, 16S rRNA primers selective for LAB and fungal ITS primers using the Fluidigm Access Array. Sequence were analyzed with QIIME v1.8 and statistical analysis with Canoco 5. Microbial ecology was distinct by company (pseudo-F 38.7) with the predominant microbes at company A being *Clostridia*, specifically *C. disporicum*, *C. paraputrificum* and *C. tertium*. Predominant bacteria at company B were *Enterobacteriaceae*, specifically those grouping with *E. coli* and *Enterobacter cloacae*. The predominant LAB at both companies were *Enterococcus faecalis* and *E. gallinarum*, confirmed by sequencing the 16S gene of colonies picked from MRS agar plate counts. The predominant fungi were *Aspergillus niger* and *Saccharomyces cerevisiae*, with *Candida sake* or *Alterneria* sp. in some flocks of company A. Intra-company variation between flocks was greater at company B where the predominant bacteria were facultatively anaerobic *Enterobacteriaceae*, while anaerobic *Clostridia* were the predominant colonizers in day-old poults at company A. Further work is necessary to determine how

this variability affects microbiota succession and impacts growth and production of the birds.

Key Words: turkey, *Clostridium*, *Enterobacteriaceae*, *Enterococcus*

52 Pectin utilization by the human colonic bacterium *Bacteroides intestinalis* DSM17393.

H. Müller Paul*, R. I. Mackie, and I. Cann,
Carl R. Woese institute for Genomic Biology, University of Illinois, Urbana, IL, USA.

Pectin is a structural heteropolysaccharide present in plant cell walls. The polysaccharide is abundant in citrus fruits, but can be found in smaller proportion in soft fruits, like strawberries and cherries, and root vegetables such as carrots and beets. In addition, it is also often present in the human diet due to its applications as a gelling agent, particularly in jams and jellies. Although pectin can vary greatly in composition and structure, the most common forms present in human diets are homogalacturonans. These are composed of linear chains of α-(1–4)-linked d-galacturonic acid, with occasional substitutions of D-xylose, D-apiose, D-galactose or L-arabinose, as well as with methyl and acetyl group substitutions. Genomic analysis of the human colonic bacterium *Bacteroides intestinalis* DSM 17393 revealed that this bacterium harbors many genes including some that encode enzymes that are potentially involved in pectin breakdown to its monomeric units. The full enzyme repertoire includes 130 glycoside hydrolases (GH), 12 polysaccharide lyases (PL) and 7 carbohydrate esterases (CE), organized in 38 polysaccharide utilization loci (PULs). Some of these PULs are conserved throughout other *Bacteroidetes*. Moreover, the genome of this bacterium also contains the genes encoding the enzymes required for the conversion of galacturonate and glucuronate, the more abundant monomeric units of pectin, into intermediates involved in central metabolism. To probe our findings based on the in silico analysis of pectin degradation by this human colonic bacterium, the organism was tested for growth on different pectin-containing substrates. *B. intestinalis* was able to grow in minimal media containing pectin from citrus peel, apple and sugar beet as the sole carbon sources, as well as galacturonate, glucuronate and glucose. Subsequent to these experiments, data from RNaseq experiments, that compare growth on pectin versus its unit sugars, and biochemical characterization of enzymes are underway to validate the predicted pathways for pectin degradation and fermentation gleaned through bioinformatics analyses of the *B. intestinalis* genome.

Key Words: pectin, *Bacteroides intestinalis*, metabolic pathway

53 Feeding the natural plant extract β-resorcylic acid reduces enteric colonization and down-regulates attachment and gene expression of the foodborne pathogen *Campylobacter* in chickens.

A. M. Donoghue*[1], B. R. Wagle[2], A. Upadhyay[2], K. Arsi[2], S. Shrestha[2], K. Venkitanarayanan[3], and D. J. Donoghue[2],
[1]*Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, AR, USA,* [2]*Department of Poultry Science, University of Arkansas, Fayetteville, AR, USA,* [3]*Department of Animal Science, University of Connecticut, Storrs, CT, USA.*

Campylobacter is a major foodborne pathogen that causes severe gastroenteritis in humans, primarily through consumption of contaminated poultry products. Chickens are the reservoir host of *Campylobacter*, where the pathogen colonizes the intestinal tract, predominantly in the ceca, thereby leading to contamination of carcasses during slaughter. A reduction in cecal colonization by *Campylobacter* would directly translate into reduced product contamination and risk of human infections. With increasing consumer demand for antibiotic free chickens, research is being conducted to discover natural, safe and economical antimicrobials that can effectively control *Campylobacter* colonization in birds. This study investigated the efficacy of in-feed supplementation of a phytophenolic compound, β-resorcylic acid (BR) for reducing *Campylobacter* colonization in chickens. In 2 separate, replicate trials, day-old-chicks (n = 10 birds/treatment) were fed BR (0, 0.25, 0.5 or 1%) in feed for 14 d (n = 40/trial). Birds were challenged with a 4-strain mixture of *C. jejuni*

(~10^6 cfu/mL; 250 µL/bird) on d 7 and cecal samples were collected on d 14 for enumerating surviving *Campylobacter* in cecal contents. In addition, the effect of BR on critical colonization factors of *Campylobacter* (motility, epithelial cell attachment) was studied using phenotypic assay, cell culture and real-time PCR (qPCR). Supplementation of BR in poultry feed for 14 d at 0.5 and 1% reduced *Campylobacter* populations in cecal contents by ~2.5 and 1.7 Log cfu/g, respectively ($P < 0.05$). Follow up mechanistic analysis revealed that sub-inhibitory concentration (SIC) of BR reduced *Campylobacter* motility, attachment to and invasion of Caco-2 cells. The expression of *C. jejuni* genes coding for motility (*motA*, *motB*, *fliA*) and attachment (*jlpA*, *ciaB*) was downregulated as compared with controls ($P < 0.05$). These results suggest that BR could potentially be used as a feed additive to reduce the intestinal colonization of *Campylobacter* in broiler chickens.

