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CONTENTS

Monday, April 15

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

Please pick up your registration materials, name tag, and welcome mixer drink tickets at the registration desk before you enter the auditorium.

Poster presenters: Please use your assigned abstract number (see page 9) to post your presentation on the corresponding numbered poster board (6th floor, room 621).

Special Session: "Bugs and Drugs" (separate registration required)

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

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

08:40–09:25 1 ***Invited talk:*** **Natural product biosynthesis by posttranslational modification.**
W. van der Donk*, *Department of Chemistry and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA*.

09:25–10:10 2 ***Invited talk:*** **A genetic approach to uncovering host–microbe metabolic interactions in the gut.**
D. Dodd*, *Stanford University School of Medicine*, *Stanford, CA, USA*.

10:10–10:40 **Tea break**

10:40–11:25 3 ***Invited talk:*** **High-throughput approaches to study microbial–drug interactions.**
N. Typas*, *EMBL Heidelberg*, *Heidelberg, Germany*.

11:25–12:10 4 ***Invited talk:*** **Systematic identification of drug-metabolizing gut microbes and their gene products.**
M. Zimmermann*, M. Zimmermann-Kogadeeva, R. Wegmann, and A. L. Goodman, *Department of Microbial Pathogenesis and Microbial Sciences Institute, Yale University School of Medicine*, *New Haven, CT, USA*.

12:10 **Workshop close**

12:15–13:30 **Lunch** (please make your own arrangements)

2019 Opening Session
Invited Presentations and Bryant Memorial Lecture

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

13:30–13:40 **Welcome.**
Rod Mackie, Chair, *University of Illinois at Urbana-Champaign, USA*.

13:40–14:25 5 ***Invited talk:*** **Harnessing the gut microbiome to improve the treatment of chronic disease.**
P. J. Turnbaugh*[1,2], [1]*Department of Microbiology and Immunology, University of California, San Francisco, CA, USA*, [2]*Chan Zuckerberg Biohub*, *San Francisco, CA, USA*.

14:25–15:10 6 ***Invited talk:*** **Potential drivers of plasticity and persistence in the animal gut microbiome.**
I. Mizrahi*, *The Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev*, *Beer-Sheva, Israel*.

15:10–15:40 **Coffee break**

15:40–16:25 7 ***Bryant Memorial Lecture***
Not all methane is created equally: Concepts and consequences for a diet-induced inflammatory microbiota?
M. Morrison*, *Faculty of Medicine, University of Queensland Diamantina Institute, Translational Research Institute*, *Woolloongabba, Australia*.

16:25 **Presentation of Honorary Plaque.**
Rod Mackie, Chair, *University of Illinois at Urbana-Champaign, USA.*

16:30–18:30 **Welcome Mixer** (please wear your name tag)
Gleacher Center, Sixth Floor (Room 621)
Refreshments: drink tickets, hors d'ouevres, cash bar
Informal poster viewing

Tuesday, April 16

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

Podium presentations: Session 1

Chair: Isaac Cann, University of Illinois, USA
Gleacher Center, First Floor, Tiered Classroom

09:00–09:45 8 ***Invited talk:*** **Perinatal impacts, microbiome, and health.**
M. G. Dominguez-Bello*, *Rutgers University*, *New Brunswick, NJ, USA*.

09:45–10:05 9 **Variation in butyrate-production pathways across human and nonhuman primates.**
E. K. Mallott* and K. R. Amato, *Northwestern University*, *Evanston, IL, USA*.

10:05–10:25 10 **Procyanidin–cell wall interactions within apple matrices decrease the metabolization of procyanidins by the human gut microbiota and the anti-inflammatory effect of the resulting microbial metabolome *in vitro*.**
C. Le Bourvellec[1], P. Bagano Vilas Boas[2], P. Lepercq[3], S. Comtet-Marre[3], P. Auffret[3], P. Ruiz[3], R. Bott[1], C. Renard[1], C. Dufour[1], J.-M. Chatel[2], and P. Mosoni*[3], [1]*UMR408 SQPOV (Sécurité et Qualité des Produits d'Origine Végétale), INRA*, *Avignon, France*, [2]*Micalis, INRA, AgroParisTech, Université Paris-Saclay*, *Jouy-en-Josas, France*, [3]*UMR454 MEDIS (Microbiologie, Environnement Digestif et Santé) INRA, Université Clermont Auvergne*, *Clermont-Ferrand, France*.

10:30–11:00 **Coffee break**

11:00–11:20 11 **The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans.**
S. L. La Rosa*[1], M. Leth[2], L. Michalak[1], M. Ejby Hansen[2], N. Pudlo[3], R. Glowacki[3], G. Pereira[3], C. Workman[2], M. Ø. Arntzen[1], P. B. Pope[1], E. C. Martens[3], M. Abou Hachem[2], and B. Westereng[1], [1]*Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences*, *Ås, Norway*, [2]*Department of Systems Biology, Danish Technical University*, *Lyngby, Denmark*, [3]*Department of Microbiology and Immunology, University of Michigan Medical School*, *Ann Arbor, Michigan, USA*.

11:20–11:40 12 **Sulfatases and fucosidases play critical roles in mucin utilization by *Bacteroides*.**
A. Luis*[1,3], A. Cartmell[2], R. Glowacki[1], J. Chunsheng[3], N. Karlsson[3], G. Hansson[3], and E. Martens[1], [1]*University of Michigan*, *Ann Arbor, MI, USA*, [2]*Newcastle University*, *Newcastle upon Tyne, United Kingdom*, [3]*Gothenburg University*, *Gothenburg, Sweden*.

11:40–12:00 13 **Conserved putative RNA binding proteins regulate carbohydrate utilization in *Bacteroides thetaiotaomicron*.**
A. N. D. Adams*[1], Z. A. Costliow[2], C. K. Vanderpool[1], and P. H. Degnan[3], [1]*Department of Microbiology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*The Broad Institute of MIT and Harvard*, *Cambridge, MA, USA*, [3]*Department of Microbiology and Plant Pathology, University of California Riverside*, *Riverside, CA, USA*.

12:00–13:00 **Business meeting: CGIF 2021** (open to all attendees)

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

Podium presentations: Session 2

Chair: Isaac Cann, University of Illinois, USA
Gleacher Center, First Floor, Tiered Classroom

13:30–14:10 14 ***Invited talk:*** **Learning from the locals: Linking digestion in wild ruminants to improved diets in livestock.**
B. Westereng[1], J. C. Gaby[1], L. M. Solden[2], A. E. Naas[1], S. L. La Rosa[1], L. Michalak[1], L. H. Hagen[1], B. J. Kunath[1], F. Delogu[1], M. Hess[3], V. G. H. Eijsink[1], M. Ø. Arntzen[1], T. R. Hvidsten[1], K. C. Wrighton[2,4], P. B. Pope*[1,5], [1]*Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences*, *Ås, Norway*, [2]*Department of Microbiology, The Ohio State University*, *Columbus, OH, USA*, [3]*Department of Animal Science, University of California*, *Davis, CA, USA*, [4]*Department of of Soil and Crop Sciences, Colorado State University*, *Fort Collins, CO, USA*, [5]*Faculty of Biosciences, Norwegian University of Life Sciences*, *Ås, Norway*.

14:10–14:30 15 **Rumen microbial composition and fermentation profiles through divergent dietary interventions during early life in calves.**
O. C. Carballo*[1,2], S. A. McCoard[1], K. Lowe[1], S. Ganesh[1], S. J. Lewis[1], and S. Muetzel[1], [1]*AgResearch*, *Palmerston North, New Zealand*, [2]*Massey University*, *Palmerston North, New Zealand*.

14:30–14:50 16 **Early-life rumen microbiota manipulation effect on calf's bacterial community development.**
H. Huuki*[1,5], S. Ahvenjärvi[2], M. Popova[3], J. Vilkki[4], A. Vanhatalo[5], and I. Tapio[1], [1]*Natural Resources Institute Finland, Production systems, Animal genetics*, *Jokioinen, Finland*, [2]*Natural Resources Institute Finland, Production systems, Milk production*, *Jokioinen, Finland*, [3]*National de la Recherche Agronomique, UMR1213 Herbivores, Clermont Université, VetAgro Sup, UMR Herbivores*, *Clermont-Ferrand, France*, [4]*Natural Resources Institute Finland, Service groups*, *Jokioinen, Finland*, [5]*University of Helsinki, Faculty of Agriculture and Forestry, Department of Agricultural Sciences*, *Helsinki, Finland*.

14:50–15:20 **Coffee break**

15:20–15:40 17 ***Lactobacillus reuteri*** **attenuates colonic mucosal disruption and inflammation following broad-spectrum antibiotic treatment.**
J. Allen*[1], C. Ladaika[1], L. Mashburn Warren[1], G. Besner[2], S. Goodman[1], and M. Bailey[1], [1]*Center for Microbial Pathogenesis, Research Institute at Nationwide Children's Hospital*, *Columbus, OH, USA*, [2]*Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital*, *Columbus, OH, USA*.

15:40–16:00 18 **Combined low dietary fiber and mucus-degrading symbiotic gut bacteria cause lethal colitis in IL-10 deficient mice.**
G. Pereira*[1], M. Wolter[2], M. Ostrowski[1], A. Luis[1], R. Glowacki[1], N. Pudlo[1], K. Eaton[1], G. Chen[1], M. Desai[2], J. Chunseng[3], N. Karlsson[3], G. Hansson[3], and E. Martens[1], [1]*University of Michigan Medical School*, *Ann Arbor, MI, USA*, [2]*Luxembourg Centre for Systems Biomedicine*, *Esch-sur-Alzette, Luxemburg*, [3]*Gothenburg University*, *Gothenburg, Sweden*.

16:00–16:20 19 **The use of chicken enterocyte culture to understand intestinal competency.**
N. C. Rath*[1], R. Liyuanage[2], A. Gupta[3], and M. Acharya[1,3], [1]*USDA-Agricultural Research Service, Fayetteville, AR, USA*, [2]*Statewide Mass Spectrometry Facility, University of Arkansas*, *Fayetteville, AR, USA*, [3]*Poultry Science Department, University of Arkansas*, *Fayetteville, AR, USA*.

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

Wednesday, April 17

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

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

09:00–09:20 20 **Comparative diversity of microbiomes and resistomes in beef feedlots, downstream environments and urban sewage influent.**
R. Zaheer*[1], S. Lakin[2], R. Ortega-Polo[1], S. Cook[3], F. Larney[1], P. Morley[2], C. Booker[4], G. Van Domselaar[5], R. Read[6], and T. McAllister[1], [1]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*, [2]*Department of Clinical Sciences, Colorado State University*, *Fort Collins, CO, USA*, [3]*Alberta Agriculture and Forestry*, *Lethbridge, AB, Canada*, [4]*Feedlot Health Management Services*, *Okotoks, AB, Canada*, [5]*National Microbiology Laboratory, Public Health Agency of Canada*, *Winnipeg, MB, Canada*, [6]*Cumming School of Medicine, University of Calgary*, *Calgary, AB, Canada*.

09:20–09:40 21 **Comparative genomics of *Clostridium perfringens* isolated from dairy cows with hemorrhagic bowel syndrome.**
R. R. Geier*, J. S. Thompson, A. H. Smith, and T. Rehberger, *Church & Dwight Co. Inc.*, *Waukesha, WI, USA*.

09:40–10:00 22 **Reduction in tetracycline-resistant bacteria during grain challenge by the red clover isoflavone biochanin A.**
M. Flythe*[1,2] and B. Harlow[1,2], [1]*USDA, ARS*, *Lexington, KY, USA*, [2]*University of Kentucky*, *Lexington, KY, USA*.

10:00–10:30 **Coffee break**

10:30–11:10 23 ***Invited talk:* Dietary carbohydrate metabolism by the human gut microbiota.**
P. Louis*, *University of Aberdeen Rowett Institute*, *Aberdeen, United Kingdom*.

11:10–11:30 24 **Energy conservation in the rumen bacterium *Pseudobutyrivibrio ruminis*.**
A. Katsyv*[1], M. C. Schölmerich[2], J. Dönig[1], T. J. Hackmann[3], and V. Müller[1], [1]*Goethe University Frankfurt, Molecular Microbiology and Bioenergetics*, *Frankfurt/Main, Germany*, [2]*University of Hamburg, Microbiology and Biotechnology*, *Hamburg, Germany*, [3]*University of Florida, Department of Animal Sciences*, *Gainesville, FL, USA*.

11:30–11:50 25 ***Clostridium scindens* ATCC 35704: Integration of nutritional requirements, the complete genome sequence, and global transcriptional responses to bile acids.**
S. Devendran[1,2], R. Shrestha[3], J. M. P. Alves[4], P. G. Wolf[2], L. Ly[1,5], C. Méndez-García[7], A. G. Hernandez[6], C. L. Wright[5], C. J. Fields[5], S. L. Daniel[3], and J. M. Ridlon*[8,9], [1]*Microbiome Metabolic Engineering Theme, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Department of Animal Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [3]*Department of Biological Sciences, Eastern Illinois University*, *Charleston, IL, USA*, [4]*Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo*, *São Paulo, Brazil*, [5]*Division of Nutritional Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [6]*Keck Center for Biotechnology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [7]*Independent investigator*, *Boston, MA, USA*, [8]*Cancer Center of Illinois, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [9]*Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University*, *Richmond, VA, USA*.

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

Podium presentations: Session 4

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

13:00–13:20 26 **Impact of organic rearing on the gastrointestinal microbiota of swine.**
A. Steinberger*, F. Assadi-Porter, W. Porter, and G. Suen, *University of Wisconsin-Madison*, *Madison, WI, USA*.

13:20–13:40 27 **Hydrolyzed casein enhances gastrointestinal chemosensing and gastric acid secretion in pigs fed low-crude-protein diets.**
J. Shen[1,2], H. Wang[1,2], Y. Pi[1,2], K. Gao[1,2], Y. Cheng[1,2], W. Jin[1,2], and W. Zhu*[1,2], [1]*Laboratory of Gastrointestinal Microbiology, Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, College of Animal Science and Technology, Nanjing Agricultural University*, *Nanjing, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, China*.

13:40–14:00 28 **Effect of feeding chlorophyll on *Escherichia coli* and enterococci in the pig gut.**
R. C. Anderson*[1], R. C. S. Mendonça[1,2], M. A. Rasmussen[3], H. He[1], K. J. Genovese[1], R. B. Harvey[1], R. C. Beier[1], and D. J. Nisbet[1], [1]*United States Department of Agriculture/Agricultural Research Service*, *College Station, TX, USA*, [2]*Viçosa Federal University*, *Viçosa, Brazil*, [3]*Iowa State University*, *Ames, IA, USA*.

14:00–14:30 **Coffee break**

14:30–14:50 29 **Methyl-compound production by rumen bacteria.**
W. J. Kelly[1], S. C. Leahy[2], J. Kamke[3], P. Soni[2], S. Koike[4], R. I. Mackie[5], R. Seshadri[6], G. M. Cook[7], C. Greening[8], and G. T. Attwood*[2], [1]*Donvis Ltd.*, *Palmerston North, New Zealand*, [2]*AgResearch Ltd., Grasslands Research Centre*, *Palmerston North, New Zealand*, [3]*Horizons Regional Council*, *Palmerston North, New Zealand*, [4]*Hokkaido University*, *Sapporo, Japan*, [5]*University of Illinois*, *Urbana, IL, USA*, [6]*Department of Energy Joint Genome Institute*, *San Francisco, CA, USA*, [7]*University of Otago*, *Dunedin, New Zealand*, [8]*Monash University*, *Melbourne, Australia*.

14:50–15:10 30 **Innate variability in animal performance across seasonal changes in a northern Australian grazing system.**
S. Denman*[1], G. Martinez Fernandez[1], E. Charmley[2], G. Bishop-Hurley[1], and C. McSweeney[1], [1]*CSIRO Agriculture and Food*, *Brisbane, Qld, Australia*, [2]*CSIRO Agriculture and Food*, *Townsville, Qld, Australia*.

15:10–15:30 31 **Temporal stability of the ruminal bacterial communities in beef steers.**
B. Clemmons*[1], C. Martino[2], L. Schneider[1], M. Embree[2], and P. Myer[1], [1]*Department of Animal Science, University of Tennessee Institute of Agriculture*, *Knoxville, TN, USA*, [2]*ASCUS Biosciences Inc*, *San Diego, CA, USA*.

15:30–15:40 **Presentation of Russell Awards.**
Best oral and poster presentations by graduate students and young investigators.

15:40 **Closing remarks and invitation to CGIF 2021.**
CGIF 2021 will mark the 35th biennial meeting and 70 years of research in gut microbiology and its scientific communication.

