2024 CONGRESS ON GASTROINTESTINAL FUNCTION

2024 CONGRESS ON GASTROINTESTINAL FUNCTION
APRIL 8–10

SCIENTIFIC PROGRAM AND ABSTRACTS

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CONTENTS

Monday, April 8

All times listed in central daylight time (CDT)

Special Session: Maximizing the impact of next generation approaches

Chair: Phil Pope, Centre for Microbiome Research—QUT, Australia
Beckman Institute Room 1025 (Beckman Auditorium)
08:45 – 13:30

There is currently rapid progressions in third-gen molecular technologies as well as high-throughput cultivation-based approaches, but many long-standing knowledge gaps in gut microbiology still elude us. This session will bring forward case studies and discussions regarding how new tech can be better coupled to meaningful experimental frameworks that are guided by efforts to overcome technical shortcomings and answer both new- and old-school biological mysteries.

08:45 **Introduction**
Phil Pope, *Centre for Microbiome Research—QUT, Australia*.

09:00 1 ***Invited talk:*** **Intertwining plasmids, microbial interactions and adaptations to gut environments.**
I. Mizrahi*, *The Department of Life Sciences & The School of Sustainability & Climate Change, Ben-Gurion University of the Negev*, *Beer-Sheva, Israel*.

09:45 2 ***Invited talk:*** **Contributions of dietary fiber and mucin-degrading bacteria to inflammatory bowel disease.**
E. Martens*, *University of Michigan Medical School, USA*.

10:30 **Break**

11:00 3 ***Invited talk:*** **Microbial microproteins as mediators of microbe-microbe and microbe-host communication and warfare.**
A. Bhatt*, *Department of Medicine and Genetics, Stanford University*, *Stanford, CA, USA*.

11:45 **Discussion and Synthesis**

12:00 **Workshop Close**

12:01 **Lunch Break, Room 1005 Beckman Center**

2024 Opening Session: Invited presentations

Chair: Itzik Mizrahi, Ben Gurion University of the Negev, Israel
Beckman Institute Room 1025 (Beckman Auditorium)
13:30 – 16:00

13:30 **Welcome and Solar Eclipse (weather permitting).**
Itzik Mizrahi, *Co-Chair, University of Illinois at Urbana-Champaign, USA*.

14:15 4 ***Invited talk:*** **The yin and yang of microbiome signatures in early life.**
L. Hall*, *University Birmingham*, *Birmingham, United Kingdom*.

15:00 5 ***Invited talk:*** **Journey through time: Unraveling the evolution of the human gut microbiome.**
A. Kostic*, *Harvard Medical School*, *Boston, MA, USA*.

15:45 **Break**

Bryant Memorial Lecture

Chair: Rod Mackie, University of Illinois, USA
Beckman Institute Room 1025 (Beckman Auditorium)
16:00 – 19:00

16:00 6 ***Invited talk:*** **Cellulosome-producing bacteria from the environment to the rumen and human-gut microbiomes.**
Ed Bayer*[1,2], [1]*The Weizmann Institute of Science*, *Rehovot, Israel*, [2]*Ben-Gurion University of the Negev*, *Beersheva, Israel*.

17:00 **Mixer and informal poster session, Room 1005 Beckman Center**

Tuesday, April 9

Podium presentations: Session 1

Chair: Josh McCann, University of Illinois, USA
Beckman Institute Room 1025 (Beckman Auditorium)
08:30 – 13:30

08:30 7 ***Invited talk:*** **Metaproteomics to investigate functional diet-microbiota interactions.**
Manuel Kleiner*, *North Carolina State University*, *Raleigh, NC, USA*.

09:15 8 **Intestinal microbiota consume specific dietary proteins that escape host digestion.**
Ayesha Awan*, Alexandria Bartlett, Alfredo Blakeley-Ruiz, Tanner Richie, Casey Theriot, and Manuel Kleiner, *North Carolina State University*, *Raleigh, NC, USA*.