Key Words: *Campylobacter*, β-resorcylic acid, chicken, colonization factor, gene expression

54 Elucidating the ecology of *Fibrobacter* spp. in the herbivore gut using comparative genomics.

A. Neumann* and G. Suen,
University of Wisconsin-Madison, Madison, WI, USA.

Herbivores rely on symbioses with fiber-degrading microbes living in their gut because these animals alone cannot efficiently digest the plant cell wall polysaccharides that make up their diet. One example is bacteria in the genus *Fibrobacter*, which includes important cellulose-degraders from the rumen. However, other members of this genus are poorly understood and are known to be conserved members of the gut microbiota of a diversity of herbivores. Culture-independent studies indicate that *Fibrobacter* populations in hindgut-fermenting herbivores (e.g., horses) are phylogenetically distinct from those populations in the rumen, but our ability to investigate their physiology has been hampered by a lack of representative axenic cultures. To address this knowledge gap, we developed a novel method for recovering axenic *Fibrobacter* cultures from herbivore gastrointestinal samples, and applied it toward the isolation of 45 novel *Fibrobacter* strains from 11 different hosts. These 45 strains represent 9 different phylotypes, and include the first confirmed representatives of phylogenetically distinct *Fibrobacter* populations common in the horse hindgut. Based on our phylogenetic analysis, we hypothesized that these *Fibrobacter* phylotypes occupy distinct ecological niches *in vivo*. We tested this by performing whole genome sequencing on a subset of our cultured isolates using an Illumina HiSeq 2500 sequencer. In total, 23 high-quality draft genomes were produced, and an analysis of their predicted proteins identified potential functional differences in both carbon and nitrogen metabolism that corresponded to their phylogenetic placement. The availability of these novel *Fibrobacter* strains and their genomic sequences promises to greatly expand on our knowledge of these enigmatic, yet important, fiber-degrading gut bacteria.

Key Words: *Fibrobacter*, herbivory, cellulose, microbiota

55 Functional analyses of an esterase-enriched polysaccharide utilization locus conserved in diverse human colonic *Bacteroidetes*.

G. V. Pereira*, D. Wefers, T. Natof, R. I. Mackie, and I. Cann,
University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, USA.

The human gastrointestinal tract (GIT) microbiome, through co-evolution, has generated a complex and stable community capable of modulating host physiology. *Bacteroides* are a predominant group of commensal bacteria within the colon responsible for a network of metabolic interactions with the host and other colonic microbes. Pharmaceutical interest in natural phenolic compounds, like ferulic acid, results from potential therapeutic uses to prevent or treat diseases. *Bacteroides* are primary degraders of diet-derived carbohydrates that escape host metabolism, and they possess an extensive array of carbohydrate degrading enzymes. Carbohydrate esterases (CEs) are widely conserved through members of the GIT, highlighting the importance of ester-

bound moieties in degradation of dietary fiber. Metagenomic and metatranscriptomic studies provide insight into microbe/host dynamics in the GIT environment. However, functional information is key for interpretation and downstream applications. Toward this aim, understanding the activity of CEs increases the knowledge of carbohydrate degradation and the phenolic compounds released by the human colonic microbiota. In this study, we analyzed the activity of several CEs encoded within a single polysaccharide utilization locus (PUL) in the human symbiont *B. intestinalis* DSM 17393. These CEs possess synergistic activity with several glycoside hydrolases produced by *B. intestinalis*, as well as enzymes from a related organism, *Prevotella bryantii*. This synergistic activity increases activity of the CEs toward wheat arabinoxylan, allowing release of larger amounts of ferulic acid. The hypothetical proteins encoded in this PUL, BACINT_01034 and BACINT_01040, also possess acetyl-xylan esterase and feruloyl esterase activities, respectively. Moreover, the hypothetical BACINT_01040 was the only feruloyl esterase to release ferulic acid from sugar beet pulp. Analysis of this PUL revealed insight into carbohydrate metabolism as well as metabolic interactions between the colonic members and the potential to interact with the human host.

Key Words: *Bacteroides*, esterase, gastrointestinal microbiome, glycoside hydrolase

56 Sequence-based analysis of the genus *Ruminococcus* resolves its phylogeny and reveals strong host association.

A. J. La Reau*[1], J. P. Meier-Kolthoff[2], and G. Suen[1],
[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.*

It has become increasingly clear that the composition of mammalian gut microbial communities is substantially diet driven. These microbiota form intricate mutualisms with their hosts, which have profound implications on overall health. For example, many gut microbes are involved in the conversion of host-ingested dietary polysaccharides into host-usable nutrients. One group of important gut microbial symbionts are bacteria in the genus *Ruminococcus*. Originally isolated from the bovine rumen, ruminococci have been found in numerous mammalian hosts, including other ruminants, and non-ruminants such as horses, pigs, and humans. All ruminococci require fermentable carbohydrates for growth, and their substrate preferences appear to be based on the diet of their particular host. Most ruminococci that have been studied are those capable of degrading cellulose, much less is known about non-cellulolytic non-ruminant-associated species, and even less is known about the environmental distribution of ruminococci as a whole. Here, we capitalized on the wealth of publicly available 16S rRNA gene sequences, genomes and large-scale microbiota studies to both resolve the phylogenetic placement of described species in the genus *Ruminococcus*, and further demonstrate that this genus has largely unexplored diversity and a staggering host distribution. We present evidence that ruminococci are predominantly associated with herbivores and omnivores, and our data supports the hypothesis that very few ruminococci are found consistently in non-host-associated environments. This study not only helps to resolve the phylogeny of this important genus, but also provides a framework for understanding its distribution in natural systems.