Poster Presentations

Gleacher Center, 6th Floor (Room 621)

Computational approaches and applications

32 **Accurate estimation of microbial sequence diversity with Distanced.**
T. Hackmann*, *University of California, Davis*, *Davis, CA, USA*.

33 **Utilization of assembled long read amplicons to improve taxonomic resolution.**
J. Rehberger* and A. Smith, *Arm & Hammer Animal and Food Production*, *Waukesha, WI, USA*.

34 **Comparative genome analyses of human gut methanogens.**
J. G. Volmer[1], B. J. Woodcroft[2], G. W. Thompson[2], and M. Morrison*[1], [1]*Faculty of Medicine, University of Queensland Diamantina Institute, Translational Research Institute*, *Woolloongabba, Australia*, [2]*School of Chemistry and Molecular Biosciences and Australian Centre for Ecogenomics, University of Queensland*, *Saint Lucia, Australia*.

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

35 **Identifying bacteria involved in nitrogen cycling in dairy cow manure on farms across California.**
J. V. Hagey*[1], S. Bhatnagar[2], J. M. Heguy[3], B. M. Karle[3], P. L. Price[1], D. Meyer[1], and E. M. Maga[1], [1]*University of California*, *Davis, CA, USA*, [2]*University of Calgary*, *Calgary, Alberta, Canada*, [3]*University of California Cooperative Extension*, *CA, USA*.

36 **Refining direct-fed microbials and silage inoculants for reduction of methane emissions from ruminants.**
P. Mbandlwa*[1,2], N. Doyle*[1,2], C. Hill[1,3], C. Stanton[2,3], and P. Ross[1,3], [1]*School of Microbiology, University College Cork*, *Cork, Munster, Ireland*, [2]*Teagasc Moorepark Food Research Centre, Fermoy*, *Cork, Munster, Ireland*, [3]*APC Microbiome Ireland, University College Cork*, *Cork, Munster, Ireland*.
*These two authors contributed equally to this work.

37 **Evaluation of the potential of two common Pacific Coast macroalgae for mitigating methane emissions from ruminants.**
C. Shaw*[1], C. Brooke[1], B. Roque[1], N. Najafi[1], M. Gonzalez[1], A. Pfefferlen[1], V. DeAnada[1], D. Ginsburg[2], M. Harden[2], S. Nuzhdin[2], J. Salwen[3], E. Kebreab[1], and M. Hess[1], [1]*University of California, Davis*, *Davis, CA, USA*, [2]*University of Southern California*, *Los Angeles, CA, USA*, [3]*Stanford University*, *Stanford, CA, USA*.

Immunology (including host–microbe interactions)

38 **Low-protein diets supplemented with casein hydrolysate enhance small intestinal barrier functions and inhibit ileal pro-inflammatory cytokine expressions in pigs.**
H. Wang*[1,2], J. Shen[1,2], Y. Pi[1,2], K. Gao[1,2], Y. Cheng[1,2], W. Jin[1,2], and W. Zhu[1,2], [1]*Laboratory of Gastrointestinal Microbiology, Jiangsu Key Laboratory of Gastrointestinal Nutrition and Animal Health, College of Animal Science and Technology, Nanjing Agricultural University*, *Nanjing, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, China*.

39 **Chemotherapy disrupts microbial-enterohepatic bile acid metabolism in mice.**
B. R. Loman*[1], K. Jordan[2], L. M. Pyter[2], and M. T. Bailey[1,2], [1]*Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital*, *Columbus, OH, USA*, [2]*Institute for Behavioral Medicine Research, The Ohio State University*, *Columbus, OH, USA*.

40 **Steroid-17,20-desmolase (DesAB) activity from gut and urinary microbes forms 11-oxy-androgens from glucocorticoids *in vitro*.**
L. K. Ly*, J. L. Rowles, S. Devendran, J. W. Erdman, I. K. O. Cann, and J. M. Ridlon, *University of Illinois at Urbana-Champaign*, *Urbana-Champaign, IL, USA*.

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

41 **Cellulosome assembly revealed by cryoelectron microscopy.**
M. Tatli*[1], S. Moraïs[2], I. Mizrahi[2], and O. Medalia[1], [1]*University of Zurich, Department of Biological Chemistry, University of Zurich*, *Zurich, Switzerland*, [2]*BenGurion University of the Negev, The Faculty of Natural Sciences, Ben-Gurion University of the Negev*, *Beer-Sheva, Israel*.

42 **Functional and structural study of 20β-hydroxysteroid dehydrogenase from *Bifidobacterium adolescentis* strain L2-32.**
H. Doden*[1], S. Mythen[1], R. Pollet[2], Z. Wawrzak[3], S. Devendran[1], I. Cann[1], N. Koropatkin[2], and J. Ridlon[1,4], [1]*University of Illinois*, *Urbana-Champaign, IL, USA*, [2]*University of Michigan Medical School*, *Ann Arbor, MI, USA*, [3]*Northwestern University*, *Evanston, IL, USA*, [4]*Virginia Commonwealth University*, *Richmond, VA, USA*.

43 **Characteristics of fecal microbiota in captive Antillean manatees *Trichechus manatus manatus*.**
A. Suzuki*[1], K. Ueda[2], T. Segawa[3,1], and M. Suzuki[1], [1]*Nihon University*, *Fujisawa, Kanagawa, Japan*, [2]*Okinawa Churashima Foundation*, *Kunigami, Okinawa, Japan*, [3]*Mie University*, *Tsu, Mie, Japan*.

44 **The hibernating squirrel microbiome responds to seasonal dietary shifts by altering its functional potential.**
E. Chiang*, H. V. Carey, and G. Suen, *University of Wisconsin-Madison*, *Madison, WI, USA*.

45 **Isolation, identification, and phenotypic evaluation of bacteria from infant feces.**
H. Kim*, R. Mackie, I. Cann, and S. Donovan, *University of Illinois*, *Urbana-Champaign, IL, USA*.

46 **Genome-wide transcriptional responses of sensitivity and adaptation of ruminal bacteria to monensin.**
N. Kim*, I. K. O. Cann, and R. I. Mackie, *University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

47 **Evaluation of intra-species diversity of *Oxalobacter formigenes* strains using pulsed-field gel electrophoresis.**
N. Pareek* and S. Daniel, *Eastern Illinois University*, *Charleston, IL, USA*.

48 **Dynamics of feed particle colonization by anaerobic fungi.**
N. McElhinney*[1], R. Evans[1], T. M. Callaghan[1], S. Huws[2], N. R. McEwan[3], and G. W. Griffith[1], [1]*Aberystwyth University, Institute of Biological, Environmental and Rural Sciences (IBERS)*, *Aberystwyth, United Kingdom*, [2]*School of Biological Sciences, Queen's University*, *Belfast, United Kingdom*, [3]*School of Pharmacy and Life Science, Robert Gordon University*, *Aberdeen, United Kingdom*.

49 **Identifying novel antimicrobials from anaerobic rumen fungi.**
R. Evans*[1], G. W. Griffith[1], T. M. Callaghan[1], B. Thomas[1], and S. A. Huws[2], [1]*IBERS, Aberystwyth University*, *Aberystwyth, United Kingdom*, [2]*School of Biological Sciences, Queen's University*, *Belfast, United Kingdom*.

50 ***Methanobrevibacter boviskoreanii* JH1T growth on alcohols allows development of a high-throughput bioassay.**
Y. Li[1], W. Kelly*[2], G. Attwood[1], P. Reid[1], and S. Leahy[1], [1]*AgResearch Limited, Grasslands Research Centre*, *Palmerston North, Manawatu, New Zealand*, [2]*Donvis Ltd*, *Ashhurst, Manawatu, New Zealand*.

51 **Digestive tract microbiota of beef cattle that differed in feed efficiency.**
H. C. Freetly*[1], J. Wells[1], A. Dickey[1], A. Lindholm-Perry[1], J. Keele[1], and A. Foote[2], [1]*USDA, ARS, US Meat Animal Research*, *Clay Center, NE, USA*, [2]*Oklahoma State University, Stillwater*, *Stillwater, OK, USA*.

Nutrition and metabolism of livestock, humans, and companion animals

52 **Influence of an organic acid–based feed additive on intestinal parameters of weaned pigs.**
V. Ocelova*[1], S. Stelzhammer[1], N. Roth[1], F. Chen[2], C. Fu[2], and J. He[2], [1]*BIOMIN Holding GmbH, Erber Campus* 1, *Getzersdorf, Austria*, [2]*College of Animal Science and Technology, Hunan Agricultural University*, *Changsha, Hunan, China*.

53 **Impact of sub-therapeutic Carbadox feeding on growth performance and the fecal microbial population of newly weaned swine.**
R. S. Hampton*, M. J. Azain*, J. Lourenco, C. R. Dove, C. E. Edmunds, and T. R. Callaway, *Department of Animal and Dairy Science, University of Georgia*, *Athens, GA, USA*.

54 **Effect of camphor concentrations on caprine *in vitro* mixed ruminal microorganism fermentations.**
D. S. Seidel*[1], T. R. Whitney[2], J. W. Walker[2], J. M. Musser[3], and T. R. Callaway[1], [1]*University of Georgia Department of Animal and Dairy Science*, *Athens, GA, USA*, [2]*Texas A&M AgriLife Research*, *San Angelo, TX, USA*, [3]*Texas A&M University Department of Veterinary Medicine*, *College Station, TX, USA*.

55 **Microbiome effects on metabolic efficiencies in easy and hard keeper horses.**
A. Johnson* and A. Biddle, *University of Delaware*, *Newark, DE, USA*.

56 **Altering the ruminal microbiota in dairy calves using rumen contents dosing.**
M. Cox*[1], P. Weimer[2], A. Steinberger[1], J. Skarlupka[1], and G. Suen[1], [1]*University of Wisconsin-Madison*, *Madison, WI, USA*, [2]*US Dairy Forage Research Center, USDA Agricultural Research Service*, *Madison, WI, USA*.

57 **Characterization of the epimural microbiota across four geographical locations within the rumen of dairy cows.**
D. Sbardellati*[1], A. Fischer[2], K. Kalscheur[2], and G. Suen[1], [1]*Department of Bacteriology, University of Wisconsin-Madison*, *Madison, WI, USA*, [2]*USDA Dairy Forage Research Center, USDA-Agricultural Research Service*, *Madison, WI, USA*.

Prebiotics, probiotics, and DFM development

58 **Effect of inoculants of lactic acid bacteria on the fermentation quality of ryegrass at different dry matter content.**
N. T. Huyen*[1], I. Martinez[2], and W. F. Pellikaan[1], [1]*Wageningen University and Research*, *Wageningen, the Netherlands*, [2]*Via Manzoni*, *Cadorago, Italy*.

59 **Investigating the effects of direct-fed microbials on beef cattle during an acidosis challenge.**
S. Tondini* and J. McCann, *University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*.

Special Session: "Bugs and Drugs"

1 Natural product biosynthesis by post-translational modification.

W. van der Donk*,
Department of Chemistry and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

The genome-sequencing efforts of the first decade of the 21st century have revealed that ribosomally synthesized and posttranslationally modified peptides (RiPPs) constitute a very large class of peptide natural products. These molecules are produced in all 3 domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Their biosynthetic genes are present in high frequencies in the microbiomes. The defining posttranslational modification of most RiPPs is one or more macrocyclization steps. This presentation will discuss investigations of the biosynthesis of these compounds as well as discovery of unanticipated new biosynthetic reactions.

2 A genetic approach to uncovering host–microbe metabolic interactions in the gut.

D. Dodd*,
Stanford University School of Medicine, *Stanford, CA, USA.*

The human gut microbiota influences many diverse aspects of human health and disease. One of the most concrete ways that the microbiome impacts host biology is through the production of small molecules that interact with host cells in the gut, but also gain access to the bloodstream where they can have effects at distal sites in the host. These small molecules commonly reach concentrations similar to those achieved by pharmaceuticals; however, remarkably little is known about the microbial metabolic pathways that produce them. We are using a genetic approach to discover host-microbe metabolic interactions in the gut responsible for production of drug-like molecules by the gut microbiota. Using bacterial genetics, we mapped the pathway for indolepropionic acid (IPA), an important bioactive molecule produced exclusively by the microbiota. We then showed that by toggling IPA on and off in a gnotobiotic mouse model, we can influence intestinal permeability and consequently, systemic immune cell profiles. Further interrogation of mutants by untargeted metabolomics yielded the unexpected observation that this pathway produces one of the most abundant mammalian urine metabolites, hippuric acid. Using a combination of untargeted metabolomics, stable isotope tracing, bacterial genetics, and gnotobiotic colonization, we validated the role of this pathway in hippuric acid production. This work provides mechanistic insight into how the gut microbiota contributes to the host metabolome and produces drug-like molecules that may be harnessed to promote human health and treat disease.

3 High-throughput approaches to study microbial–drug interactions.

N. Typas*,
EMBL Heidelberg, *Heidelberg, Germany.*

In collaboration with other labs at EMBL, we have recently developed a suite of experimental frameworks for high-throughput microbiomics. As proof of principle, we have used this platform to map and study the interface of gut bacteria with drugs and xenobiotics, monitoring both how drugs impact gut bacteria and how bacteria modulate drug bioavailabilities. Here, I will be presenting recent developments on these fronts and discussing future directions and opportunities.

4 Systematic identification of drug-metabolizing gut microbes and their gene products.

M. Zimmermann*, M. Zimmermann-Kogadeeva, R. Wegmann, and A. L. Goodman,
Department of Microbial Pathogenesis and Microbial Sciences Institute, Yale University School of Medicine, *New Haven, CT, USA.*

Individuals vary widely in their drug responses, which can be dangerous and expensive due to

significant treatment delays and adverse effects. Growing evidence implicates the gut microbiome in this variability; however, the molecular mechanisms remain mostly unknown. Using antiviral nucleoside analogs and clonazepam as examples, we recently reported experimental and computational approaches to separate host and gut microbiota contributions to drug metabolism. The resulting pharmacokinetic models identified measurable physiological, microbial and chemical parameters that dictate host and microbiome contributions to the metabolism of xenobiotics. To systematically map the drug metabolizing capacity of the gut microbiota and assess its potential contribution to drug metabolism, we further measured the ability of 76 diverse human gut bacteria to metabolize each of 271 oral drugs. We found that two-thirds of these drugs are chemically modified by at least one of the tested microbes. Through combination of high-throughput bacterial genetics with mass spectrometry, we systematically identified drug-metabolizing microbial gene products. These gene products better explain the drug-metabolizing capacity of bacterial strains than their phylogenetic classification. We further demonstrate that the abundance of homologs of these gene products predict the capacity of complete human gut communities to metabolize the targeted drugs. These causal links between microbiota gene content and metabolic activities connect inter-individual microbiome variability to interpersonal differences in drug metabolism, which has translatable potential on medical therapy and drug development across multiple disease indications.

Key Words: microbiome, drug metabolism, pharmacokinetics, gnotobiotics, mass spectrometry

2019 Opening Session
Invited Presentations and Bryant Memorial Lecture

5 Harnessing the gut microbiome to improve the treatment of chronic disease.

P. J. Turnbaugh*[1,2],
[1]*Department of Microbiology and Immunology, University of California, San Francisco, CA, USA,*
[2]*Chan Zuckerberg Biohub*, *San Francisco, CA, USA.*

Although the importance of human genetic polymorphisms in therapeutic outcomes is well established, the role of specific genotypic or copy number variants in our “second genome” (the microbiome) has been largely overlooked. Our research group takes a microbiome-centric approach to pharmacology, working to elucidate the direct and indirect mechanisms through which the human microbiome shapes the efficacy and toxicity of small molecule and biologic therapies. I will discuss our ongoing studies focused on the impact of human gut bacteria on the efficacy of anti-inflammatory drugs used for rheumatoid arthritis. Together, these results emphasize the broad impact of gut bacteria metabolism on drug pharmacokinetics and pharmacodynamics, and a starting point for more systematic studies of the complex interactions between pharmaceuticals and human-associated microbes. This work was supported by the National Institutes of Health, the National Cancer Institute, the Searle Scholars Program, and the Damon Runyon Cancer Research Foundation.

6 Potential drivers of plasticity and persistence in the animal gut microbiome.

I. Mizrahi*,
The Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, *Beer-Sheva, Israel.*

Relationships between gut microbial ecosystems and their vertebrate hosts have been shown in

recent years to play an essential role in the well-being and proper function of their hosts. I will discuss some of our recent findings regarding such ecosystems stability, development, and interaction with the host.

7 Bryant Memorial Lecture: Not all methane is created equally: Concepts and consequences for a diet-induced inflammatory microbiota?