09:35 9 **Gut microbial production of epitestosterone driving metastatic prostate cancer.**
T. Wang*[1,2], S. Ahmad[3], K. Yovani Olivos Caicedo[4], F. Fernández[1,2], B. Binion[1,2], J. W. Lee[5], J. D. Kang[6], S. C. Harris[6], H. R. Gaskins[1,7], J. W. Erdman[7,8], S. L. Daniel[1], P. B. Hylemon[9], S. E. Ernst[10], A. Cruz-Lebrón[10], K. S. Sfanos[10], Karthik Anantharaman[11], Joao M. P. Alves[4], Joseph Irudayaraj[3], Jason M. Ridlon[1,2,7,8,9]*, [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Microbiome Metabolic Engineering Theme, Carl R. Woese Institute for Genomic Biology*, *Urbana, IL, USA*, [3]*Department of Bioengineering, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [4]*Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo*, *São Paulo, Brazil*, [5]*Department of Biotechnology, Sungshin Women's University*, *Seoul, South Korea*, [6]*Stravitz-Sanyal Institute for Liver Disease & Metabolic Health, Virginia Commonwealth University, School of Medicine*, *Richmond, VA, USA*, [7]*Division of Nutritional Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [8]*Cancer Center at Illinois, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [9]*Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine*, *Richmond, VA, USA*, [10]*Departments of Pathology, Oncology, and Urology, Johns Hopkins University School of Medicine*, *Baltimore, MD, USA*, [11]*Department of Bacteriology, University of Wisconsin–Madison*, *Madison, WI, USA*.

09:55 10 **The healthy microbiome in an unhealthy context: Chronic pouchitis.**
S. M. Dall*[1], S. J. Kousgaard[1], O. Thorlacius-Ussing[1,2], and M. Albertsen[1], [1]*Aalborg University*, *Aalborg, Denmark*, [2]*Aalborg University Hospital*, *Aalborg, Denmark*.

10:15 **Break**

10:30 11 **Exploring the link between socioeconomic environment, microbial diversity and colonization of multi-drug resistant bacteria in the human gut microbiome.**
I. Zuniga-Chaves*[1], S. Eggers[2], A. Kates[1], N. Safdar[1], G. Suen[1], and K. Malecki[3], [1]*University of Wisconsin–Madison*, *Madison, WI, USA*, [2]*University of Iowa*, *Iowa City, IA, USA*, [3]*University of Illinois Chicago*, *Chicago, IL, USA*.

10:50 12 **Gut microbiota and bile salt hydrolase activity in heavy and light broiler chickens.**
H. W. Kim*[1], N. K. Kim[1], J. S. Thompson[3], J. M. Rehberger[3], T. G. Rehberger[3], A. H. Smith[3], and R. I. Mackie[1,2], [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Carle R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [3]*Arm & Hammer Animal and Food Production*, *Waukesha, WI, USA*.

New this year at the 2024 CGIF Congress is our 2-minute Fire Poster Pitch session. During this session, presenters will captivate the audience with a 2-minute talk and direct you to their posters for deeper discussions during the dedicated poster session.

11:10 13 ***Fire Poster Pitch:*** **Utilizing the COVID-19 pandemic as an opportunity to examine the role of mass gatherings in the emergence of antimicrobial resistance: A wastewater-based surveillance from 2020 to 2022.**
Changzhi Wang*[1], Yevhen Myshkevych[1], Tiannyu Wang[1], Mohammad Monjed[2], and Pei-Ying Hong[1], [1]*King Abdullah University of Science and Technology*, *Thuwal, Makkah, Saudi Arabia*, [2]*Umm Al-Qura University*, *Makkah, Makkah, Saudi Arabia*.
This Fire Poster Pitch describes abstract 44 in the poster session.

11:12 14 ***Fire Poster Pitch:*** **Genome analysis of ruminal *Selenomonas* reveals multiple pathways for hydrogen production and utilization.**
G. T. Attwood*, L. Crouzet, P. Soni, and W. J. Kelly, *AgResearch Ltd*, *Palmerston North, New Zealand*.
This Fire Poster Pitch describes abstract 46 in the poster session.

11:14 15 ***Fire Poster Pitch:*** **Anaerobic fungal carbohydrate-binding modules possess prominent preference to assist the enzymatic hydrolysis of hemicellulose.**
Q. C. Shi[1], D. Y. Wang[1], C. Duan[1], Y. Q. Li[1], Z. Y. Sun[1], W. Y. Zhu[1], Y. F. Cheng*[1], and I. Cann[2], [1]*Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Champaign, IL, USA*.
This Fire Poster Pitch describes abstract 52 in the poster session.

11:16 16 ***Fire Poster Pitch:*** **Microbiome-mediated colonization resistance against necrotic enteritis.**
Jing Liu, Isabel Tobin, Joy Scaria, and Glenn Zhang*, *Oklahoma State University*, *Stillwater, OK, USA*.
This Fire Poster Pitch describes abstract 53 in the poster session.