Key Words: phylogenomics, phylogeny, host range, 16S rRNA, *Ruminococcus*

57 The effects of nitrate and nitrite on rumen protozoal chemotaxis and yeast growth.

C. M. Welty[1], S. Waits[1], A. M. Gehman[2], Y. Roman-Garcia*[1], J. Plank[1], R. Meller[1], and J. L. Firkins[1],
[1]*The Ohio State University, Columbus, OH, USA,* [2]*Alltech Inc., Nicholasville, KY, USA.*

Nitrates can decrease methane emissions in cattle while potentially contributing to bacterial N assimilation. Bacterial reduction of NO_3 to NO_2 to NH_3 competes for H_2 used in methanogenesis. Alternatively, NO_3 or NO_2 could inhibit the chemotaxis of protozoa, which also produce H_2. Because of the risk of methemoglobinemia

caused by NO_2 absorption, we studied the potential for live *Saccharomyces cerevisiae* to decrease NO_2 accumulation by using it as an electron sink when transitioning from aerobic to anaerobic conditions. The first study assessed protozoa viability and chemotaxis toward glucose, saline, and peptides when pre-treated with either low (5.68 m*M*) or a high (17.03 m*M*) doses of NO_3 or NO_2 compared with a negative control or to a positive control with sodium nitroprusside (SNP), which activates directional motility in ciliates. The second study measured *S. cerevisiae* 1026 growth (OD 600 nm fit to an exponential growth model) on liquid YPD media either aerobically, in transition to anaerobic phase, or anaerobically. Media peptone was substituted with NO_3, NO_2, or NH_3 at 25, 50, or 75%. Both studies had 3 replicates. Protozoal motility and viability decreased ($P < 0.01$) with increasing NO_2. Contrary to our previous study, SNP inhibited ($P < 0.01$) motility and viability. Incubation treatments (NO_3, NO_2, or SNP) interacted with source of chemoattractant for entodiniomorphids but not for isotrichids. Chemoattraction by isotrichids to glucose or peptides was inhibited ($P < 0.05$) by SNP and NO_2 but not by NO_3. Entodiniomorphid chemotaxis toward glucose was unaffected by incubation treatment but decreased ($P < 0.01$) for peptides when incubated with NO_2 or SNP, which probably prevented detection of the peptide gradient. Yeast growth was inhibited with increasing doses of NO_2 but was unaffected by NO_3 or NH_3. Results did not support yeast supplementation to prevent NO_2 accumulation when feeding NO_3 to mitigate methane emission.

Key Words: nitrate, nitrite, methane, yeast, protozoa

58 Conversion of 12-ketolithocholate to deoxycholate by *Clostridium scindens*, *Clostridium hylemonae*, and *Clostridium hiranonis*, major bile acid-metabolizing anaerobes in the human gut.

L. Sallam*[1], J. Ridlon[2], G. Doden[2], H. Doden[2], and S. Daniel[1],
[1]*Eastern Illinois University, Charleston, IL, USA,* [2]*University of Illinois Urbana-Champaign, Champaign, IL, USA.*

The human intestinal microbiota represents an organ that has endocrine function. The gut microbiota is also responsible for converting primary bile acids to toxic secondary bile acids which play a role in colon cancer and gallstone disease. Secondary bile acids are produced by 7α-dehydroxylating bacteria such as *Clostridium scindens*, *Clostridium hylemonae*, and *Clostridium hiranonis*. In addition to their removal of the 7α-hydroxyl group of primary bile acids, these anaerobes metabolize bile acid derivatives via 3α- and 7α-hydroxysteroid dehydrogenases (HSDH). Our preliminary studies, based on a published protein sequence for 12α-hydroxysteroid dehydrogenase (HSDH; conversion of 12-ketolithocholate to deoxycholate) from *Clostridium* group P, identified a putative 12α-HSDH gene in *C. hylemonae*. This gene has now been cloned, expressed, purified, and found to encode an active 12α-HSDH. Given these results, the focus of this study was to determine if *C. scindens* ATCC 35704, *C. hylemonae* DSM 15053, and *C. hiranonis* DSM 13275 were capable of converting 12-ketolithocholate to deoxycholate during growth. All organisms were grown anaerobically at 37°C in brain heart infusion (BHI) broth. 12-Ketolithocholate and deoxycholate were prepared in methanol and added to BHI broth to a final concentration of 0.1 m*M*. Following growth, cultures were extracted with ethyl acetate; extracts were dried, resuspended in methanol, and subjected to thin-layer chromatography (TLC) for the detection of bile acids. Based on TLC analysis, all of the organisms tested converted 12-ketolithocholate to deoxycholate. None of the organisms metabolized deoxycholate, and growth was not affected by methanol, 12-ketolithocholate, or deoxycholate. Efforts are now underway to characterize the enzyme(s) responsible for the conversion of 12-ketolithocholate to deoxycholate in these important gut bacteria.