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

Methanogenic archaea are ubiquitous in anaerobic habitats, including the microbial communities of the human and animal gastrointestinal tract. Historically, most attention has been directed toward *Methanobrevibacter* spp., which are numerically predominant within these communities and are capable of autotrophic growth via the hydrogenotrophic pathway of methane formation. More recently, the seventh order of methanogenic archaea—the *Methanomassilliicoccales*—were recognized and characterized with respect to their hydrogen-dependent growth with methylated amines and/or methanol. The genus *Methanosphaera* was created based on a single isolate recovered from human stool in the mid-1980s (*M. stadtmanae*). The *Methanosphaera* have received relatively little attention, beyond Thauer and colleagues' model of energy conservation during hydrogen-dependent methanol reduction to methane, which is consistent with recent findings that *Methanosphaera* spp. abundances are remarkable in "low hydrogen/methane" producing herbivores. In humans, the gut archaea are principally composed of methane producers, and irrespective of health status or diagnostic measure, subjects with methane/methanogen positivity experience longer transit times and reduced laxation frequency, which is most pronounced in constipation-related disorders. Recent clinical studies have provided further evidence that the relative abundance of *Methanobrevibacter* and breath methane concentrations are increased in MS patients compared with healthy controls; and these changes correlate (positively) with the expression of host genes indicative of a proinflammatory response. To date however, detailed studies of host immune response to methanogenic archaea have only been performed with 3 type strains, with the responses to *Methanobrevibacter smithii* PS^T and *Methanomassiliicoccus luminyensis* strain $B10^T$ found to be benign relative to the responses elicited by *Methanosphaera stadtmanae* $DSMZ3091^T$. In this presentation, I will provide an overview of our recent studies of *Methanosphaera* spp., which have used a combination of culture-based and metagenomics-based approaches to expand the genus and examine its evolutionary depth, functional properties and physiological ecology across animal and human hosts. These findings suggest that not all methane is created equally: that the inflammatory potential of diet × microbiome interactions in immune-mediated inflammatory diseases such as IBD and MS warrants greater consideration of the archaebacteria, and specific groups of methanogens in particular.

Podium presentations: Session 1

8 Perinatal impacts, microbiome, and health.

M. G. Dominguez-Bello*,
Rutgers University, *New Brunswick, NJ, USA.*

The microbiota is transmitted vertically, within species and throughout generations. In mammals, exposure to live microbes occurs at the time of labor. Different body sites of the baby reshape the primordial source of maternal vaginal/perineal microbiota, and these microbes play a role in healthy development of each organ. Bypassing the birth canal at birth (such as in C-section) and early exposure to antibiotics have been associated with increased incidence of immune and metabolic disorders (asthma, T1D, allergies, obesity). Urban lifestyle is rich in antimicrobial practices and has been associated with a decrease in diversity of the human microbiota. If the degraded human microbiota is a result of modern practices (some of them needed, such as medical interventions) and is a factor fostering modern diseases, we will need restoration approaches to arrest and prevent the current trend in urban diseases. More research is needed to determine the functions of the lost microbes and their evolutionary history, to be used in the restorative medicine of the future.

9 Variation in butyrate-production pathways across human and nonhuman primates.

E. K. Mallott* and K. R. Amato,
Northwestern University, *Evanston, IL, USA.*

Over the course of human evolution, shifts in dietary practices such as meat eating and cooking, have resulted in reduced fiber intake, particularly in industrialized populations. This dietary shift has likely had important consequences for host–gut microbe interactions as well as human nutrition and health. Reduced fiber consumption is associated with a loss of gut microbial taxa that degrade fiber, particularly butyrate, which can compound over generations. This shift toward a low-fiber diet in humans has likely altered the abundance of genes involved in short-chain fatty acid (SCFA) production, impacting host energy sources, fat deposition, and inflammation and, therefore, influencing disease risk in modern humans. To determine if low-fiber diets in humans are associated with changes in butyrate production, we compared the gut microbiomes of humans and non-human primates, examining variation in the presence and relative abundance of 4 butyrate-producing pathways: 4-aminobutyrate, acetyl-CoA, glutarate, and lysine. Samples from industrialized human (n = 20), non-industrialized human (n = 10), ape (n = 10), Old World monkey (n = 30), New World monkey (n = 40), and lemur (n = 15) gut metagenomes were examined. Butyrate-production genes were identified using gene-targeted assembly to construct a gene catalog of butyrate synthesis pathway genes from all samples in addition to a published reference database. Humans had significantly fewer pathways present compared with other phylogenetic groups (humans = 1.90 ± 0.88 pathways, apes = 3.00 ± 1.41, Old World monkeys = 3.03 ± 0.76, New World monkeys = 2.70 ± 0.82, lemurs = 2.27 ± 0.59) ($F_{4,120}$ = 8.02, P < 0.001). Additionally, humans from industrialized populations lacked 2 of the 4 pathways, 4-aminobutyrate and glutarate, whereas non-industrialized humans and all other phylogenetic groups had all 4 pathways present, albeit at varying frequencies. This marked decrease in butyrate-producing pathway presence may increase human susceptibility to metabolic disorders, particularly in industrialized populations consuming low-fiber diets.

Key Words: gut microbiota, short-chain fatty acid, evolution, primate

10 Procyanidin–cell wall interactions within apple matrices decrease the metabolization of procyanidins by the human gut microbiota and the anti-inflammatory effect of the resulting microbial metabolome *in vitro*.

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B-type oligomeric procyanidins in apples constitute an important source of polyphenols in human diet. Their role in health is not known although it is suggested that they might generate beneficial bioactive compounds upon metabolization by the gut microbiota. During technological processing of apples, procyanidins interact with cell-wall polysaccharides and form stable complexes. These interactions need to be taken into consideration to better assess the biological effects of fruit constituents. Hence, our objectives were to evaluate the impact of these interactions on the microbial metabolization of both cell walls and procyanidins and to investigate the potential anti-inflammatory activity of the resulting metabolome, in addition to analyzing the taxonomical changes undergone by the microbiota. *In vitro* fermentation of 3 model apple matrices with microbiota from 4 healthy donors showed that the binding of procyanidins to cell-wall polysaccharides, whether covalently or non-covalently, reduced substantially procyanidin degradation. Although cell wall-unbound procyanidins negatively affected carbohydrate fermentation, they generated more hydroxyphenylvaleric acid than bound procyanidins, and increased the abundance of *Adlercreutzia* and *Gordonibacter* genera (*Actinobacteria* phylum). Best results in terms of production of anti-inflammatory bioactive metabolites were observed from the apple matrix with no bonds between procyanidins and cell wall polysaccharides; the matrix with non-covalent bonds provided a lower anti-inflammatory activity followed by that with covalent bonds.

Key Words: *in vitro* batch fermentation, polyphenol, dietary fiber, 16S metabarcoding

11 The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans.

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The human gut microbiota is responsible for the catabolism of dietary glycans to short chain fatty acids (SCFAs), which, in turn, affect host physiology and health. Among the SCFAs, butyrate exhibits multiple health-promoting effects. Accordingly, correlation studies have demonstrated increased levels of butyrate-producing bacteria in healthy individuals compared with those with inflammatory and metabolic disorders. β-Mannans constitute a ubiquitous family of plant cell-wall heteropolysaccharides present in the human diet. These polysaccharides are abundant in certain nuts, coconut and coffee beans and legume seeds. In addition, they are widely present in the human diet due to their applications as stabilizers and food-thickening agents, particularly in baked products, ice creams, condiments, and canned soups. In contrast to the extensively studied *Bacteroides*, insight into β-mannan catabolism of Firmicutes-affiliated gut commensals is limited. One prominent Firmicutes group of butyrate-producing organisms is the clostridial cluster XIVa, of which *Roseburia intestinalis* is a representative member. Here, we demonstrate that *R. intestinalis* efficiently grows on β-mannans possessing varying decorations. Transcriptomic and proteomic analyses revealed the upregulation of two gene clusters encoding a complete repertoire of enzymes for β-mannan binding, transport, and breakdown to its monomeric units. Through a systematic

biochemical characterization of each component of the machinery, we validate the predicted pathways for degradation and fermentation of this glycan. During intermittent dietary fiber deficiency, β-mannan provides a selective advantage to promote beneficial commensal bacteria, exemplified by increased *R. intestinalis*, and reduction of mucus-degraders in the gnotobiotic mouse gut. Our findings highlight that *R. intestinalis* is a primary degrader of this dietary glycan and that this catabolic capacity could be exploited to selectively promote key members of the healthy microbiota using β-mannan-based therapeutic interventions.

Key Words: β-mannan, *Roseburia intestinalis*, metabolic pathway

12 Sulfatases and fucosidases play critical roles in mucin utilization by *Bacteroides*.

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The gastrointestinal mucus layer is mainly composed of mucins glycoproteins, containing ~10^2 different *O*-linked glycan structures, and provides a critical barrier that separates gut microbes from the intestinal epithelium. The combination of increased mucus degrading bacteria and the corresponding disruption of the mucus barrier have been proposed to promote inflammatory bowel disease (IBD), which is supported by our preliminary data. *Bacteroides thetaiotaomicron* (*Bt*), a dominant member of human microbiota, has numerous polysaccharide utilization loci (PULs) encoding dozens of predicted mucin-degradation enzymes. Significantly, the enzymatic mechanisms of mucin degradation by this and other gut remain unclear. We hypothesized that “early” steps in depolymerization of *O*-glycans exist, which could block downstream metabolism of mucin glycans and may represent drug targets to block mucus degradation by the microbiota. Using biochemical and genetic approaches, we identified several key enzymes involved in *Bt* mucus degradation. Simultaneous deletion of 2 α1,2-fucosidases revealed a critical role for these enzymes in growth on gastric *O*-glycans that are enriched for this linkage. The activity of these fucosidases against *O*-glycans was confirmed by enzymatic assays. Both enzymes show a preference for blood group H trisaccharide but are unable to cleave blood group A and B linkages and therefore dependent on enzymes specific for their removal, both of which are produced by *Bt*. Investigation of 24 sulfatases revealed the specificity for 12 of these enzymes, which is supported by 6 crystal structures in complex with substrate. Consistent with an essential role of sulfatases for growth on colonic mucin, a mutant lacking anaerobic sulfatase maturating enzyme (anSME) was unable to utilize highly sulfated rectal *O*-glycans. This phenotype was also observed with a mutant lacking just 2 PULs encoding 4 sulfatases, suggesting critical roles for the enzymes encoded in these loci. The characterization of these enzymes provides novel insights into the mechanism of mucin degradation allowing the identification of potential drug targets in the treatment of IBD.

Key Words: mucin, enzyme, *Bacteroides*

13 Conserved putative RNA binding proteins regulate carbohydrate utilization in *Bacteroides thetaiotaomicron*.

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The gut microbiome is an important metabolic organ, yet the regulatory mechanisms that govern microbial community structure in response to diet change remain poorly understood. Despite the importance of RNA regulation in mediating nutrient acquisition, RNA regulatory proteins remain largely uncharacterized in the majority of gut bacteria. We sought to identify candidate RNA regulators that mediate carbohydrate utilization in prominent gut bacteria through comparative genomics of 313 human gut-

associated microbial genomes. We identified a class of putative RNA binding proteins (pRBP) containing a single RNA recognition motif (RRM) that are widely conserved in the *Bacteroidetes* and in *Proteobacteria* lacking the canonical RNA regulator host factor Q (Hfq), suggesting that RRM pRBPs may be important RNA regulators in the gut. To determine if RRM pRBPs are important for carbohydrate utilization in *Bacteroidetes*, we deleted 2 of 3 divergent pRBP genes, *rbpB* and *rbpC*, in the genetic model *Bacteroides thetaiotaomicron* VPI-5482. Failure to delete the third gene, *rbpA*, using classic allelic exchange methods, suggests that *rbpA* is essential in culture. In transcriptomic data, the Δ*rbpB* and Δ*rbpC* mutants display altered expression of RNA helicases as well as polysaccharide utilization and capsular polysaccharide synthesis genes when cultured in rich and minimal media. Both Δ*rbpB* and Δ*rbpC* grow aberrantly on structurally diverse carbon sources when cultured in phenotype microarrays. These data suggest that these pRBPs are broadly important for carbohydrate utilization in *Bacteroidetes*. These substrates include the raffinose family oligosaccharides (RFOs), which are abundant plant sugars. Small amounts of RFO monosaccharides glucose and galactose (<1 m*M*) rescue Δ*rbpB* growth defects on RFOs, suggesting that RFO monosaccharides regulate RFO utilization independently of *rbpB*. Taken together, these data support the conclusion that RRM pRBPs are important carbohydrate utilization regulators that may be important in mediating cell responses to changing carbohydrate availability in the host.

Key Words: RNA regulation, raffinose oligosaccharides, *Bacteroides*, human gut microbiome

Podium presentations: Session 2

14 Learning from the locals: Linking digestion in wild ruminants to improved diets in livestock.

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The mammalian gut is an infamous plant fiber-digesting ecosystem, which depending on its host, can harbor a complex mixture of bacteria, archaea, protozoa, fungi and viruses that coordinate breakdown of complex dietary carbohydrates into nutrients. Despite extensive efforts to functionally elucidate the gut microbiome, fiber degradation has so far been attributed to a limited number of cultivable representatives that predominantly originate from the human gut. Moreover, the majority of the gut microbiota, their complex interactions, and the enzymatic machineries they employ remain poorly understood. Here, we present an overview of our recent efforts, where we combine traditional culturing, meta-omics, bioinformatics, biochemistry, and enzymology to investigate the different saccharolytic mechanisms that microbiota use from both ruminants and monogastric animals. We demonstrate key findings from studies on well-known *Roseburia intestinalis* and *Fibrobacter succinogenes* isolates, a novel as-yet uncultured *Bacteroidetes* family ('*Candidatus* MH11'), and large-scale ruminant and monogastric (pigs) meta-omics projects that seek to decrypt plant fiber metabolism at a community level. These approaches have revealed new mechanistic information related to the hydrolytic capacity of outer membrane vesicles (OMVs), polysaccharide utilization loci (PULs) and large multi-modular enzymes. In particular, carbohydrate analytics, (meta)genome-resolved metaproteomics and

biochemical data from wild ruminants has generated deeper insights into the intricate networks of in situ plant fiber deconstruction, which have led to new sophisticated diets for production animals designed to elicit a response in beneficial butyrate-producing populations.

15 Rumen microbial composition and fermentation profiles through divergent dietary interventions during early life in calves.

O. C. Carballo*[1,2], S. A. McCoard[1], K. Lowe[1], S. Ganesh[1], S. J. Lewis[1], and S. Muetzel[1],
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Pre-weaning feeding can change the rumen microbiota of young ruminants, but very little data are available to indicate whether these changes persist beyond the treatment period. This study aimed to determine whether divergent feeding regimens in early life can produce a long-term change in rumen microbiota composition and fermentation profiles. Twenty-four female Hereford × Friesian calves (6 ± 2 d old) were randomly allocated, using a 2 × 2 factorial design, to 2 pre-weaning dietary regimens (wk 0–14) × 2 post-weaning grazing diets (wk 15–30), followed by grazing on the same forage. Pre-weaning, calves were fed either concentrates and low-milk volume with weaning at wk 8 (CO), or a forage and high-milk volume with weaning at wk 12 (FO). Post-weaning, half of each group were grazed on either a high- (HQ) or low-quality forage (LQ) to wk 30. Methane (CH_4) emissions and dry matter intake (DMI) were measured at wk 9, 19 and 41 over a 2-d period in respiration chambers, and rumen contents analyzed for short chain fatty acid (SCFA) and microbial composition. Pre-weaning, CO calves had a higher ($P < 0.01$) DMI, but a lower CH_4 yield (g/kg of DMI) and acetate:propionate ratio (A:P) than FO calves. The CO calves had lower proportions of cellulolytic bacteria ($P < 0.05$) than FO calves. The methanogens in CO calves were dominated by *Methanobrevibacter boviskoreanii*, whereas *Mbb*. *gottschalkii* dominated in FO calves. Post-weaning, LQ calves had lower DMI and higher A:P ($P < 0.05$), but similar CH_4 yield to HQ calves. The abundance of cellulolytic bacteria was higher ($P < 0.05$) in LQ than HQ calves. The archaea community was dominated by *Mbb*. *gottschalkii* in both groups with no *Mbb*. *boviskoreanii* detected. No pre-weaning effects were observed. In wk 41, all calves had similar DMI, CH_4 yield, SCFA profiles and microbial composition. We conclude that the rumen microbiota and their associated fermentation profiles are primarily driven by diet, and that dietary interventions, implemented between 4 d and 30 wk of age, do not lead to a long-term microbial imprint, nor changes in rumen function.