11:18 17 ***Fire Poster Pitch:*** **Understanding the host–microbiome interactions involved in liver abscess formation in beef cattle.**
R. J. Gruninger*[1], E. O. O'Hara[1], Y. Wang[2], R. Zaheer[1], N. Chomistek[1], G. O. Ribeiro[3], L. L. Guan[2,4], and T. A. McAllister[1], [1]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*, [2]*Faculty of Agriculture, Life and Environmental Sciences, University of Alberta*, *Edmonton, AB, Canada*, [3]*College of Agriculture and Bioresources, University of Saskatchewan*,

Saskatoon, SK, Canada, [4]*Faculty of Land and Food Systems, University of British Columbia*, *Vancouver, BC, Canada*.
This Fire Poster Pitch describes abstract 54 in the poster session.

11:20 18 ***Fire Poster Pitch:*** **Transcriptomic insights into the anti-ruminal *Streptococcus* activity of natural compound betulin.**
M. Adachi*[1], K. Ito[1], R. Yano[1], R. Hiyama[2], K. Seki[2], D. Kondoh[1], M. Hanada[1], T. Nishida[1], and N. Fukuma[1], [1]*Obihiro University of Agriculture and Veterinary Medicine*, *Obihiro, Hokkaido, Japan*, [2]*Forest Research Department, Forest Products Research Institute, Hokkaido Research Organization*, *Asahikawa, Hokkaido, Japan*.
This Fire Poster Pitch describes abstract 58 in the poster session.

11:22 19 ***Fire Poster Pitch:*** **Hydrogenotrophic methanogens facilitate microbial energy harvesting from plant polysaccharides in breed-determined obese pigs.**
Xuan Li*[1,2], Haiqin Wu[1,2], Chunlong Mu[3], Erwin G. Zoetendal[4,2], Ruihua Huang[1,2], and Weiyun 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, Jiangsu, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [3]*Food Informatics, AgResearch, Te Ohu Rangahau Kai*, *Palmerston North, New Zealand*, [4]*Laboratory of Microbiology, Wageningen University & Research*, *Wageningen, the Netherlands*.
This Fire Poster Pitch describes abstract 59 in the poster session.

11:24 20 ***Fire Poster Pitch:*** **Effect of succinate on the metabolic activity of *Selenomonas ruminantium*.**
L. Miyazaki, Y. Suzuki, and S. Koike*, *Hokkaido University*, *Sapporo, Hokkaido, Japan*.
This Fire Poster Pitch describes abstract 61 in the poster session.

11:26 21 ***Fire Poster Pitch:*** **Characterization of a dual steroid 3β-, 17β-oxidoreductase in the gut bacterium *Eggerthella lenta*.**
Briawna Binion* and Jason Ridlon, *University of Illinois Urbana-Champaign*, *Urbana, IL, USA*.
This Fire Poster Pitch describes abstract 63 in the poster session.

11:28 22 ***Fire Poster Pitch:*** **Optimizing the recovery of prokaryote metagenome-assembled genomes (MAGs) from the challenging cow rumen microbiome.**
R. D. Wollenberg*[1], E. Aa. Sørensen[1], P. B. Pope[2,3], C. M. Koble[3], and M. T. Søndergaard[1], [1]*DNASense*, *Aalborg, Denmark*, [2]*Center for Microbiome Research, Queensland University of Technology*, *Woolloongabba, QLD, Australia*, [3]*Norwegian University of Life Sciences*, *Ås, Norway*.
This Fire Poster Pitch describes abstract 64 in the poster session.

11:30 23 ***Fire Poster Pitch:*** **Methanogenesis inhibition stimulates acetogenesis by novel microbes in ruminants.**
Gaofeng Ni*[1], Nicola Walker[2], André Fischer[2], René Stemmler[2], Rhys Grinter[1,3], Mick Watson[4], Emiel Ver Loren van Themaat[2], Maik Kindermann[2], and Chris Greening[1], [1]*Department of Microbiology, Biomedicine Discovery Institute, Monash University*, *Melbroune, Victoria, Australia*, [2]*DSM Nutritional Products, Animal Nutrition and Health*, *Kaiseraugst, Switzerland*, [3]*Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne*, *Melbourne, Victoria, Australia*, [4]*Centre for Digital Innovation, DSM Biotechnology Center*, *Delft, the Netherlands*.
This Fire Poster Pitch describes abstract 69 in the poster session.