Key Words: bile acids, 12-ketolithocholate, deoxycholate, *Clostridium scindens*

Nutrition and metabolism of livestock, humans, and companion animals

59 See Oral Abstracts (page 29)

60 Assessment of the effect of high quality hay on rumen health through epithelial gene expression and epimural microbiota.

R. M. Petri*, M. T. Kleefisch, Q. Zebeli, and F. Kleverhusen,
University of Veterinary Medicine, Vienna, Austria.

Diets based on large amounts in starch and low in fiber are useful to enhance energy supply of cattle but increase the risk of ruminal acidosis with major consequences for rumen microbiome and the host health. The inclusion of energy-rich hay has recently been suggested as an alternative to starch-rich diets however, it is not clear if these diets are harmful for rumen microbiome and rumen epithelium. The aim of this research was to quantify the effect of titrated levels of high energy, high fiber hay on the rumen epimural population, ruminal pH and epithelial gene expression. Eight ruminally cannulated nonlactating and non-pregnant Holstein cows were used in a replicated 4 x 4 Latin square design with 3 of the 4 dietary treatment groups containing high-quality hay, with either 0% concentrate (100% HQH), 25% concentrate (75% HQH) or 40% concentrate (60% HQH). The fourth group (CON), the control treatment, was fed with 60% normal fiber-rich hay and 40% concentrate. Samples for rumen pH, papillae samples for microbiota and gene expression were taken in each run. The relative expression of genes targeting barrier function (Claudin 1 and 4) and sodium/hydrogen exchange (NHE3) were significantly higher ($P \leq 0.05$) in the 60% HQH diet. Analysis of the most abundant operational taxonomic units (OTU; >0.01% total sequences) showed a significant decrease ($P = 0.004$) in the relative abundance of an OTU identified as a member of the *Comamondaceae* family of gram-negative *Proteobacteria* in HQH diets compared with the CON. This OTU also showed a significant negative correlation ($r = -0.55$; $P = 0.002$) to rumen pH. A dietary impact on phyla was only seen for *Spirochaetes* with the lowest percent abundance in the CON diet, which corresponded with genus SJA88 of the *Spirochaetes* phylum. The substitution of high quality hay in place of concentrate in high producing animal diet in this experiment showed no negative effects on rumen pH, gene expression targets for barrier function and pH regulation as well as epimural microbiota.

Key Words: epimural, rumen, gene expression, high quality hay

61 Feed efficiency phenotypes in lambs involve changes in ruminal, colonic, and small intestine-located microbiota.

K. Perea, K. Perz, S. Olivo, A. Williams, M. Lachman, S. Ishaq*, J. Thomson, and C. Yeoman,
Montana State University, Bozeman, MT, USA.

Several studies have revealed differences in rumen-located microbes between greatly efficient and inefficient animals, however, how the microbiota vary in the hind gastrointestinal tract (GIT) has only been sparsely explored and how they vary in the small intestine remains to be determined. We therefore sampled the microbiota of the duodenum, jejunum, ileum, colon, and colorectally obtained feces, in addition to the rumen of 12 lambs that, in a residual feed intake trial were found to be at either extreme of feed efficiency phenotypes. The 16S rRNA gene (V3-V4 region) profiles of all samples were analyzed and revealed unique microbiota in all GIT locations except the jejunum and ileum (ANOSIM $R > 0.2$, $P < 0.001$). Measures of β-diversity revealed greater dissimilarity between more anatomically distant GIT locations (e.g., Rumen – Duodenum, ANOSIM $R = 0.365$, $P < 0.001$; Rumen – Colon, ANOSIM $R = 1$, $P < 0.001$) with the nearest distal region typically more similar than the nearest proximal location. The relative abundances of 13 operational taxonomic units (OTUs) from the duodenum, jejunum, colon, and feces, as well as the rumen, differed between efficient and inefficient animals (Bonferroni corrected $P < 0.05$), while another

2 OTUs trended toward significance. These OTUs were classified as taxa with known roles in fibrolysis (*Fibrobacteres*, *Ruminococcaceae*, and *Saccharofermentans*) and others that are commonly associated with health (*Bifidobacteriaceae* and *Christensenellaceae*) and dysbiosis (*Proteobacteria*). Our findings show biogeographical delineations of microbiota throughout the GIT and suggest that feed efficiency extends beyond the rumen, transcending these regions, and involves increases in both rumen-, and colon-located fibrolytic taxa, increases in bifidobacterial species in the small intestine, and reductions in small intestine and distal GIT-located *Proteobacteria*.