Key Words: calf, early life, rumen microbiome, dietary management, fermentation profile

16 Early-life rumen microbiota manipulation effect on calf's bacterial community development.

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The factors influencing microbial community colonization and functional development of calf's rumen are still poorly understood. The aim of this project was to study whether an adult animal rumen liquid inoculum, given to young calves, would have positive effect on the developing rumen microbial community structure and function. Six pairs of twins were produced by splitting Nordic Red dairy cow embryos. Members of each pair were randomly allocated to treatment (TR, n = 6) and control (C, n = 6) groups. TR group received oral dose of rumen liquid from a feed efficient cow 3 times a week during 2–8 weeks of age. Rumen samples were collected on wk 2, 4, 6, and 8. The calves were handled and fed similarly. The quantitation of rumen bacteria was performed using targeted qPCR of 16S

rRNA gene region V4, and bacterial community structure was determined by sequencing 16S rRNA gene V4 region. Preliminary qPCR analysis shows that the number of bacteria decreased from the second week until wk 6 and remained at a similar level thereafter (P = 0.01). The trend was similar in both groups during the whole period (P = NS) and there were no significant differences in the average number of bacteria between the groups (TR: $1.16 \times 10^6 \pm 63{,}074$; C: $1.28 \times 10^6 \pm 63{,}075$ copies/ng DNA, P = NS). The number of bacterial OTUs increased until wk 6, after which the level stabilized (wk 2: 446 ± 87, wk 4: 788 ± 276, wk 6: 899 ± 377, wk 8: 931 ± 416, mean ± std number of OTUs). Results follow the changes in feeding regimen and rumen development, as on wk 2 the diet comprised mainly of milk replacer and solid feed intake started to rapidly increase on wk 6. Molar proportions of individual volatile fatty acids in the rumen and increases in VFA concentrations from wk 2 to wk 8 were not affected by the treatment. The weighted UniFrac distances indicate, that the bacterial community composition differed significantly between sampling weeks (Adonis: P = 0.001) and between groups (P = 0.014). The number of observed OTUs was on average higher in TR (899 ± 458, mean ± std number of OTUs) than in C group (652 ± 194; P = 0.02) indicating that the inoculum affected the rumen community composition.

Key Words: rumen microbiota, 16S rRNA gene sequencing, microbial quantitation

17 *Lactobacillus reuteri* attenuates colonic mucosal disruption and inflammation following broad-spectrum antibiotic treatment.

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Disruptions in the endogenous microbiota induced by broad-spectrum antibiotic (Abx) predisposes individuals to infection or chronic inflammatory conditions. *Lactobacillus reuteri* (*Lr*) is a probiotic that has potential to limit Abx-induced disturbances in gut homeostasis. Here, we investigate whether *Lr* can limit Abx-induced stem cell dysregulation, mucosal disruption, and inflammation in mice. C57Bl/6 mice (female, 6–8 weeks, n = 36) were provided a cocktail of Abx (kanamycin, gentamycin, colistin, metronidazole, and vancomycin) in drinking water for 5 d. Twelve mice remained unexposed to Abx throughout the experiment (noAbx). After 2 d, Abx mice were injected with clindamycin [10 mg/kg]. After 24 h, all mice were gavaged with PBS-vehicle (n = 12, Abx-vehicle; n = 12 noAbx-vehicle) or *Lr* (10^8; n = 12, Abx-*Lr*). Mice were killed at either 24 h (n = 18) or 96 h (n = 18) after *Lr*/vehicle inoculation. Gene expression for markers of colonic stem cell proliferation (Wnt-3a, Lgr5) and inflammation (IL-6, TNF-α) were assessed with rt-PCR. Immunofluorescence (IF) and fluorescence in situ hybridization (FISH) was implemented to determine mucosal layer thickness. Statistical significance was set to $P < 0.05$. Twenty-four hours post inoculation, colonic expression of Wnt3-a and Lgr5 were upregulated in Abx-vehicle treated mice compared with noAbx-vehicle treated mice. *Lr* treatment attenuated Abx-induced upregulation in Lgr5 expression. Indicative of a delayed inflammatory response, colonic expression of IL-6 and TNF-α were highly upregulated in Abx-vehicle treated mice compared with noAbx-vehicle treated mice at 96h post inoculation, a response that was attenuated by *Lr* treatment. FISH combined with IF indicated Abx-induced thinning of the mucosal layer at 96 h; an effect that was also attenuated by *Lr* ($P < 0.05$). Broad spectrum Abx initiate a delayed, but robust inflammatory response in the mouse colon that is preceded by early disruptions in the stem cell pool. *Lr* limits mucosal disruption and colonic inflammation making it a candidate therapy for limiting side effects associated with antibiotic usage.

Key Words: antibiotics, *Lactobacillus reuteri*, inflammation, probiotics, mucus

18 Combined low dietary fiber and mucus-degrading symbiotic gut bacteria cause lethal colitis in IL-10 deficient mice.

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The precise etiology of inflammatory bowel disease (IBD) remains unknown. Despite >100 genetic polymorphisms being associated with IBD, host genetics does not fully explain disease risk, leading to the postulate that environmental factors such as diet and gut microbes play critical, causal roles in IBD progression. Using a gnotobiotic mouse model, in which germfree wild-type and interleukin 10 (IL-10) knockout animals are colonized with synthetic human gut microbial communities composed of fully sequenced and metabolically characterized commensal bacteria, we have begun to elucidate the mechanistic interactions between dietary fiber, the gut microbiota and the colonic mucus barrier, which serves as a primary defense against encroachment by intestinal bacteria. During dietary fiber deficiency, the gut microbiota resorts to host-secreted mucus glycoproteins as a nutrient source, leading to erosion of this protective layer. Dietary fiber deprivation, together with a fiber-deprived, mucus-eroding microbiota, promotes severe, often lethal, spontaneous colitis in mice lacking IL-10, a cytokine for which loss of function is associated with early and very early onset IBD. In contrast, both isogenic wild-type mice and colonized IL-10 knockout mice fed high fiber do not experience disease, revealing that inflammation precipitates develops in a diet, microbiota and host genotype specific fashion. Selectively removing mucus-degrading species from the synthetic microbiota abrogates disease in IL-10 knockout, fiber-deprived mice, providing an additional link to microbial causation. Recombinant expression of bacterial mucus-degrading enzymes (sulfatases, fucosidases, mucin proteases, and others) that are hypothesized to be involved in causing mucus erosion and eventual inflammation have begun to reveal the molecular pathways involved in this disease model and provide targets for therapeutics to block disease progression.

Key Words: inflammatory bowel disease (IBD), mucus degrading, microbiome, fiber, colitis

19 The use of chicken enterocyte culture to understand intestinal competency.

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Enterocytes are both absorptive and protective components of intestine that come in close contact with a variety of dietary, microbial, parasitic, and toxic factors which passage through it. Increased epithelial permeability is one of the problems that can lead to enteric diseases because it allows easy access of pathogens and toxins into the system. In poultry, the restrictions in the use of antibiotic growth promoters has increased the chances of birds prone to enteric problems. Therefore, we have used chicken enterocyte cultures to understand their behavior and interaction with different agent including the pathogens and screen antibiotic alternatives. We have used enterocytes from intestinal villi of chickens and immunochemically characterized and studied their interactions with different chemical modulators and toxins (retinoic acid, calcitriol, cAMP, thyroxin, Na-butyrate, phorbol myristate acetate (PMA), aflatoxin B1, deoxynivalenol, and LPS, using the changes in their morphology and viability. Whereas some chemicals (retinoic acid, thyroxin) showed trophic effects, others (LPS, Na-butyrate) had negligible morphological effects, and some (aflatoxin, deoxynivalenol) were toxic. PMA, particularly, caused discernible dystrophic changes resulting in cellular cachexia, mimicking increased epithelial permeability. We followed the effect of PMA using their cellular proteomes to find whether any regulatory changes could underlie their morphological disposition. The enterocytes

were treated with PMA (500 ng/10^5 cells/mL) and the cellular proteins were extracted, analyzed by mass spectrometry, and compared with their control counterparts. Our results show that PMA affects several biological processes, largely, negatively affecting energy and nuclear metabolism pathways and differentially regulating several stress proteins, chaperon, cytoskeletal, and signal transduction proteins, which can be the basis of their dystrophy. It also altered certain signal transduction proteins that could increase their chances to pathogen infection. We surmise that the enterocyte culture provides opportunity to study intestinal interaction with different chemicals, pathogen, and their disease predispositions and can be useful to screen antibiotic alternatives.

Key Words: chicken, enterocytes, chemicals, proteomics

Podium presentations: Session 3

20 Comparative diversity of microbiomes and resistomes in beef feedlots, downstream environments and urban sewage influent.

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Antimicrobial use in livestock may contribute to increased prevalence of antimicrobial resistance (AMR) in bacteria within animal production systems and surrounding environments. Comparative knowledge of microbiomes and resistomes across environmental interfaces between animal production and urban settings is lacking. In this study, we executed a comparative analysis of the microbiomes and resistomes of metagenomes isolated from feedlot beef cattle feces (FC; n = 12), runoff catch basins adjacent to feedlots (CB; n = 13), manured agricultural soil (n = 4) from fields near feedlots, and urban sewage influent (SI; n = 6). Metagenomic DNA were subjected to Illumina HiSeq2000 sequencing, generating on average ~54 million 100-bp paired-end reads per sample. *Firmicutes* exhibited the highest prevalence (40%) in FC, whereas *Proteobacteria* were most abundant in CB (64%), soil (60%) and SI (83%). Among sample types, SI had the highest diversity of AMR and MBR classes (13 and 15) followed by FC (10 and 8), CB (8 and 4), and soil (6 and 1). Highest AMR gene abundance was harbored by FC whereas soil samples had a very small, but unique resistome that did not overlap with FC and CB resistomes. In the beef production system, tetracycline resistance predominated followed by macrolide resistance, consistent with use patterns. One of the feedlots contributed both conventional (cattle raised with antibiotics) and natural (raised without antibiotics) pen samples. Although natural FC samples exhibited a microbial ecology that was similar to conventional, their resistome was less complex. Similarly, the SI resistome was indicative of drug classes used in humans, including β-lactam, macrolide, tetracycline, aminoglycoside fluoroquinolone and fosfomycin resistance determinants. Metal and biocide resistance accounted for 26% of the SI resistome with a predominance of mercury resistance that may be associated with contamination of municipal water with household and industrial products. This study demonstrates an increasing divergence of the microbiome and resistome as distance from the feedlot increases.

Key Words: microbiome, antimicrobial resistance, cattle production, wastewater

21 Comparative genomics of *Clostridium perfringens* isolated from dairy cows with hemorrhagic bowel syndrome.

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Hemorrhagic bowel syndrome (HBS) is a devastating illness in cattle which leads to death within days. The cause of HBS is not known, but often *Clostridium perfringens* are present in high numbers. In this study, cattle diagnosed with HBS upon death were autopsied and intestinal sections were shipped on ice from the farm to the lab for processing. Samples were selectively plated and colonies were struck for isolation and typed by 16S sequencing. Twenty-two diverse HBS-associated isolates were selected using RAPD fingerprints as a proxy for genomic dissimilarity. Genomes were sequenced on the iSeq100 to an average depth of 84-fold coverage. Genomes were assembled in PATRIC and annotated using prokka. HBS genomes were compared with all 107 RefSeq *C. perfringens* genome assemblies obtained from NCBI. Genome assemblies were clustered using nucleotide k-mers with PopPUNK into 53 groups. HBS genomes were present in 13 of the 53 PopPUNK clusters. Roary was used used to determine the *C. perfringens* core genome (95% blastp identity), which consisted of 1,739 genes of 19,015 genes identified (9%), indicating a variable pangenome. Known virulence protein sequences were aligned to assemblies using tblastn; protein sequences were aligned to each other and manually curated for accuracy. Alpha toxin, collagenase, and the precursor to α-clostripain were present in all assemblies analyzed (e = 0.0). All HBS isolates were type A. Only 10 of all *C. perfringens* strains (8%) were other types (B, C, D, or E) in all genomes analyzed, indicating these types are rare. PopPUNK cluster 5 was missing many of the known virulence factors including sialidases, hyaluronoglucoasminidases, and perfringolysin O, which were common in the other assemblies. NetB was not identified in any ruminant isolates and was only detected in chicken, equine, and canine isolates. HBS genomes contained between 48 to 55 CAZyme families, 45 of which were conserved among all strains. Many of these CAZymes are used to degrade host carbohydrates. Overall, genomic diversity within *C. perfringens* is high, rendering it capable of causing disease in a variety of hosts.

Key Words: *Clostridium perfringens*, hemorrhagic bowel syndrome, comparative genomics

22 Reduction in tetracycline-resistant bacteria during grain challenge by the red clover isoflavone biochanin A.

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Red clover (*Trifolium pratense*) produces a selectively antimicrobial isoflavone called biochanin A (BCA). Previous *in vitro* and in vivo experiments showed that BCA inhibited the growth of particular rumen hyper-ammonia-producing bacteria (HAB) and amylolytic bacteria (e.g., streptococci). However, cellulolytic bacteria and fiber digestion were not inhibited, and animal average daily gain was enhanced. Pure culture experiments suggested that the mechanism of action was potentiation of endogenous antimicrobials (*i.e.* bacteriocins), and other studies indicated potentiation was via inhibition of multidrug efflux pumps (e.g., TetA). Based on this mechanism of action, we hypothesized that BCA would also reduce the number of rumen bacteria resistant to the antibiotic, tetracycline. An 8-d grain challenge was used to induce subacute rumen acidosis (SARA) and increase the prevalence of tetracycline-resistant (TR) rumen bacteria. Rumen fistulated steers (n = 12) received 1 of 2 diets: corn silage only (n = 3) or silage and corn (added in 2 steps; 40% corn, 4 d; 70% corn 4 d). Challenged steers received monensin (200 mg d^{-1}; n = 3), BCA (6 g d^{-1}; n = 3) or no intervention (n = 3). TR bacteria were enumerated by broth dilution and plating on 2 media types (nutrient-glucose agar or bile esculin azide agar). Silage-only steers had approximately 10^5 cfu TR bacteria mL^{-1} rumen fluid throughout the study. Steers in SARA had approx. 10^7 cfu TR bacteria mL^{-1}, which was greater than silage-only ($P < 0.05$). Steers receiving BCA had TR levels as low as the silage-only group throughout the experiment ($P > 0.05$). Monensin did not prevent the corn-associated

increase in TR bacteria. These results suggest that BCA negated the fitness advantage of the drug efflux pumps that typically make bacteria resistant to tetracycline, which is consistent with the hypothesis that the isoflavone interferes with drug efflux pumps. There are several limitations to this experiment. Further research is needed to determine if forage legumes or extracted isoflavones can be used to reduce shedding of antibiotic-resistant pathogens.

Key Words: antibiotic alternative, culture, phytochemical, rumen

23 Dietary carbohydrate metabolism by the human gut microbiota.

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The human large intestinal microbiota plays a crucial role in the degradation of dietary carbohydrates that remain undigested in the upper gut (non-digestible carbohydrates or fiber). Many different microbes contribute to fiber breakdown in the colon and cross-feeding of carbohydrate intermediates and fermentation products such as lactate and succinate between microbes plays an important role in this process. Microbial fiber fermentation leads to the production of health-promoting metabolites, especially the short-chain fatty acids acetate, propionate and butyrate. To modulate short-chain fatty acid production by the gut microbiota through diet, there is a need to better understand the role of different microbes in the fermentation of the multitude of carbohydrate types that constitute fiber. Many gut microbes have the necessary enzymes to degrade soluble carbohydrates, but the ability to perform the initial breakdown of insoluble fiber into more soluble fractions appears to be restricted to fewer species. Thus, these species may play a keystone role in making insoluble fiber available to the wider microbial community. *Ruminococcus bromii* performs this role for the fermentation of resistant starch and the absence of this species in human volunteers was shown to coincide with the fecal excretion of large amounts of resistant starch. Its superior starch degradation ability may be due to the presence of extracellular multienzyme complexes, so-called amylosomes. *In vitro* work indicates that, once solubilized, some types of non-digestible carbohydrates tend to promote propionate production, whereas others result in higher relative levels of butyrate. Similar responses were shared between microbiotas of different individuals, demonstrating a certain level of functional redundancy in propionate- and butyrate-producing species. However, other factors such as the gut pH influence fiber fermentation, and the cooperative and competitive relationships between microbes are not fully understood. Mathematical modeling is another valuable tool to study fiber breakdown by the gut microbial community.