Key Words: feed efficiency, residual feed intake, microbiota, biogeography, lamb

Prebiotics, probiotics, and DFM development

62 See Oral Abstracts (page 31)

63 Effect of antibiotics, Diamond V Original XPC and *Lactobacillus plantarum* on broiler performance.

S. Rasoulzadeh[1], S. Rahimi*[1], and K. Akbari[2],
[1]*Tarbiat Modares University, Tehran, Tehran, Iran,* [2]*National Institute of Genetic Engineering and Biotechnology, Tehran, Tehran, Iran.*

A comparative study was carried out to evaluate the effect of antibiotic, Diamond V Original XPC and *Lactobacillus plantarum* on broiler performance. A total of 480 one-day-old chicks of mixed sex were assigned in 4 treatments with 8 replications (15 birds in each pen) as completely randomized design. The diet treatments included (1) control (basal diet), (2) basal diet plus antibiotic (oxytetracycline 20%), (3) basal diet plus Diamond V Original XPC, 4) basal diet plus *Lactobacillus plantarum* (LP). One milliliter of LP (2×10^7 cfu/mL at OD = 0.1) was used for LP treatment. The basal diets were formulated according to National Research Council (NRC). Experiment carried out for 42 d and the data were analyzed by ANOVA using the GLM procedure of SAS. Duncan's multiple range tests was used to determine the statistical significance among the means. There was no statistically significant difference between the treatment groups in body weight, body weight gain, feed intake and feed conversion ratio ($P \leq 0.05$). It was assumed that Diamond V Original XPC and LP can improve microbiota of broiler gastrointestinal tract, which thereby have an important role in enhancement of intestinal function and production performance. Data analysis of this experiment did not show a significant effect on production performance of broilers. Further studies are needed to investigate the effect of the above-mentioned treatments on gut health and growth performance of broiler chickens.

Key Words: Original XPC, *Lactobacillus plantarum*, broiler chicken

64 Comparison the effect of antibiotic, probiotic, prebiotic, phytobiotic, and *Bacillus subtilis* on broiler performance.

M. Hamidi[1], S. Rahimi*[1], and N. Mojgani[2],
[1]*Tarbiat Modares University, Tehran, Tehran, Iran,* [2]*Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.*

In this study, the effects of antibiotic (oxytetracyclin), probiotics (type 1 and type 2), Bactocell, prebiotic (Diamond V Original XPC), phytobiotic (licorice extract with thyme), phytobiotic plus probiotic type 2, and *Bacillus subtilis* on broiler performance were evaluated. Experiment was performed in a completely randomized design with 10 treatments, 4 replicates, and 14 chicks in each pen. The experimental diets were fed from d 1 to d 42. Feed intake, weight gain and feed conversion ratio were measured on a weekly basis. Statistical analysis of the data showed significant effect of treatments on FI, WG, and FCR ($P \leq 0.05$). Administration of probiotics, phytobiotic, and *Bacillus subtilis* to the diet resulted in reduce of FI and FCR compared with the antibiotic and control groups ($P \leq 0.05$). Higher weight gain was seen in probiotic and phytobiotic treatments compared with prebiotic group ($P \leq 0.05$). In conclusion, using probiotic, phytobiotic and

Bacillus subtilis in broiler diets improve growth performance of these birds; therefore, can be considered as antibiotic alternatives in poultry production

Key Words: antibiotic, *Bacillus subtilis*, prebiotic, phytobiotic, broiler

65 Functional properties of lablab bean husk and soybean husk in hindgut fermentation and microbiota of rats.

H. Myint*, H. Kishi, Y. Iwahashi, W. Saburi, S. Koike, and Y. Kobayashi,
Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, Japan.

Bean husk is rich in fiber and can be considered as feed material for various animals, expecting potential nutritive and health impacts. This experiment was conducted to evaluate the functional properties of lablab bean (*Dolichos lablab*) husk and soybean (*Glycine max*) husk in hindgut fermentation and microbiota of rats. Twenty male rats at 5 week of age were equally divided into 4 groups and fed either of the following diets for 3 weeks: purified diet (AIN 93G) containing 5% cellulose (Cellulose), same diet in which cellulose was replaced by corn starch (Starch), lablab bean husk (Lab) or soybean husk (Soy). Growth performance of rats fed 4 different diets was not different among the groups. Rats on Lab and Soy showed higher ($P < 0.05$) concentrations of cecal short chain fatty acids including acetate and butyrate, and lactate than those on Cellulose, accompanying with lower ($P < 0.01$) pH. Cellulose, Soy and Lab, especially Lab, lowered ($P < 0.05$) cecal indole and skatole levels. MiSeq analysis of cecal microbiota revealed increased ($P < 0.05$) abundance of *Lactobacillus* on Soy, *Bifidobacterium* on Lab and Soy, *Akkermansia* on Lab, and *Dorea* on Starch. The qPCR analysis revealed increased ($P < 0.05$) proportions of *Dorea* on Starch, *Lactobacillus* on Soy, and *Akkermansia* on Lab. Both husks were indicated to contain oligosaccharides (raffinose, stachyose and others) by TLC, mass spectrometry and nuclear magnetic resonance analysis and these might selectively stimulate the growth of beneficial bacteria in the gastrointestinal tract of rats. Based on these, bean husks tested in the present study are considered to be functional fiber for promoting the health of monogastric animals.

Key Words: lablab bean husk, soybean husk, fermentation, microbiota

66 Characterization of probiotic abilities of lactobacilli isolated from Iranian Koozeh traditional cheese.

M. Tavakoli[1], Z. Hamidi-Esfahani*[1], M. A. Hejazi[2], M. H. Azizi[1], and S. Abbasi[1],
[1]*Tarbiat Modares University, Tehran, Tehran, Iran,* [2]*Agricultural Biotechnology Research Institute of Iran, Tabriz, E. Azarbayjan, Iran.*

Eight lactic isolates including *Lactobacillus plantarum* (MT.ZH193, MT.ZH293, MT.ZH393, and MT.ZH593), *L. casei* (MT.ZH493), *L. pentosus* (MT.ZH693), and *L. fermentum* (MT. ZH893, and MT.ZH993) were identified from an Iranian traditional cheese, Koozeh paneer, using the morphological, phenotypical, biochemical and molecular characterization and then their probiotic characteristics were compared. Results showed that the lactic isolates of *L. plantarum* (MT.ZH293) and *L. fermentum* (MT.ZH893 and MT.ZH993) were resistant to all the used bile salts up to concentrations of 0.3 to 2.0%. All the strains showed low sensitivity to the presence of conjugated bile salts. *L. plantarum* MT.ZH293 exhibited the highest enzymatic activity of β-galactosidase and survival rate in a simulated stomach duodenum passage. *L. casei* MT.ZH493 generated the highest amount of hydrogen peroxide, followed by *L. fermentum* MT.ZH993 and *L. plantarum* MT.ZH593. Although the most selected LAB isolates had a moderate cell surface hydrophobicity, *L. plantarum* MT. ZH593 expressed the highest cell surface hydrophobicity. *L. fermentum* MT.ZH893 had strong resistance to all the antibiotics tested such as amoxicillin, ceftriaxon, chloramphenicol, erythromycin, gentamicin, streptomycin, tetracycline and vancomycin. Five lactic strains of *L. plantarum* (MT.ZH193, MT.ZH393, and MT.ZH593) and *L. fermentum* (MT.ZH893 and MT.ZH993) inhibited the growth of the tested foodborne pathogens including *Escherichia coli* PTCC5052, *Salmonella*

enterica, *Enterococcus hirea*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Key Words: probiotic cheese, lactic acid bacteria, antibacterial, antibiotic resistance, gastric resistance

67 Antagonistic effects of lipids against the bactericidal activity of thymol-β-D-glucopyranoside.

R. C. Anderson*[1], G. Levent[1,2], B. Petrujkic[1,3], G. Ciftcioglu[2], R. B. Harvey[1], M. H. Hume[1], H. He[1], K. J. Genovese[1], R. C. Beier[1], C. L. Swaggerty[1], T. R. Callaway[1], and D. J. Nisbet[1],
[1]*USDA/ARS, College Station, TX, USA,* [2]*Istanbul University, Istanbul, Turkey,* [3]*University of Belgrade, Belgrade, Serbia.*

The gut of food-producing animals is a reservoir for zoonotic pathogens. Thymol is bactericidal against *Salmonella*, *E. coli*, and *Campylobacter* but its rapid absorption from the proximal gut reveals a need for protective technologies to deliver effective concentrations to the lower gut where the pathogens mainly colonize. Thymol-β-D-glucopyranoside (hereafter named β-D-thymol) is resistant to absorption in everted jejunal segments because of its β-glycosidic bond and thus could be used as a prebiotic, being resistant to absorption and degradation in the proximal gut but hydrolysable by microbial β-D-thymol-hydrolyzing enzymes in the distal gut. Results from *in vitro* dose titration studies have identified efficacious doses of β-D-thymol against *Salmonella*, *E. coli* and *Campylobacter* during culture with porcine gut bacteria, with concentrations of β-D-thymol needed to achieve efficacious reductions of *Salmonella* or *E. coli* being 6 to 9 times higher than that (1 m*M*) needed to effectively kill *Campylobacter* species. The increased susceptibility of *Campylobacter* to β-d-thymol may be a consequence of its dependence on amino acid fermentation as free thymol is thought to inhibit this activity. Oral administration of β-D-thymol to pigs did not achieve significant reductions in cecal and rectal concentrations of *Salmonella*, *E. coli* or *Campylobacter*. Among several hypotheses for the lack of activity *in vivo*, sequestration of β-D-thymol, a lipophilic compound, in dietary lipids may limit access to hydrolytic enzymes thereby preventing release of free thymol. In support of this latter hypothesis, we found that the bactericidal effect of β-D-thymol (6 m*M*) against *Salmonella* Typhimurium and *E. coli* K88 (4.50 and 4.69 $\log_{10}$-fold reductions in cfu, respectively), achieved after 24 h *in vitro* culture with porcine fecal microbes, was diminished ($P < 0.05$; SEM = 0.448 and 0.497; respectively) more than 68 and 91% by 3% (wt/vol) added vegetable or olive oil (n = 3 cultures/treatment). Additional research is warranted to learn how to overcome obstacles diminishing bactericidal activity of β-D-thymol in the lower gastrointestinal tract.

Key Words: *Escherichia coli*, *Salmonella*, thymol, zoonotic pathogens

68 Identification and characterization of a 20β-hydroxysteroid dehydrogenase from the human gut microbe *Bifidobacterium adolescentis*.