Key Words: human gut microbiota, diet, non-digestible carbohydrates, butyrate, propionate

24 Energy conservation in the rumen bacterium *Pseudobutyrivibrio ruminis*.

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The mesophilic rumen bacterium *Pseudobutyrivibrio ruminis* converts different sugars (C_6 and C_5) to butyrate and conserves ATP through substrate-level phosphorylation. Recently, the decryption of rumen *Butyrivibrio* genome sequences revealed that several species within these genera contain both *rnf* and *ech* clusters. Interestingly, genome sequences revealed that these class of organisms harbor gene clusters encoding Ech, Rnf, and 2 highly similar F_1F_O ATP synthases. The crucial difference is that one is apparently specific for Na^+, because it harbors a characteristic Na^+ binding motif, whereas the other is probably H^+-dependent. We suggest that Ech and Rnf could function in concert with the NifJ protein and Bcd-Etf complex, permitting unprecedented ATP yield during glucose fermentation to butyrate. We aimed to elucidate whether these organisms indeed produce and use Rnf and Ech simultaneously as coupling sites. Glucose and

xylose utilization and product formation under Na^+-containing and Na^+-deprived conditions were studied in growth experiments. Biochemical and molecular experiments were performed to identify the key enzymes: Rnf, Ech and ATPases during metabolism. Growth experiments revealed that *P. ruminis* grows on C_6 (glucose) or C_5 (xylose) sugars and both growth rate and final optical density were decreased to approximately 50% in the absence of Na^+ on either energy source. Gene expression analyses also revealed differences in the expression level of *Ech*, *Rnf* and both *ATPase* gene clusters under Na^+-containing and Na^+-deprived conditions. In addition, the $Fd:NAD^+$ oxidoreductase-, ATP-hydrolyse-activity and Fd^{2-}-dependent H_2 evolution were detected and compared in membrane fractions of *P. ruminis*. The experimental and bioinformatic data substantiate the presence of membrane-embedded Ech, Rnf and ATPases. In addition, it was revealed that *P. ruminis* uses Na^+ bioenergetics for additional ATP synthesis.

Key Words: energy conservation, bioenergetics, coupling site, metabolism

25 *Clostridium scindens* ATCC 35704: Integration of nutritional requirements, the complete genome sequence, and global transcriptional responses to bile acids.

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In the human gut, *Clostridium scindens* is one of a few identified gut bacterial species capable of converting host primary bile acid, like cholic acid, into disease-associated secondary bile acids such as deoxycholic acid. The current work represents the nutritional requirements and response to bile acids of the medically important human gut bacterium *C. scindens* ATCC 35704. To determine the amino acid and vitamin requirements of *C. scindens*, the "leave one out" technique was used to eliminate the non-essential amino acids and vitamins. With this approach, the amino acid tryptophan and 3 vitamins (riboflavin, pantothenate, and pyridoxal) were found to be required for the growth of *C. scindens*. In the newly developed defined medium, *C. scindens* fermented glucose mainly to ethanol, acetate, formate, and H_2. The genome of *C. scindens* ATCC 35704 was completed through PacBio sequencing. Pathway analysis of the genome sequence coupled with RNA-Seq analysis of gene expression under defined culture conditions revealed consistency with the growth requirements and end products of glucose metabolism. Induction with bile acids revealed complex and differential responses to cholic acid and deoxycholic acid, including the expression of potentially novel bile acid-inducible genes involved in cholic acid metabolism. Responses to toxic deoxycholic acid included expression of genes predicted to be involved in DNA repair, oxidative stress, cell wall maintenance/metabolism, chaperone synthesis, and downregulation of one-third of the genome. These analyses provide valuable insight into the overall biology of *C. scindens*, which may be important in treatment of disease associated with increased colonic secondary bile acids.

Key Words: *Clostridium scindens*, bile acid, deoxycholic acid, RNA-Seq, defined medium

Podium presentations: Session 4

26 Impact of organic rearing on the gastrointestinal microbiota of swine.

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Swine are important agricultural animals as they are a major source of meat protein globally. Demand for organic pork has caused organic swine farming to become commonplace, but there are significant challenges raising swine without antibiotics. Gut microbial communities within mammalian gastrointestinal tracts are crucial to immune development and host nutrient acquisition. An improved understanding of how organic rearing affects swine gut bacterial communities may provide insight into the susceptibility of organically raised swine to pathogens and swine productivity. To address this, 4 cohorts of 7 to 10 swine were raised organically or conventionally from birth on one of 4 diets. Organically raised swine were either fed an organic diet or an organic diet supplemented with hay. Conventionally raised swine were either fed a conventional diet or a genetically modified organism (GMO)-based diet. Swine were killed at market weight (~280 lbs, or 6 mo of age) at which cecum contents were collected. Cecum bacterial communities were assessed using Illumina 16S rRNA V4 amplicon sequencing. We found the cecum bacterial communities to be significantly different by rearing method in both community richness and composition. Cecum bacterial communities from organic swine were less diverse than those from swine raised by conventional methods. There was no significant diet effect on community richness or composition between the 2 organic diets. There was also no difference in community richness between swine fed either of the conventional diets; however, the bacterial community composition of swine fed the GMO-based diet was significantly distinct from swine fed the conventional diet. These results demonstrate that organic methods of swine production do affect the cecum microbiota composition of swine differently than conventional production methods.

Key Words: swine, 16S rRNA gene, organic, diet, genetically modified organism (GMO)

27 Hydrolyzed casein enhances gastrointestinal chemosensing and gastric acid secretion in pigs fed low-crude-protein diets.

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Low-crude-protein (CP) diets supplemented with hydrolyzed casein (HC) rather than intact casein (IC) improve gastrointestinal (GI) function thereby achieving a better growth performance for pigs. Considering that peptides from HC play a critical role in upper GI chemosensing, whether this HC-induced chemosensing has an improvement on gastric function, especially gastric acid secretion, still remains unclear. This study was designed to investigate the effects of HC supplementation in low-CP diets on oral and gastric sensing of nitrogen nutrients, activation of gastric acid secretion (GAS), and pepsin activity and gastric microbiota. A total of 16 barrows (18.40 ± 0.89 kg of initial BW and 63 ± 2 d of age) were randomly assigned to 1 of 2 dietary treatments of 13% CP plus IC or HC. Pigs in the 2 groups were fed with equal quantity of diets daily for 28 d. Results showed that compared with IC, HC upregulated ($P < 0.05$) mRNA levels and (or) protein levels of nitrogen nutrient sensor CaSR, GPR92 and T1R1 in dorsum of tongue, gastric corpus and gastric antrum. In addition, HC increased ($P < 0.05$) free hydrochloric acid level in gastric lumen, and decreased ($P < 0.05$) gastric pH value. Moreover, HC increased ($P < 0.05$) parietal cell number and H^+, K^+-

ATPase activity in gastric corpus, which were accompanied by higher ($P < 0.05$) levels of GAS activation-extracellular key stimulus histamine, gastrin and Ach and their receptors (H2R, CCK2R and M3R), as well as higher ($P < 0.05$) levels of the related intracellular cAMP, PKA, PKC and CaMK 2. As a result, HC increased ($P < 0.05$) the pepsin activity, and it altered the gastric microbiota with decreasing ($P < 0.05$) total bacterial number and bacterial richness. The abundance of *Lactobacillus* tended to be increased ($P = 0.066$). Taken together, under condition of low CP-diets, HC enhances upper GI chemosensing of nitrogen nutrients and gastric acid secretion. These findings suggest that upper GI chemosensing of nitrogen nutrients is crucial to the regulation of gastric function under condition of low-CP diets, and HC could enhance this chemosensing.

Key Words: peptides, chemosensing, gastric acid secretion, low-CP diet, swine

28 Effect of feeding chlorophyll on *Escherichia coli* and enterococci in the pig gut.

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Natural efflux pump inhibitors, such as digestive metabolites of chlorophyll (CPL), impede efflux pumps contributing to antimicrobial resistance of bacteria. Farm-reared pigs normally consume little if any CPL and thus we hypothesized that feeding pig diets supplemented with CPL may affect the fitness of resistant bacteria in their gut. Nine pigs (75 ± 7.5 kg) were randomly assigned to receive a nonmedicated commercial finisher diet individually fed twice daily (07:30 and 16:30) without or with added CPL (5 and 4 pigs, respectively), the latter top-dressed (300 mg) on each meal. CPL did not affect feed intake ($P > 0.05$), averaging 4.3 + 0.11 kg feed (as-feed basis)/day. Freshly voided feces collected after morning feedings at 4-d intervals were cultured on MacConkey and M Enterococcus agar supplemented without (NO) or with 8 µg penicillin (PEN)/mL or 16 µg chlortetracycline (CTC)/mL for enumeration of generic *Escherichia coli* and enterococci. Log_{10} transformations of colony forming units (cfu/g feces) were analyzed using a repeated-measures ANOVA with a Tukey's multiple comparison of means. Main effects of CPL treatment and day on diet ($P < 0.05$) were observed on fecal *E. coli* recovered on NO antibiotic medium, ranging from 6.5 to 4.9 $\log_{10}$ cfu/g, but differences were less than 1 $\log_{10}$ cfu/g. *E. coli* numbers did not differ ($P = 0.98$) whether recovered on medium without or with PEN or CTC. Main effects of CPL treatment, day on diet and their interaction were observed ($P < 0.05$) on fecal enterococci recovered on NO antibiotic medium during d 0 and 4, with numbers being 1.0 $\log_{10}$ cfu/g lower in feces collected from CPL-treated pigs than non-treated pigs (ranging 5.6 to 6.0 $\log_{10}$ cfu/g) but not on later days, where populations ranged 4.4 to 4.7 $\log_{10}$ cfu/g. A difference was observed ($P < 0.05$) between enterococci recovered on medium containing PEN, 1.8 to 3.0 $\log_{10}$ cfu/g lower, than those recovered on NO antibiotic medium or medium containing CTC. Results demonstrate that feeding CPL to pigs reduced carriage of PEN-sensitive but not CTC-sensitive enterococci and had no effect of numbers of PEN or CTC-sensitive *E. coli*.

Key Words: antibiotic resistance, *E. coli*, enterococci, pig

29 Methyl-compound production by rumen bacteria.

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Methylamines and methanol are formed in the rumen by the action of bacteria, and are used by methyltrophic methanogens to form methane, an important agricultural greenhouse gas. Methylamines come from plant phosphatidylcholine degradation, via the action of choline trimethylamine lyase, while methanol comes from demethoxylation of dietary pectins by pectin methylesterase activity. We used the protein domains of choline trimethylamine lyase (CutC), and activator protein (CutD) to screen rumen metagenomic and metatranscriptomic data sets, metagenome assembled genomes, and the Hungate1000 genomes to identify organisms capable of producing methylamines. In the metagenome and metatranscriptome data sets, we found good matches only to *Olsenella umbonata* and to *Cecibacter*, whereas the Hungate1000 genomes set found bacteria within the phyla *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. The *cutC* and *cutD* genes clustered with genes that encode structural components of bacterial microcompartment proteins. *Prevotella* was the dominant genus encoding pectin methyl esterases responsible for methanol release, while some large pectin methyl esterases (>2100 aa) were encoded in *Butyrivibrio* genomes. We also analyzed metagenomic sequences from an enrichment of pectin-degrading and methane-forming microbes from sheep rumen contents. The consortium was composed of 3 organisms: a putative pectin-degrading bacterium (phylum *Tenericutes*, class *Mollicutes*), a galacturonate-using *Sphaerochaeta* sp. predicted to produce acetate, lactate, and ethanol, and a methylotrophic methanogen, *Methanosphaera* sp., with the ability to form methane via a primary ethanol-dependent, hydrogen-independent, methanogenesis pathway. These analyses have identified the main bacteria that produce methyl-compounds in ruminants, and their enzymatic activities can now be targeted with the aim of finding ways to reduce the supply of methyl-compound substrates to methanogens, and thereby limit methylotrophic methanogenesis in the rumen.

Key Words: rumen bacteria, methylamines, methanol

30 Innate variability in animal performance across seasonal changes in a northern Australian grazing system.

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In the grazing animal, efficiency is a function of how effective the animal can harvest the feed resource, the optimum fermentation conditions in the rumen and the genetic potential of the animal for intake and growth. All of these aspects are interdependent and influenced by many variables. Thus improving cattle feed efficiency is not trivial. The northern Australian grazing system is highlighted by a dry winter season with poor quality feed followed by a wet summer season with relatively higher quality forage material. A grazing herd of 90 composite *Bos indicus* × *Bos taurus* animals were monitored for 18 mo across multiple dry and wet seasons. Average daily weight gains were collected in real time using walk-over weighers (WoW) at watering points to monitor live weight change throughout the grazing trial. Animals that were ranked as the best and poorest performers based on average daily weight gain (ADWG) in the wet season on higher quality pastures were more consistent in their ranking across both seasons, while animal live weight rankings based on the dry season were not. Rumen fermentation and microbiota parameters were significantly affected by seasons and quality of feed, with observed increases in the VFA average chain length (ACL), A:P, Butyrate, branched chain VFAs and ammonia between the dry and wet seasons. Total VFA measurements were observed to be lower in better performing animals and during the wet season samplings. Bacterial species assigned to *Bacteroidetes* and *Butyrivibrio* were relatively more abundant in the wet season but lower in the better performing animals, while *Firmicutes* were lower in the wet season and higher in lower ranked animals. *Fibrobacter* and *Ruminococcus* species were relatively less abundant in the wet season when the animals were on better quality forage. There

is a confirmed variance in the data linked to ADWG ranking of animals across the seasons; however, selection of the best performers during the wet season on higher quality pastures would select for animals that consistently produce better than average ADWG across the grazing periods. Feed intake was not available, due to the difficulty of measuring this on grazing animals, yet current research involving the deployment of collar sensors with algorithms that estimate feed intake are attempting to overcome this limitation and provide data in relation to feed consumption.

31 Temporal stability of the ruminal bacterial communities in beef steers.

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Historically, nutritional studies have incorporated a 2- to 4-week diet acclimation period, which may not be sufficient for ruminal microbiome stabilization. Although rumen microbiota are responsible for providing nutrients vital to ruminants, little is known about adequate acclimation periods required to reach stabilization of the rumen microbiome that could provide more accurate results from nutritional studies. This study analyzed the rumen bacterial communities and rumen environment parameters over 10 weeks following transition from a forage- to concentrate-based diet following a traditional 2-week adaptation period. Each week, rumen content was collected via orogastric tubing before morning feeds and the V1-V3 hypervariable region of the 16S rRNA gene was sequenced for bacterial community analysis. Data were analyzed using mixed model one-way ANOVA and random forests. Metrics for richness were often greater at wk 5 of the trial, but lower by the final week of the trial, whereas measurements of equitability fluctuated but were similar between the first and last weeks of the trial ($P > 0.05$). Several α-diversity metrics, including observed OTU and Simpson's Evenness E fluctuated throughout the trial, but were typically either greatest (observed OTU) or lowest (Simpson's) at wk 5 of the trial compared with wk 1 and 10 ($P < 0.05$). Using Bray-Curtis distances, PCoA illustrated shifts in β-diversity from wk 1 to wk 10, with shifts beginning at wk 4. Week 1 phylogenetic diversity was different than that of wk 10 according to ANOSIM ($P = 0.01$). At wk 4, several orders were associated with the shift to the final bacterial community composition as determined by spectral co-clustering, including *Pasteurellales*, *Aeromonadales*, and *Bacteroidales*, which may be the result of changes in substrates. Rumen microbiome stability did not occur until approximately 9 weeks following adaptation to the diet, and was associated with changes in specific bacterial populations and rumen environment. The results of this study suggest that adaptation and wash-out periods need to be re-evaluated to accommodate necessary rumen microbiome acclimation.

Key Words: temporal stability, rumen microbiome, steers

POSTER PRESENTATIONS

Computational approaches and applications

32 Accurate estimation of microbial sequence diversity with Distanced.

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Microbes from the gut and other environments are diverse. Deep sequencing of ribosomal DNA suggests thousands of different microbes may be present in a single sample. Errors in sequencing have made any estimate of diversity uncertain, however. Here we developed a computational tool that can accurately estimate diversity of ribosomal DNA sequences, regardless of sequencing errors. Our tool, Distanced, calculates how different (distant) sequences would be without sequencing errors. It does this using a Bayesian approach. To determine the accuracy of this approach, we used Distanced to estimate diversity of artificial communities of bacteria and fungi. These artificial communities have known ribosomal DNA sequences. Distanced estimated their diversity more accurately than did state-of-the-art tools (DADA2 and Deblur). Distanced was accurate because it kept all sequence reads, whereas state-of-the-art tools discarded up to 90% of them. We subsequently used Distanced to estimate diversity of real communities of bacteria from feces and soil. Its estimates of diversity differed from those of state-of-the-art tools. This finding is problematic for state-of-the-art tools, presuming Distanced is as accurate on real communities as artificial ones. Our findings suggest Distanced will be a useful tool for estimating microbial diversity in the gut and other environments.