S. Mythen*, S. Devendran, and J. Ridlon,
University of Illinois at Urbana-Champaign, Urbana-Champaign, IL, USA.

The gut microbiota consists of a complex network of distinct bacterial genera that together affect the physiology of the human host. Of the many facets affected by gut microbiota, their impact on the endocrine system is both significant and understudied. *Clostridium scindens* was previously shown to encode for steroid 17,20-desmolase (desAB), an enzyme that cleaves carbon-carbon bonds in steroid side chains; in turn converting C21 glucocorticoids into C19 androgens. *C. scindens* also encodes a 20α-hydroxysteroid dehydrogenase (HSDH) that is hypothesized to regulate the rate of side-chain cleavage. *Butyricicoccus desmolans* and *Clostridium cadaveris* have also been found to express steroid 17,20-desmolase and 20β-HSDH activity. We recently identified the gene encoding 20β-HSDH in *B. desmolans*. A phylogenetic analysis of *B. desmolans*' amino acid sequence of 20β-HSDH activity led to the identification of bifidobacteria strains with the potential to encode for 20β-HSDH enzymes as well. *Bifidobacteria* are regularly used as probiotics in products such as milk, yogurt, and dietary supplements. There are more than 30 species of bifidobacteria, of which only a few

strains, including *B. adolescentis* strain L2–32, express 20β-HSDH activity. The enzyme encoded by the 20β-HSDH gene is capable of metabolizing cortisol into 20β-dihydrocortisol and may alter androstane formation and potentially other steroid modifications such as steroid 21-dehydroxylation by *Eggerthella lenta*. In the laboratory, the gene encoding a 294 amino acid 20β-HSDH enzyme from *B. adolescentis* was cloned, overexpressed in *E. coli* and purified. The deduced molecular weight is 31.7 kDa and observed molecular weight is 32 ± 2.2 kDa. The enzymatic activity was measured by monitoring the oxidation of NADH at 340 nm using spectrophotometry techniques, quantifying the metabolism of cortisol to 20β-dihydrocortisol. The K_m and V_{max} were determined to be 26.9 μ*M* and 49.5 μmol/min, respectively. The optimal reaction direction and pH were determined as well as substrate specificity.

Key Words: bifidobacteria, 20β-hydroxysteroid dehydrogenase, probiotic, glucocorticoid

69 Inhibitory effect of two indigenous *Bacillus* strains on growth of some plant pathogenic fungi and mycotoxins reduction.

F. Siahmoshteh[1], Z. Hamidi-Esfahani*[1], and M. Razzaghi Abyaneh[2],
[1]*Tarbiat Modares University, Tehran, Tehran, Iran,* [2]*Pasteur Institute, Tehran, Tehran, Iran.*

Food and agricultural products are always exposed to fungal contamination in the field or during storage. These fungi could produce hazardous toxins such as aflatoxin, the most carcinogenic mycotoxin and ochratoxin A as a nephrotoxic and carcinogenic mycotoxins. In the present research, the growth of some plant pathogenic and food-spoiling fungi were studied at 25°C and 30°C and also the biocontrol of the fungi by 2 pistachio orchard-soil isolate bacteria were investigated. After proving of being antagonist against broad spectrum of pathogenic fungi, aflatoxin (AFB1 and AFG1) and ochratoxin A (OTA) reduction in liquid medium was investigated at different intervals. According to results, both *Bacillus* strains were able to inhibit the growth of *A. parasiticus*, *A. carbonarius*, *P. expansum*, *F. graminearum, F. verticilloides* and *F. oxysporum*. The bacterial strains also could reduce the toxins in liquid medium. The toxin reduction was time-dependent phenomenon. The order of reduction rate in liquid medium was OTA > AFG1 > AFB1.

Key Words: inhibitory effect, pathogenic fungi, aflatoxin, ochratoxin A, biocontrol

70 The effect of antibiotic, probiotic and prebiotic (Diamond Original XPC) in reducing colonization of *Campylobacter jejuni* in intestine of broilers.

N. Soltani[1], S. Rahimi*[1], and P. Khaki[2],
[1]*Tarbiat Modares University, Tehran, Tehran, Iran,* [2]*Razi Vaccine and Serum Research Institute, Karaj, Alborz, Iran.*

The research was conducted to evaluate the effect of antibiotic, probiotic and prebiotic (Diamond Original XPC) on growth performance and reducing colonization of *Campylobacter jejuni* in intestine of broiler chickens challenged with this bacterium. In this study 560 d-old male and female (equally from each sex) broiler chicks (Ross 308) were divided into the 10 treatments with 4 replicates of 14 chicks per replicate in a completely randomized design. Treatments 1 to 8 received following additives: 1: antibiotic (0.5 g/kg); 2: probiotic (1 g/kg in starter and grower diets, and 0.5 g/kg in finisher diet); 3,4: prebiotic (0.3, 0.15 g/kg). Treatments 5 to 8 were challenged (gavaged) with 1×10^7 (cfu/ mL) of *Campylobacter jejuni* at 21 d of age. Treatments 9 and 10 were negative and positive control, respectively. Probiotic group showed significant reduction of *C. jejuni* in intestine of broilers compared with positive control group ($P \leq 0.05$). The highest FCR was observed in treatment 7 and the lowest in treatments 3 and 5 ($P \leq 0.05$). The lowest and highest feed intake were seen in groups 4 and 6, respectively ($P \leq 0.05$). Treatment 6 had the highest and treatments 4 and 9 had the lowest body weight gain ($P \leq 0.05$). The results of this study indicated that probiotic and prebiotic could be effective in reduction of *C. jejuni* colonization in intestine of broilers and improve growth performance of these birds.

Key Words: *Campylobacter jejuni*, broiler, probiotic, prebiotic, antibiotic

71 Improving gut microbiome function via high-throughput screening of biological and chemical compounds.

L. Marsh and M. Hess*,
University of California, Davis, CA, USA.

Microbial communities, commonly referred to as microbiomes, associated with the gastrointestinal tract of animals and humans are complex ecosystems that play a key role in host health and performance. Diet is an important factor in shaping the phylogenetic composition and functionality of a microbiome and subsequently on the health and performance of the host organism. Unquestionably, *in vivo* (human or animal) trials to determine the sustainability and efficiency of dietary ingredients and supplements or pharmaceuticals are essential and have to be conducted before any of these products can be approved for human or animal consumption. However, there are ethical and economic limitations involved in conducting *in vivo* trials. These limitations restrict our basic understanding of host-microbiome interactions and make it extremely challenging to screen large numbers of new dietary factors that may increase host health and performance. To address these limitations, we designed and established an artificial (*in vitro*) rumen system that allowed us to monitor various biological, physical, and chemical parameters during the digestion process. Since this system facilitates to study how this suite of parameters responds to the addition of biological and chemical compounds, it represents an economical platform to quickly screen and evaluate the effect of feedstock composition as well as the effect of biological and chemical feed additives on gut and host health and performance.

Key Words: artificial rumen system, gut health and performance, rumen microbiome, prebiotic, probiotic

72 Composting of laying hen litter with the addition of a yeast probiotic.

Y. Castillo-Castillo*[1], J. D. Rivera[1], O. Ruiz-Barrera[2], M. Itza[1], C. Arzola[2], M. E. Hume[3], R. C. Anderson[3], J. Salinas[4], and A. Corral[2],
[1]*Universidad Autonoma de Ciudad Juarez, Ciudad Juarez, Chihuahua, Mexico,* [2]*Universidad Autonoma de Chihuahua, Chihuahua, Chihuahua, Mexico,* [3]*USDA/ARS, College Station, TX, USA,* [4]*Universidad Autonoma de Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico.*

Poultry litter has a high content of non-protein nitrogen and minerals, which make it suitable as a ruminant ration. However, poultry litter represents a prime source of pathogenic microorganisms if it is not given proper treatment. The objective of this study was to assess the effect of inclusion of Prozoot15 on the composting of laying hen litter and its effect on the elimination of potential pathogenic microorganisms. A complete random experimental design with a factorial arrangement (3 × 3) was conducted, where factor A was the dosage of Prozoot15 (0, 7.5 and 15% wet weight) and factor B was the fermentation time (0, 7 and 23 d), with 4 repetitions for each time. Prozoot15 was mixed into the poultry litter according to treatment plus 20% molasses. Four bags per treatment were removed at each sample time. Sample pH as well as lactobacilli, total aerobes (TA), total coliforms (TC), *E. coli* and *Salmonella* counts were measured. Results revealed an interaction between *Salmonella* and pH ($P < 0.0001$). The lowest ($P < 0.05$) *Salmonella* counts were obtained at d 7 of composting in treatments containing 7.5 and 15% of Prozoot15 and at d 23 in all 3 Prozoot15 treatments. The lowest ($P < 0.05$) values for pH were obtained at d 23 in treatments containing 0 and 15% of Prozoot15 (5.65 and 5.69, respectively). Lactobacilli, *E. coli*, CT and AT counts were affected by fermentation time ($P < 0.05$). In all treatments, lactobacilli increased ($P < 0.05$) by d 7 then decreased by d 23. *E. coli* and CT counts decreased ($P < 0.05$) across fermentation time and AT remained constant for the first 7 d then decreased ($P < 0.05$) by d 23. In conclusion, anaerobic fermentation of poultry litter over 23 d is sufficient to lower pH and to eliminate pathogenic microorganisms *E. coli* and *Salmonella* in laying hen litter. The inclusion of the probiotic Prozoot15 at levels of 7 and 15% resulted in the reduction of *Salmonella* to undetectable levels by d 7. Treatment of poultry litter compost with Prozoot15 renders

it pathogen-free and safe as a nitrogen- and mineral-rich ruminant ration.

Key Words: probiotic, poultry litter, pathogenic microorganism, composting

73 Influence of a direct-fed microbial on growth performance and digestibility of broiler chicks fed commercially available diets.

J. Barnes*, J. Ison, and R. Carpenter,
BiOWiSH Technologies Inc., Cincinnati, OH, USA.

Direct-fed microbials (DFMs) provide a viable alternative to antibiotics for growth enhancement, improved digestibility and gut health across species. The effects of a DFM containing bacteria from the genera *Bacillus* and *Lactobacillus* were evaluated in 2 broiler chick studies using standard poultry diets. In the first study, 1,296 Ross 508 straight-run broiler chicks were used. Dietary treatments were Control – high protein pelleted diet, high protein diet with DFM added, Control – low protein pelleted diet, and low protein diet with DFM added. Diets were formulated based on information from commercial broiler companies. Feed conversions for broiler chicks on both the high protein and low protein DFM containing-pelleted diets improved by 3.5% and 6.6% versus the respective controls. This benefit derives from improved feed ingredient utilization. In the second study, 1,800 Cobb 500 straight-run broiler chicks were used in a 60-pen trial using a low protein pelleted diet with or without a DFM. Feed conversions for broiler chicks receiving the DFM were significantly lower ($P < 0.10$) than their control counterparts. Day 0-42 mortality-adjusted FCR and mortality-adjusted FCR corrected to a common body weight of 4.5 lb were significantly lower ($P < 0.10$) for birds receiving the DFM than control birds. These data illustrate that DFM supplementation improves broiler performance and digestibility through increased feed conversion and nutrient utilization.

Key Words: direct-fed microbial (DFM), *Bacillus*, *Lactobacillus*, broiler, performance

NOTES

Author Index

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