Key Words: diversity, bacteria, fungi, DNA, sequencing

33 Utilization of assembled long read amplicons to improve taxonomic resolution.

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As sequencing technologies advance, it is important to compare new methods to those currently accepted to evaluate the benefits and costs. Loop Genomics utilizes Illumina sequencing and barcoding to generate assembled long read amplicons. Sequencing coverage created while generating long amplicons could minimize sequencing errors while the amplicon length created would allow for more accurate taxonomic assignments as compared with traditional paired end amplicon sequencing. We compared assembled long read amplicons and traditional Illumina V4 16S rRNA across 5 gastrointestinal samples from breeder hens and 7 d-old chicks (DOC). The V4 data were filtered using DADA2 and taxonomy assigned by closed reference clustering at 97% against the EZBioCloud database to the species level and α diversity analysis performed. The assembled long read amplicons were imported into QIIME2 for taxonomic assignment and α diversity analysis but no further quality filtering was performed. Breeder gastrointestinal tracts had an average of 121 unique species as determined by assembled amplicons while V4 sequencing detected an average of 39. In DOC, assembled amplicon data detected 32 unique species while sequencing of the V4 region detected only 6. Even though the richness was greater in the assembled amplicon samples, the Shannon Diversity Index was greater in the V4 amplicon samples, indicating that these samples were more even. Although V4 amplicons were identified to the species level the 300 base pairs generated is not sufficient to identify taxonomy to that level. The most abundant genera from the V4 amplicon samples were *Enterococcus*, *Clostridium*, *Paeniclostridium*, *Lactobacillus* and *Enterobacteriaceae*. By assembled amplicon sequencing, the most abundant species were found to be *Enterococcus faecalis*, *Clostridium tertium*, *Paeniclostridium sordellii*, and *Lactobacillus aviarus* followed by several *Clostridiales*. The assembled amplicons allowed

for the assignment of *Enterobacteriaceae* at lower taxonomic levels. The similarity in results coupled with the added taxonomic resolution of the assembled amplicons holds promise for further studies.

Key Words: Illumina, taxonomy, poultry

34 Comparative genome analyses of human gut methanogens.

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Methanogens and methane positivity are more prevalent in patients and subjects with slower gut transit times and/or constipation-related digestive disorders, typically, with a decrease in the abundance of *Methanobrevibacter* spp. observed in patients with diarrhea-like symptoms and/or active inflammatory bowel disease (IBD). Although such findings seem ecologically intuitive, not all methanogenic archaea appear to be susceptible to the inflammatory milieu in IBD patients and may in fact contribute to this host response. Here, we report our initial findings into the phylogenetic and genomic features of human *Methanobrevibacter* and *Methanosphaera* spp. isolates, as well as metagenome assembled genomes (MAGs) recovered from human subjects with different health status. We isolated new strains of *M. smithii* (JC9) and *M. stadtmanae* (PA5) from healthy Australian subjects. These strains possess bile salt hydrolase genes but their growth kinetics differ compared with those observed with the type strains for each genus. The MAGs recovered from publically available data sets of healthy individuals and patients with ulcerative colitis and Crohn's disease, were mainly affiliated with the genus *Methanobrevibacter*. The genome alignments show a high degree of genetic synteny between the MAGs and isolate genomes within each genus. However, a small subset of genes differentiate between the genomes recovered from strains present in either healthy subjects or IBD patients; but the great majority of these genes are currently annotated as encoding "hypothetical" proteins. Despite the documented shift away from *Methanobrevibacter* spp. in IBD patients, MAGs affiliated with this genus are indeed recoverable from such patients. Furthermore, our preliminary analysis has identified candidate genes that differentiate between strains favored by the ecological landscape characteristic of health and disease (IBD). To that end, we are currently undertaking further culture-based and metagenomics studies from methane-positive subjects with different health status, to better define the genetic adaptations, and clinical relevance, of different methanogen strains.

Key Words: methanogenic archaea, bile salts, physiological ecology

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

35 Identifying bacteria involved in nitrogen cycling in dairy cow manure on farms across California.

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Manure from cattle can be used as an organic fertilizer for crops meant for livestock feed. Its application changes the composition of the soil and its microbial communities. Although fecal nitrogen in the form of ammonium is important for plant growth, nitrogen in other forms contributes to global warming through volatile emissions of nitric oxide and ammonia, and eutrophication by leaching of nitrate into ground water. In recent

years, increased nitrate concentration in the ground water of the Central Valley has increased pressure on California's dairies to reduce their contributions of this pollutant. Up to this point, bacteria contributing to nitrogen cycling in cow manure have yet to be identified. Elucidating the fecal microbiome and their functions contributes to understanding the fertilization potential of manure and aids in waste management decisions. This study aims to determine the composition and functional capacity of the fecal microbiome of commercial dairy cattle (n = 12) on farms across northern/central California. Ten farms representing a variety of feeding and management systems were enrolled, and fecal samples were taken from individual cows for metagenomic sequencing. Cleaned metagenomic reads were queried against a custom database of Hidden Markov Models for genes involved in nitrogen cycling. All genes used to predict for nitrogen fixation (nifHDK and nifENB) were found in all fecal samples. Reads were assembled with MegaHit and Anvi'o was used to identify contigs containing genes involved in nitrogen cycle. Taxonomy was assigned to these contigs with Kaiju, thus identifying *Clostridiaceae* and *Lachnospiraceae* as the most common families to contain members putatively involved in nitrogen fixation. Farms showed variation in the abundance of genes involved in nitrogen cycling and abundance of genera involved in these processes. These findings will inform future methods to manage the fecal communities to improve the fertilization potential of manure while mitigating the taxa that contribute to eutrophication of water waste.

Key Words: cattle, dairy, rumen, metagenomics, nitrogen fixation

36 Refining direct-fed microbials and silage inoculants for reduction of methane emissions from ruminants.

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The Agricultural industry currently produces 14% of the world's annual greenhouse gas emissions (GHGs), and by 2050, developed countries are required to reduce their emissions by 80 to 95% while producing 70% more food. Domesticated ruminants, such as livestock, contribute around 86 million metric tonnes of methane per annum. This poses a problem for the agricultural industry, as the ability of methane to trap infrared radiation is significantly higher compared with other GHGs. Methane production is largely the result of resident microbial interactions where complex organic matter is fermented within the rumen. It is therefore expected that methane can be reduced by targeting the bio-network of methanogenesis in the rumen i.e., methanogens or other precursors in the methanogenic pathway. To determine whether methane mitigation can be achieved through direct microbial intervention, presumed lactic acid bacteria (LAB) were isolated on Lactic Minimal Media, from various agricultural sources; that is, silage, rumen fluid, and dietary fibers (in total mixed ration, TMR). Well diffusion assays confirmed inhibitory activity of some isolates against the sulfate reducing (*Desulfovibrio desulfuricans*) and acetogenic (*Blautia schinkii*) bacteria. Subsequently, cell-free supernatants (26% of the total volume) were used to directly inhibit various species of methanogens (*Methanobrevibacter boviskoreanii, Methanobrevibacter gottschalkii) in vitro*. Gas chromatography was used to analyze methane produced during 15 d incubation in closed anaerobic conditions. The best performing isolate impeded methane production of *M. boviskoreanii*, decreasing measurements from 100,000 ppm to 60,000 ppm by d 6 of co-incubation. Methane production by *M. gottschalkii* was reduced to <2,000 ppm in the presence of the supernatant, compared with 50,000 ppm measured in the untreated control. These results suggest that LAB inhibit methanogens and have potential to reduce methane emissions from ruminants when incorporated into feed as either direct-fed microbials or silage inoculants.

Key Words: ruminant, direct-fed microbial, lactic acid bacteria, methane mitigation, silage inoculants

*These two authors contributed equally to this work.

37 Evaluation of the potential of two common Pacific Coast macroalgae for mitigating methane emissions from ruminants.

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With increasing interest in feed-based methane mitigation strategies, fueled by local legal directives aimed at methane production from the agricultural sector in California, identifying local sources of biological feed additives will be critical in keeping the implementation of these strategies affordable. In a recent study, the red alga *Asparagopsis taxiformis* stood out as the most effective species of seaweed to reduce methane production from enteric fermentation. Due to the potential differences in effectiveness based on the location from where *A. taxiformis* is collected and the financial burden of collection and transport, we tested the potential of *A. taxiformis*, as well as the brown seaweed *Zonaria farlowii* collected in the nearshore waters off Santa Catalina Island, California, USA, for their ability to mitigate methane production during *in vitro* rumen fermentation. At a dose rate of 5% dry matter, *A. taxiformis* reduced methane production by 74% (P = 0.01) and *Z. farlowii* reduced methane production by 11% (P = 0.04) after 48 h and 24 h of *in vitro* rumen fermentation respectively. The methane reducing effect of *A. taxiformis* and *Z. farlowii* described here make these local macroalgae promising candidates for biotic methane mitigation strategies in the largest milk producing state in the United States. To determine their real potential as methane-mitigating feed supplements in the dairy industry, their effect in vivo requires investigation.

Key Words: *in vitro* rumen fermentation, greenhouse gas mitigation, macroalgae, feed supplementation

Immunology (including host–microbe interactions)

38 Low-protein diets supplemented with casein hydrolysate enhance small intestinal barrier functions and inhibit ileal pro-inflammatory cytokine expressions in pigs.

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Greatly reducing the dietary crude protein (CP) level (>4% reduction vs. NRC), even supplemented with all amino acids (AAs), negatively affects intestinal environment in pigs, which might be due to the excessive lack of protein-derived peptides. This study investigated the effects of addition of casein hydrolysate (peptide source) in low-protein (LP) diets, in comparison with crystalline AA addition, on small intestinal bacterial community, mucosal barrier and immunity in pigs. Twenty-one pigs (initial BW 19.90 ± 1.00 kg, 63 ± 1 d of age) were assigned to 3 groups and fed with control diet (16% CP, CON), LP diet (13% CP) supplemented with crystalline AAs (LPA), or casein hydrolysate (LPC) for 4 weeks. In comparison with pigs fed the CON and LPA diets, those on LPC had a significantly greater average daily gain. Compared with CON diet, LPA diet significantly decreased the protein expression level of jejunal ZO-1 and stem cell proliferation factor Lgr-5, whereas LPC diet enhanced intestinal barrier function by increasing the protein expression of jejunal occludin and ZO-1 and ileal MUC-2.

Compared with CON diet, LPA diet significantly decreased *Lactobacillus* counts, while LPC diet increased *Lactobacillus* counts and decreased *E.coli* counts in the ileum. LPA diet significantly increased ileal pro-inflammatory cytokine IL-6 and IL-22 expressions, whereas LPC diet decreased ileal pro-inflammatory IL-1β, IL-17A, and TNF-α expressions. In conclusion, relative to AAs addition, the addition of casein hydrolysate in LP diets produces favorable effects in growth performance and small intestinal environment. Interestingly, ileum but not jejunum may be a key site responsible for LPC function. These findings provide novel insights into nutritional intervention in pigs.

Key Words: casein hydrolysate, low-protein diets, mucosal immunity, pig, intestinal barrier

39 Chemotherapy disrupts microbial-enterohepatic bile acid metabolism in mice.

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Although often an effective component of cancer treatment, chemotherapy is associated with gastrointestinal (GI) side effects, including pain, nausea, and diarrhea. These effects are often attributed to chemotherapy-induced apoptosis of highly proliferative intestinal epithelial cells, but interactions with intestinal microbiota and bile acid (BA) metabolism are poorly understood. Furthermore, metabolism of taxane chemotherapeutics such as paclitaxel (PAX) and BA are intimately intertwined due to shared enterohepatic metabolism. Therefore, the objective of this study is to understand how chemotherapy alters BA and PAX metabolism in the liver, intestine, and microbiota of mice. Adult female BALB/c mice received PAX (30 mg/kg, n = 8) or vehicle (n = 10) via IP injection every 2 d for a total of 6 injections. Intestinal morphology was assessed by H&E staining. The intestinal bacteriome was assessed via 16S rRNA gene sequencing of DNA extracted from contents of the duodenum (DUO), jejunum (JEJ), ileum (ILE), cecum (CEC), proximal colon (PC), distal colon (DC), and longitudinally in feces. Total fungus was assessed by qPCR of the ITS gene. Expression of genes related to inflammation and BA and PAX metabolism was assessed via qPCR. BA were quantified via HPLC. PAX reduced body mass and food intake but increased masses of all intestinal segments, except for ILE. Villus height was decreased in DUO. Crypt depth was decreased in DUO and CEC, but increased in DC. Expression of matrix metalloproteinases was increased in liver, JEJ, CEC, and PC; TNF was decreased in the liver and DC; BA and PAX metabolism-related genes was decreased in liver, DUO, JEJ, CEC, and DC. Bacterial diversity was decreased in all segments except for DUO, marked by increased *Mucispirillum*, and decreased *Lachnospiraceae NK4A136*, *Eubacterium*, and *Roseburia*. Fungi were increased in DC. In conclusion, PAX alters BA metabolism, influencing microbial composition and intestinal morphology and inflammation, which likely affects the ability of the body to efficiently metabolize and excrete chemotherapeutic drugs, thus contributing to GI side effects.

Key Words: chemotherapy, microbiome, bile, fungi

40 Steroid-17,20-desmolase (DesAB) activity from gut and urinary microbes forms 11-oxy-androgens from glucocorticoids *in vitro*.

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Members of the gastrointestinal microbiota are known to convert glucocorticoids to potent 11-oxy-androgens. Our lab has previously deduced the steroid-17,20-desmolase pathway (*desABCD*), which converts cortisol into 11b-hydroxyandrostenedione, with *Clostridium scindens* ATCC 35704 *in vitro*. Recently, our lab discovered an additional gene in this pathway, the *desE* gene that encodes a 20β-hydroxysteroid dehydrogenase

(20β-HSDH), in 2 gut microbes, *Butyricicoccus desmolans* and *Clostridium cadaveris*. Unexpectedly, a phylogenetic analysis of *desE* revealed that *Propionimicrobium lymphophilum*, a urinary microbe, also encodes the steroid-17,20-desmolase pathway. Here, we determined that both purified recombinant DesAB and *P. lymphophilum* whole cells are capable of side-chain cleaving both endogenous and exogenous (semi-synthetic) glucocorticoids, forming 11-oxy-androgens. These glucocorticoids include cortisol, 5α-dihydrocortisol, 9-fluorocortisol, allo-tetrahydrocortisol, cortisone, prednisolone, and prednisone. Because both the urethra and colon are in physical contact with the prostate in humans, we hypothesize that these microbes may provide an additional source of androgens. This may be significant because androgen receptor (AR) stimulation plays a critical role in the development and progression of prostate cancer. We show that microbial 11-oxy-androgen metabolites are capable of stimulating AR and proliferation in LNCaP cells, androgen-sensitive human prostate adenocarcinoma cells, *in vitro* through luciferase reporter and MTS proliferation assays, respectively. Taken together, this suggests that both gut and urinary microbes may contribute to prostate cancer via their production of 11-oxy-androgens from glucocorticoids.

Key Words: 11-oxy-androgen, desmolase, *Propionimicrobium lymphophilum*, prostate cancer

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

41 Cellulosome assembly revealed by cryoelectron microscopy.

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Cellulose found in the plant cell wall, is the most abundant carbohydrate on earth. The anaerobic gut bacteria are the key players in efficient enzymatic conversion of cellulose to its sugar monomers, which are readily available in the gastrointestinal tract of various mammals. Cellulosomes are multi-enzymatic complexes composed of a variety of scaffoldins occupying numerous enzymatic subunits on them to work synergistically with proximity effect. An increasing number of 3-dimensional structures revealed several enzymatic subunits and scaffoldin domains belonging to different families. However, the organization of the multi-enzyme complex and the molecular mechanisms of the proximity effect remain to be elucidated. In the present study, we explored the structure of the most prominent catalytical subunit of the well-studied gut bacterium *Clostridium thermocellum*, Cel48S bound to CipA scaffoldin designer cellulosome complex *in vitro* by cryoelectron microscopy. We also investigated the display of the complex on the cell surface in situ by means of cryoelectron tomography. Our data revealed a maximum cellulosomal density on the cell surface of *C.thermocellum*. Lignocellulosic materials are attractive renewable bioenergy sources as an alternative to unsustainable fossil fuels. Cellulosomes emerged as one of the most effective enzymatic degraders of the lignocellulosic materials into soluble sugars, which are also great interest of the fermentation industry. Understanding the structure of multi-enzyme cellulosome complex provides insight into its intricate molecular mechanism and its performance.

Key Words: lignocellulose degradation, *Clostridium thermocellum*, Cel48S, CipA, cryoelectron microscopy

42 Functional and structural study of 20β-hydroxysteroid dehydrogenase from *Bifidobacterium adolescentis* strain L2-32.

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Microbes have evolved various enzymes to modify host-derived steroids in the human gut. The steroid-17,20-desmolase pathway is responsible for biotransforming cortisol into pro-androgens which may play a role in host physiology and pathophysiology. The 20β-hydroxysteroid dehydrogenase (HSDH) catalyzes the reduction of the position 20 carboxyl group on cortisol, producing 20β-dihydrocortisol. This enzyme is capable of regulating the steroid-17,20-desmolase pathway by redirecting cortisol metabolism toward 20β-dihydrocortisol at the expense of pro-androgens. Recently, the gene encoding a 20β-HSDH in *Butyricicoccus desmolans* ATCC 43058 was reported. Through a non-redundant protein search with this enzyme, a gene with 59% homology was identified in *Bifidobacterium adolescentis* strain L2–32. The putative 20β-HSDH from *B. adolescentis* was cloned into *E. coli*, overexpressed, and purified. 20β-HSDH activity was confirmed through whole cell and enzymatic assays. The enzyme displayed specificity for NADH over NADPH, and cortisol was the preferred substrate. The recombinant 20β-HSDH was crystallized in both the apo and holo forms and diffracted at 2.2 and 2.5Å, respectively. Interestingly, the structure contains a large, flexible extended N-terminal region. This N-terminal peptide likely plays an important role in protein stability because varying length deletions resulted in corresponding decreases in enzymatic activity.

Key Words: cortisol, 20β-hydroxysteroid dehydrogenase, *Bifidobacterium adolescentis*

43 Characteristics of fecal microbiota in captive Antillean manatees *Trichechus manatus manatus*.

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Captive environments such as given diet, antimicrobial administration, and interaction with keepers potentially affect the structure of intestinal microbiota of animals. Antillean manatees in Ocean Expo Park (Okinawa, Japan) were mainly fed with several kinds of terrestrial plants available, unlike wild manatees consuming freshwater plants and seagrasses. The modification in the diet may change the components of intestinal microbial communities in captive manatees. This study aimed to characterize fecal microbiota of captive manatees by comparing with that of wild Florida manatees *Trichechus manatus latirostris* (subspecies of Antillean manatee) reported previously. Fecal samples were collected from captive Antillean manatees immediately after defecation. After extracting DNA from the feces, the V3–V4 region of bacterial 16S rRNA genes were amplified and sequenced using an Illumina MiSeq platform. High-quality reads were assigned to 16 bacteria phyla with dominance of *Firmicutes* (84.05 ± 3.50%) followed by *Bacteroidetes* (8.60 ± 1.71%), and a small number of sequences (0.37 ± 0.09%) could not be classified. In top 20 genera, bacteria groups responsible for hydrolyzing cellulose and metabolizing bile acid were listed, as consistent with previous studies on sirenians. The compositions of microbial communities at the genus level varied remarkably between captive and wild manatees as reported previously, and genera occupying a high proportion of wild manatee microbiota hardly contributed to fecal microbiota of captive manatees. nMDS plots analysis of intestinal microbial community between captive and wild manatees showed a clear segregation. Among the captive individuals, there were no considerable differences in α diversity indices (Simpson and Shannon–Wiener) with small variations in microbial richness (Chao 1) and number of OTUs. Our results showed unique intestinal microbiota of captive Antillean manatees, possibly affected by their feeding habit, diet, and/or interactions with keepers in captivity.

Key Words: fecal microbiota, manatee, captive environment, marine herbivore

44 The hibernating squirrel microbiome responds to seasonal dietary shifts by altering its functional potential.

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Hibernating animals undergo dramatic seasonal shifts in diet that alter substrate availability for their associated gut microbiota. The circannual hibernation cycle involves periods of summer host hyperphagia when the microbiota has access to plentiful dietary substrates for energy. In contrast, during winter the host fasts and hibernates, forcing the microbiota to rely solely on host-derived substrates. While microbial taxonomic composition has been shown to change in response to these seasonal substrate fluctuations, it is unknown how functional potential changes. To examine seasonal shifts in the functional potential of hibernator microbiomes, we generated metagenomes from cecal contents of 13-lined ground squirrels (*Ictidomys tridecemlineatus*). We hypothesize that seasonal metagenomes have distinct features that reflect microbial adaptation to changes in substrate availability. Samples were sequenced from 3 squirrels in each season (summer, winter, and spring) and 2 groups of functional annotations were analyzed (clusters of orthologous groups, COGs, and metabolic pathways). We found that hibernator metagenomes clustered separately by season, indicating distinct functional potentials. We observed significant differences between seasonal metagenomes based on COG annotations (P = 0.010) and a non-significant trend based on metabolic pathways (P = 0.075). To determine functional annotations that drive these seasonal differences, we identified genes and pathways that were significantly over- or under-represented in different seasons. These distinct features were present in both COGs and metabolic pathways, with the former being more prominent. Taken together, our results demonstrate that the hibernator microbiome responds to host dietary shifts by altering functional potential, highlights the importance of microbe-host symbioses in animals that live in environments with fluctuating energy and nutrient availability, and advances understanding of the hibernation phenotype for applications in biomedicine and deep space travel.

Key Words: hibernation, metagenomics, microbiome, diet, fasting

45 Isolation, identification and phenotypic evaluation of bacteria from infant feces.

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There is a renewed interest in the isolation and cultivation of commensal bacteria from the gut microbial community to better understand the physiological and metabolic functions contributing to human health. Despite this growing interest, few recent studies have described the isolation and characterization of bacteria from the gut. In this study, bacteria isolated from infant feces were isolated, sequenced and initial metabolic phenotyping was conducted. To isolate bacterial strains, fresh fecal samples were collected from 3-mo-old infants who were either exclusively breast (BF) or formula-fed (FF) and either cesarean (CD) or vaginally delivered (VD). Samples were diluted, plated, and cultured on GMM and MRS media. Also, the samples were inoculated into mBY medium for the isolation of methanogens. The isolated colonies were picked and purified for 16S rDNA sequencing and identification. The isolated strains were screened for their ability to utilize various carbon substrates, including human milk oligosaccharides and prebiotics, in 24- to 36-h incubations. After the growth, the supernatant was analyzed for metabolites. To date, >170 bacterial strains have been isolated and identified. The fecal communities from BF/VD samples were predominantly from the phylum *Actinobacteria* (dominated by *Bifidobacterium* spp.), followed by *Firmicutes*, and *Proteobacteria*. In contrast, isolates from FF/VD or FF/CD infants were primarily from *Firmicutes*, with relatively few *Actinobacteria* and *Proteobacteria* isolates. Overall, most isolates represented the genera *Bifidobacterium*, followed by *Enterococcus*, and *Ruminococcus*. Ongoing phenotypic evaluation of the isolated strains for their ability to utilize various carbon sources and the resulting metabolic profiles will provide insight into

species- and strain-specific utilization of common prebiotics in human milk and infant formula. This comprehensive phenotypic database based on our collection will provide novel insights into how the physiological and metabolic functions of the infant gut microbiota are influenced by route of delivery and early infant nutrition.

Key Words: microbiota, infant gut, human milk, formula

46 Genome-wide transcriptional responses of sensitivity and adaptation of ruminal bacteria to monensin.

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Monensin is widely used in ruminant animal diets to improve feed efficiency by manipulating the ruminal microbial population. Monensin modifies the movement of ions across the membranes of rumen microbes, selectively suppressing growth of gram-positive bacteria compared with gram-negative bacteria that are resistant to the effects of monensin. However, the cellular and molecular mode of action remain unknown. To analyze the sensitivity and the mechanism of adaptation to monensin, whole genome transcriptional profiling in the presence and absence of monensin was first analyzed using high-throughput RNA-Seq, based on minimum inhibitory concentrations determined with representative ruminal strains, G− (*Prevotella bryantii* $B_1$4 and *Fibrobacter succinogenes* S85), G+ (*Ruminococcus albus* 7 and *Streptococcus bovis* JB1), and G-intermediate (*Selenomonas ruminantium* HD4, *Megasphaera elsdenii* T81, and *Butyrivibrio fibrisolvens* D1). The transcriptional responses of strains sensitive to monensin (*S. bovis* JB1, *R. albus* 7, and *B. fibrisolvens* D1) commonly upregulated genes encoding energy metabolism, ABC transporters, and protein damage repair, whereas the expression of those genes was unchanged in the resistant strains. After each culture was subjected to growth in the presence of increasing monensin concentrations, the changes of gene expression profiles of the monensin-adapted cultures were investigated. Interestingly, while the susceptibility of the naïve cultures to monensin was in accordance with their cell wall types, their adaptability to monensin varied. The transcriptional profile of the monensin-adapted cultures shifted toward overexpression of genes related to transmembrane secretion, modification of cell wall proteins, and response to chemiosmotic gradients. Comparison of end product metabolites between naïve and adapted cultures of *S. bovis* JB1 and *P. bryantii* $B_1$4 indicated that the alteration of the primary fermentation products caused by monensin was alleviated in the adapted cultures. The findings enhance our understanding of population based ecological studies and long-term efficacy of adding ionophores to animal diets.

Key Words: monensin, ruminal bacteria, transcriptome, adaptation

47 Evaluation of intra-species diversity of *Oxalobacter formigenes* strains using pulsed-field gel electrophoresis.

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Oxalobacter formigenes is a beneficial gut bacterium that plays an important role in the prevention of kidney stone disease. To date, 21 strains of *O. formigenes* have been isolated and further divided into 2 groups based on differences in cell membrane lipids, cellular proteins, and nucleic acid fragments. The aim of this project was to evaluate intra-species diversity of *O. formigenes* strains using pulsed-field gel electrophoresis (PFGE). Nine pure cultures of *O. formigenes* strains (5 strains group 1 specific; 4 strains group 2 specific), 5 *O. formigenes* human isolates (2 isolates group 1 specific; 3 isolates group 2 specific), and 3 *O. formigenes* mice isolates (group 1 specific) were grown anaerobically at 37°C in undefined oxalate broth. Upon reaching late exponential growth, cells were harvested, suspended to an optical density (600 nm) of ~2.0, and added to melted agarose to make plugs. Agarose plugs were incubated (55°C) in proteinase K solution to lyse cells, sliced, and then digested with *Xba*I at 37°C. Digested agarose slices were loaded onto 1% Seakem Gold agarose gels and subjected to PFGE for 18–19 h. All *O. formigenes* strains and isolates produced reproducible banding patterns

yielding between 12 and 20 bands. These profiles were further analyzed by BioNumerics software. Four group 1 specific strains (isolated from human feces, sheep rumen, and fresh water lake sediment) showed a tight clustering with each other while other group 1 specific strain (isolated from wild rat) was tightly associated with group 1 specific human isolates. Three group 2 specific strains (isolated from human feces and guinea pig) showed high similarity with each other but one group 2 specific strain (isolated from human feces) was in proximity with group 1 specific strains. Mice isolates were compactly grouped with each other but did not show any closeness with rest of the strains. This study was in overall agreement with the grouping of the strains with some exceptions indicating PFGE is a useful tool to study the phylogenetic relationship among *O. formigenes* strains.

Key Words: oxalate, pulsed-field gel electrophoresis, kidney stones, gut microbiome

48 Dynamics of feed particle colonization by anaerobic fungi.

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Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of many herbivorous animals, and play an essential role in degrading food with a range of powerful hydrolytic enzymes. The dynamics of feed particle colonization is not well understood, so we incubated Dacron bags containing fresh grass forage in the rumen of cannulated cows to explore the dynamics of colonization. The succession of fungi colonizing the forage particles over a 24-h period was explored using DNA metabarcoding (D1 region of the LSU locus). A range of anaerobic fungi were also isolated from the rumen feces of a range of large herbivores. After roll tube culture to isolate single-zoospore cultures, sequencing of LSU was undertaken to identify the fungi to the species level. We also obtained genome sequence data from several of these and other cultures, allowing genomic comparisons to be made between members of different genera. In addition to elucidating the mechanisms of feed particle degradation, such analyses permit exploration of fungal genomes for novel enzymes and potential antimicrobial compounds.

Key Words: rumen, anaerobic, fungi

49 Identifying novel antimicrobials from anaerobic rumen fungi.

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Anaerobic rumen fungi (phylum *Neocallimastigomycota*) occupy the gastrointestinal tract of several herbivorous animals, and by using their powerful hydrolytic enzymes and mechanical forces they degrade plant material in the rumen, essential for rumen efficiency. The rumen microbiome represents an underexplored resource for the discovery of novel microbial enzymes and metabolites, including antimicrobial peptides (AMPs). AMPs are promising drug candidates, and are necessary for targeting the worldwide issue of antimicrobial resistance. Rumen fluid and fecal samples were collected from various large herbivores, and fungal cultures were grown and maintained under anaerobic conditions. After roll tube culture to isolate single-zoospore cultures, sequencing of the large subunit (LSU) rRNA genes was undertaken to identify the fungi to species level. Analysis of genomic data from these cultures, alongside published data, was undertaken to explore the diversity of AMPs within these fungal genomes. Using functional and computational screening, potentially novel AMPs have been discovered, with isolates showing encouraging activity against some strains of bacteria. Findings indicate that the rumen microbiome may provide

alternative antimicrobials for future therapeutic application.

Key Words: anaerobic fungi, rumen, microbiology, antimicrobial peptide, antibiotic resistance

50 *Methanobrevibacter boviskoreanii* JH1^T growth on alcohols allows development of a high throughput bioassay.

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Rumen methanogens use by-products of fermentation to carry out methanogenesis for energy generation. A key fermentation by-product used by rumen methanogens is hydrogen, which acts as the source of reducing potential for methane formation in hydrogenotrophic methanogens. The *in vitro* cultivation of hydrogenotrophic rumen methanogens requires hydrogen supplied under pressure to achieve the level of dissolved hydrogen encountered in the rumen. The requirement of pressurized hydrogen for growth of hydrogenotrophic methanogens limits the ability to conduct high-throughput screening experiments. *Methanobrevibacter* sp. AbM4 is a slowly growing rumen methanogen that has been shown to grow without H_2 in the presence of 20 m*M* methanol and 20 m*M* ethanol. Similar to AbM4, the genome sequence of *Methanobrevibacter boviskoreanii* JH1^T harbors genes encoding an NADP-dependent alcohol dehydrogenase and a F_{420}-dependent NADP reductase, which may facilitate the transfer of reducing potential from ethanol to F_{420} via NADP. The aim of this study was to explore the anaerobic culturing of JH1^T without using pressurized hydrogen, using a variety of short chain alcohols. The results demonstrate that in the absence of hydrogen, JHI^T can utilize ethanol, 1-propanol and 1-butanol but not methanol, as a source of reducing potential for methanogenesis. The ability to use ethanol to drive methane formation in JH1^T has been used to develop a high throughput bioassay enabling screening of this methanogen against anti-methanogen compounds.

Key Words: cultivation, methanogen, *Methanobrevibacter*, rumen, hydrogen

51 Digestive tract microbiota of beef cattle that differed in feed efficiency.

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We hypothesized cattle that differed in body weight gain (BW) had different digestive tract microbiota. Sixty-six steers (age = 396 ± 1 d; BW = 456 ± 5 kg) were individually fed for 84 d. Steers received a ration that as dry matter consisted of 8.0% chopped alfalfa hay, 20% wet distillers grain with solubles, 67.75% dry-rolled corn, and 4.25% vitamin/mineral mix. The vitamin/mineral mix included 772 mg/kg monensin. The 8 steers with the greatest (2.39 ± 0.06 kg/d) and 8 steers with the least average daily gain (1.85 ± 0.06 kg/d) that were within ± 0.55 SD of the mean (11.9 ± 0.1 kg/d) dry matter intake were selected for the study. At slaughter, digesta was collected from the rumen, duodenum, jejunum, ileum, cecum, and colon. Total DNA was isolated and amplicon library preparation was performed by PCR amplification of the V1 to V3 region of the 16S rRNA gene. Libraries were sequenced with a 600-cycle V2 kit with 300bp paired-end reads on an Illumina MiSeq next-generation sequencer. Operational taxonomic units (OTU) were classified using 0.03 dissimilarity and identified using the Greengenes 16S rRNA Gene Database. Chao1, Shannon, Simpson, and InvSimpson diversity indexes and PERMANOVA between steer classifications groups did not differ ($P > 0.05$) for the rumen, duodenum, ileum, cecum, and colon. In the jejunum, there tended to be a difference ($P < 0.1$) in the Chao1 and Simpson diversity indexes between steer classifications, but there was no difference in the Shannon and InvSimpson diversity indexes. Classification groups differed ($P = 0.006$) in the PERMANOVA. The hierarchical dependence

false discovery rate procedure returned 9 clades as being differentially abundant between steer classifications ($P < 0.05$). The majority of the OTU were in the families *Coriobacteriaceae* and *Veillonellaceae*. This study suggests that intestinal differences in microbiota of ruminants may be associated with animal performance.

Key Words: cattle, feed efficiency, microbiota

Nutrition and metabolism of livestock, humans, and companion animals

52 Influence of an organic acid based feed additive on intestinal parameters of weaned pigs.

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The weaning period of piglets is often accompanied by impaired gastrointestinal physiology, which leads to decreased digestion and poor overall performance or a predisposition to diseases. Supplementation with feed additives in pig production is a reasonable solution to maintain or improve gastrointestinal functionality, which is linked to better growth and possibly a reduction in antibiotic use. A product which contains a blend of formic acid, propionic acid and a permeabilizing complex mixture (Biotronic Top forte, BIOMIN, Austria), was used to test the effect on intestinal parameters and overall performance of piglets receiving a diet containing mold count above 1×10^{6} cfu/g. A total of 60 healthy 28-d-old weaned piglets were assigned to 2 treatments. The pigs in control group A were fed with a basal diet without any supplementation, while group B was supplemented with an organic acid based feed additive in a dosage of 1.85 kg/ton. The data of digestive enzymes activities, pH values in gastrointestinal content and growth performance were statistically analyzed using the general linear model and *t*-test. Poisson regression and proportions test were used to statistically analyze the diarrhea score data and diarrhea rate data. Administration of the feed additive had a significant effect on the reduction of gastric pH ($P < 0.05$). The pH of digesta in duodenum, jejunum, ileum, and colon were improved numerically. The activities of pepsin in gastric digesta and pancreatic enzymes (amylase, lipase, and trypsin) in jejunal digesta were increased by the dietary treatment ($P < 0.05$). Diarrhea rate and diarrhea score were decreased ($P < 0.01$) in the group receiving the organic acids based feed additive in comparison to the control group, which indicate enhanced intestinal health. Growth performance of piglets receiving the acidifier in a diet was numerically improved with a statistical trend ($P < 0.1$). In conclusion, the administration of an organic acid based feed additive improved intestinal health and digestion leading to better growth performance of piglets.

Key Words: formic acid, propionic acid, digesta pH, digestive enzyme, diarrhea rate

53 Impact of sub-therapeutic Carbadox feeding on growth performance and the fecal microbial population of newly weaned swine.

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Antibiotics have been widely used in human and animal medicine to prevent diseases but also to improve growth performance, and has made animal-based protein more affordable. Concerns about the incidence of antimicrobial resistance has led to American retailers and consumers choosing “antibiotic-free” meat. Because we currently do not understand how antibiotics improve animal growth efficiency, this study was designed to determine the impact of feeding an antibiotic (Carbadox) to newly weaned (21 d of age) pigs (n = 48 pigs in 12 pens). Pigs

were divided on d 0 into 2 treatments (control and Carbadox fed at 50 g/ton d 0–14, and 25 g/ton d 28–42). Pigs were randomly allocated to 6 pens per treatment, and were fed through 42 d. Feces were collected from each pen on d 14, 28, and 42; and fecal microbial DNA was extracted and sequenced to determine microbiome populations. Carbadox treatment tended to improve ($P < 0.1$) average daily gain from d 14–21 (148 vs. 215 g/d). Feed intake remained similar between groups ($P > 0.1$), but was numerically higher in Carbadox-treated pigs at all time points. Carbadox did increase ($P < 0.06$) gain to feed performance from d 21–63 of age compared with untreated control pigs (0.708 vs. 0.666 kg gain/kg feed, respectively). The number of OTU's were higher (1473 vs 1183; $P < 0.05$) in control pigs compared with Carbadox pigs across all time points. Microbial α-diversity as measured by Chao1 was greater ($P < 0.1$) in control than in Carbadox-treated pigs at 28 d of age, however, this difference disappeared by d 42 ($P > 0.31$). Collectively, Carbadox treatment did not significantly affect pig growth or Body weight though Carbadox pigs were 2.5 kg heavier than controls (d 42 weights, 23.29 vs. 25.75 kg, respectively) at the end of the study, a 12% improvement in growth. Additionally, although the consumption of Carbadox significantly affected all of the microbial α-diversity metrics at d 28, such differences were gone by d 42, indicating an adaptation of the fecal microbiota after a few weeks of exposure to antibiotic, which suggests that these effects can potentially be mimicked by the use of natural compounds or pre- and probiotics.

Key Words: antibiotic, microbiome, pig

54 Effect of camphor concentrations on caprine *in vitro* mixed ruminal microorganism fermentations.

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The ruminal microbial population allows ruminant animals to utilize low quality forages, often by detoxifying phytochemicals, such as monoterpenes. For more than 15 years, 2 divergent bloodlines of meat goats have been selected for the ability to consume high or low levels of *Juniper* sp., which contains several potent monoterpenes, including camphor. The mechanism by which these goats are able to consume high levels of *Juniper* is unclear; therefore, this study was designed to determine if differences existed between the ruminal microbial populations of the low- and high-juniper-consuming bloodlines (LJC vs. HJC) by measuring fermentation end products. LJC and HJC goats were fed either a juniper-free or juniper enhanced diet in a 2 × 2 factorial pre-collection experimental design. Mixed ruminal microorganisms were collected via esophageal tube from LJC and HJC fed goats and were inoculated (33% vol/vol) in anoxic media in sealed Balch tubes containing camphor final concentrations of: 0, 0.25, 0.5, 0.99, 1.97, and 5.91 m*M*. Camphor concentrations of 1.97 and 5.91 m*M* increased ($P < 0.01$) propionate production, total volatile fatty acids, and decreased acetate:propionate ratios between goat bloodlines and diets. Propionate production increased as camphor concentrations were increased ($P < 0.05$) compared with the controls across all treatment groups. The HJC bloodline produced significantly more propionate ($P < 0.01$) at 5.91 m*M* camphor than did the LJC bloodline. Goats fed juniper-free diets produced more ($P < 0.01$) acetate, propionate, and butyrate ($P < 0.01$) than did juniper-enhanced fed goats. Overall, camphor addition altered end products of the mixed caprine ruminal microorganism fermentation *in vitro* and resulted in increased total VFA and propionate production, and decreased the acetate:propionate ratio. Results suggest that high levels of camphor more readily alter microbial fermentation by increasing volatile fatty acid production and increasing propionate, which suggests more gluconeogenesis potential in the liver of goats.

Key Words: camphor, microbial fermentation, volatile fatty acids, goat

55 Microbiome effects on metabolic efficiencies in easy and hard keeper horses.

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Horses with 2 classically differing metabolic tendencies are referred to as “easy keepers” (EK) or “hard keepers” (HK). These tendencies are often related to the Henneke Body Condition Scoring system where an EK easily maintains a BCS ≥ 6 and HK is a ≤ 4 and struggles to maintain a BCS of 5. A third classification is the “medium keepers” (MK), who can easily maintain a BCS = 5. The goal of this study is to determine how the microbiome contributes to these metabolic tendencies by identifying differentially abundant bacteria in each group and inferring functional differences. Samples of fecal matter from 97 horses (63 EK, 22 MK, and 12 HK) underwent microbiome profiling via 16S rRNA gene sequencing; the keeper status was self-reported by equine owners. No significant differences were seen in bacterial α and β diversities between keepers. Keeper status significantly correlated with differing microbial compositions with 11 genera and 5 pathways across all groups and 12 genera and 162 pathways detected between EK and HK. Differing genera found between EK and HK included *Solibacillus*, *Acinetobacter*, and *Akkermansia*. Reduced abundances of bacilli (lactic acid producers), *Gammaproteobacteria* and *Verrucomicrobiae* (utilizers of amino acids and secondary metabolites) in HK suggests that these animals are less efficient at accessing nutrients in the hindgut. KEGG functions were inferred using PICRUSt, and significant differences were determined at *P-adj* < 0.05 using negative binomial distribution in DeSeq2. Significant KEGG IDs were then mapped to KEGG pathways using KEGG mapper. Across all groups, KOs associated with starch and sucrose metabolism were most differen; however, metabolism of specific amino acids, secondary metabolites, and key transporter groups were distinct between keeper groups. HK contained the least bacterial and KO ID abundances, followed by EK and MK respectively. Based on these data, we hypothesize that MK are the most efficient at nutrient digestion and host absorption, and that reduced bacterial abundance and functionality in HK leads to insufficient nutrient levels to support a healthy microbiome and maintain horse condition.

Key Words: equine, nutrition, microbiome, gut, metabolism

56 Altering the ruminal microbiota in dairy calves using rumen contents dosing.

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A major goal in dairy research is to improve milk production efficiency (MPE). With the advent of next-generation sequencing and its use in characterizing microbial communities, efforts are underway to improve MPE by manipulating the rumen microbiota. Recent work has demonstrated that MPE is correlated with ruminal bacterial community composition (BCC). Moreover, the adult cow rumen microbiota is highly stable and returns to a baseline community structure even after extreme perturbation. We seek to influence the rumen microbiota by early intervention in pre-weaning dairy calves. Two cannulated Holstein donors of known and disparate MPE were selected. Three cohorts of 6 bull calves were established and dosed by gavage with a rumen inoculum sourced from the high-efficiency donor (HE), the low-efficiency donor (LE), or an autoclaved 50:50 as a microbe-free control (C). Dosing occurred within 3 d of birth, then every 2 weeks through 6 weeks of age. Feces were collected at each dosing as a proxy for gut BCC. Daily dry matter intake of calf starter (kg), which has been shown to predict downstream feed efficiency, was greatest in HE calves and lowest in C calves ($P < 0.05$). Calves were killed at 8 weeks to access rumen contents and rumen wall sections were collected to assess papillation. Fecal and rumen samples were subjected to 16S rRNA amplicon sequencing. We found that BCC differed by cohort in fecal and rumen samples ($P < 0.05$), with HE calf samples most similar to adult rumen samples and C calves least similar. Additionally, HE calves tended to have elongated papillae ($P = 0.06$), the development of which is

dependent on byproducts of microbial metabolism in the rumen and the long-term impact of which points to differences in absorptive capacity of the ruminal epithelium. These data demonstrate that the microbiota can be influenced by early intervention, providing a compelling avenue for future development of whole-rumen based probiotics to the end of MPE improvement.

Key Words: rumen, calf, dairy, microbiota, 16S rRNA

57 Characterization of the epimural microbiota across four geographical locations within the rumen of dairy cows.

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Ruminants such as dairy cattle are important agricultural animals. A key feature of their biology is the rumen, which houses an essential community of symbiotic microbes important for providing host nutrition and modulating tissue development. These microorganisms are known to display pronounced heterogeneity. Although much attention has been paid to the spatial variation that exists for the luminal microbiota, the epithelial (epimural) community is less well understood. The epimural tissue serves an important role as the interface between host animal and its symbiotic community. It is across this boundary that much of the nutrient exchange and microbially driven tissue differentiation takes place. To date, studies characterizing the communities associated with rumen epithelium have examined only one tissue location. Here, we characterize the bacterial community and structure associated with the rumen epimural tissue of lactating Holstein dairy cows using the Illumina MiSeq. Four different locations in the rumen were sampled via epimural tissue biopsy: the cranial sac (CS), the ventral sac (VS), the caudodorsal blind sac (CDBS), and the caudoventral blind sac (CVBS). The CDBS location was found to have the highest average species richness, while the CVBS had the lowest ($P < 0.05$). We also found significant differences in epimural bacterial community structure and composition between rumen locations ($P < 0.01$). Epimural community composition at all locations was characterized by high abundances of phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. The family *Campylobacteraceae* dropped in relative abundance in the CDBS ($P < 0.05$). Conversely, the genus *Prevotella* increased in the CDBS ($P < 0.05$). This work demonstrates that spatial differences exists in the epimural community, which may have important effects on energy harvest and animal health given the different functions of epithelial locations. To the best of our knowledge, this work represents the first characterization of the ruminal epimural microbiota across different epithelial locations for any bovine ruminant.

Key Words: rumen, epimural, microbiota, 16S rRNA

Prebiotics, probiotics, and DFM development

58 Effect of inoculants of lactic acid bacteria on the fermentation quality of ryegrass at different dry matter content.

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The experiment was to determine the fermentation quality of fresh-ryegrass (FR; 243.04 g dry matter (DM) /kg wet basis), rain-treated-ryegrass (RTR; 232.35 g DM/kg wet basis) and wilted-ryegrass (WR; 716.96 g DM/ kg wet basis) after being treated with lactic acid bacteria (LAB) at concentrations of 1×10^5, 5×10^5 and 1×10^6 cfu/ g wet basis. The treatments were no LAB addition (control), *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* (SP13), *Lc. lactis* ssp. *lactis* (SP47), *Lc. lactis* ssp. *lactis* (SL242), *Lactobacillus plantarum* (BG 112), and

L. plantarum (14D). After inoculated with LAB, grass was ensiled in plastic bags in triplicate for each treatment. The bags were sealed with a vacuum sealer and stored indoors for 60 d at room temperature. The crude protein (CP, 98.37 g/kg DM) and neutral detergent fiber (NDF, 610.56 g/kg DM) were similar among the 3 substrates. The reducing sugar content were 60.29 g/kg DM in RTR, 90.54 g/kg DM in FR and 83.49 g/kg DM in WR. After 60 d ensiling, the results showed the dry matter (DM) loss (%) during ensiling was reduced (P = 0.003) in the FR and RTR LAB treated samples. The DM loss was lowest when *L. plantarum* 14D was inoculated into FR and RTR at 1 × 10^6 cfu/g wet basis. On the contrary, the DM loss was higher in the WR LAB-treated samples, when compared with the control. The NH_3-N (g/kg DM) contents of FR and RTR decreased (P < 0.001) when LAB were inoculated. Lactic acid content (g/kg DM) was increased (P = 0.010) in FR and WR samples after treatment with 1 × 10^6 cfu/g wet basis LAB, while the lactic acid content of RTR was increased only when *L. plantarum* 14D or SL242 were used. Treatment with LAB reduced (P < 0.001) the pH value of FR, RTR and WR. The lowest pH value was recorded when *L. plantarum* 14D was inoculated at 1 × 10^6 cfu/g wet basis. The results demonstrated that the use of LAB improves the fermentation quality of FR and RTR. *L. plantarum* 14D at 1 × 10^6 cfu/g wet basis is recommended to improve fermentation quality of FR and RTR.

Key Words: lactic acid bacteria (LAB), ryegrass, silage, fermentation quality

59 Investigating the effects of direct-fed microbials on beef cattle during an acidosis challenge.

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Ruminal acidosis is a prevalent and costly metabolic disorder in beef feedlot cattle characterized by low rumen pH, which can lead to a multitude of health problems and poor animal performance. Direct-fed microbials (DFM) are live, naturally occurring microorganisms that can be used to improve rumen fermentation and alleviate nutritional disorders. The objective of this study was to investigate the effect of 2 DFM strategies on beef cattle during an acidosis challenge model. Eighteen ruminally cannulated steers (BW = 724 ± 44) were used in a randomized block design with 6 steers in each block. Two steers in each block were assigned to one of 3 treatments: negative control (NCON), DFM1, and DFM2 with both DFM treatments containing *Megasphaera elsdenii*. DFM1 (10^8 to 10^{10} cfu/mL) was dosed on d 1, and DFM2 (10^{10} cfu/mL) was cultured daily and dosed on d 1 to 15. Blocks lasted 18 d and steers were fed the basal diet (45% forage) ad libitum on d 1 to 7. Steers were fasted for 24 h on d 8 and were fed the challenge diet (10% forage) ad libitum on d 9 to 15, with an additional 3 d on the basal diet for recovery. Ruminal pH was continuously measured on d 7 to 18 using an indwelling pH probe. Rumen fluid samples were collected before, during and after the acidosis induction. Data were analyzed as repeated measures using the MIXED procedure of SAS 9.4. Dry matter intake was not affected (P = 0.76) by treatment. However, a treatment × time effect (P = 0.04) was observed for ruminal pH on d 9, the initial day of the acidosis challenge. Administration of DFM2 resulted in greater (P < 0.05) ruminal pH at 17, 18, 19, and 24 h after feeding on d 9 compared with NCON and DFM1. Ruminal ammonia concentrations were not affected (P = 0.47) by treatment. Overall, results indicate the microbial treatment used in DFM2 may decrease the risk of acidosis in feedlot cattle.

Key Words: direct-fed microbial, rumen, acidosis

NOTES

Author Index

The author index is created directly and automatically from the submitted abstracts. If an author's name is typed differently on multiple abstracts, the entries in this index will reflect those discrepancies. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies. Numbers following names refer to abstract numbers.

Key Word Index

Numbers following key words refer to abstract numbers.

NOTES