11:32 24 ***Fire Poster Pitch:*** **Extra-chromosomal elements encode essential functions in rumen *Butyrivibrio fibrisolvens* cultures.**
W. J. Kelly*[1], N. Palevich[1], F. P. Palevich[2], and G. T. Attwood[1], [1]*AgResearch Ltd., Grasslands Research Centre*, *Palmerston North, New Zealand*, [2]*AgResearch Ltd., Hopkirk Research Institute*, *Palmerston North, New Zealand*.
This Fire Poster Pitch describes abstract 70 in the poster session.

11:34 25 ***Fire Poster Pitch:*** **Lactate utilization in anaerobic rumen bacteria.**
W. J. Kelly* and G. T. Attwood, *AgResearch Ltd., Grasslands Research Centre*, *Palmerston North, New Zealand*.
This Fire Poster Pitch describes abstract 71 in the poster session.

11:36 26 ***Fire Poster Pitch:*** **Effects on monensin on growth and nitrate/nitrite metabolism of a hypernitrite-metabolizing *Paenibacillus*.**
Robin C. Anderson*[1], Gordon E. Carstens[2], and William E. Pinchak[3], [1]*United States Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit*, *College Station, TX, USA*, [2]*Department of Animal Science, Texas A&M University*, *College Station, TX, USA*, [3]*Texas AgriLife Research*, *Vernon, TX, USA*.
This Fire Poster Pitch describes abstract 78 in the poster session.

11:38 27 ***Fire Poster Pitch:*** **Native rumen-derived probiotics alter the rumen microbiome and improve production and feed efficiency when fed to lactating dairy cows.**
B. Anderson*[1], C. Marotz[1], I. Farag[1], C. Martino[1], J. Lefler[1], A. Washburne[2], K. Calapa[1], J. Drackley[3], T. Overton[4], M. Vandehaar[5], J. Santos[6], J. Osorio[7], E. Uddin[7], A. Lago[8], M. Embree[1], [1]*Native Microbials*, *San Diego, CA, USA*, [2]*Selva Analytics*, *Bozeman, MT, USA*, [3]*University of Illinois Urbana-Champaign*, *Urbana, IL, USA*, [4]*Cornell University*, *Ithaca, NY, USA*, [5]*Michigan State University*, *East Lansing, MI, USA*, [6]*University of Florida*, *Gainesville, FL, USA*, [7]*South Dakota State University*, *Brookings, SD, USA*, [8]*DairyExperts*, *Tulare, CA, USA*.
This Fire Poster Pitch describes abstract 79 in the poster session.

11:40 **Break**

12:00 **Business Meeting: CGIF 2022 (open to all registrants)**

12:00 **Lunch, Beckman Institute Room 1005**

Podium presentations: Session 2

Chair: Rod Mackie, University of Illinois, USA
Beckman Institute Room 1025 (Beckman Auditorium)
13:30 – 16:00

13:30 28 **Holo-omic network analysis reveals bistability in the rumen microbiome.**
Carl M. Kobel*[1], Velma T. E. Aho[1], Arturo V. P. de Leon[1], Ove Øyås[1], Laura Nicoll[2], Andy Leu[3], Gene W. Tyson[3], Simon J. McIlroy[3], Ianina Altshuler[4], Torgeir R. Hvidsten[1], Rainer Roehe[2], and Phil B. Pope[1,3], [1]*Microbial Ecology and Meta-Omics group (MEMO), Norwegian University of Life Sciences*, *Ås, Norway*, [2]*Scotland's Rural College*, *Edinburgh, United Kingdom*, [3]*Centre for Microbiome Research (CMR), Queensland University of Technology*, *Woolloongabba, Australia*, [4]*Microbiome Adaptation to the Changing Environment laboratory (MACE), École Polytechnique Fédérale de Lausanne*, *Sion, Switzerland*.

13:50 29 **Exploring the influence of plant fiber on gut microbiome diversity.**
S. Winkler*, *Department of Life Sciences and School of Sustainability & Climate Change, Ben-Gurion University of the Negev*, *Beer-Sheva, Israel*.

14:10	30	**Unveiling rumen microbial responses to seaweed supplementation for sustainable livestock nutrition and methane mitigation.** A. Ferrillo[1], J. P. Tingley[2,3], A. Kidane[1], P. B. Pope[1,4], D. W. Abbott[3], and L. Heldal Hagen*[1], [1]*Norwegian University of Life Sciences (NMBU)*, *Ås, Norway*, [2]*University of Lethbridge*, *Lethbridge, AB, Canada*, [3]*Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*, [4]*Centre for Microbiome Research (CMR), Queensland University of Technology*, *Woolloongabba, Australia*.
14:30	31	**Metagenome analysis revealed supplementation with 3-nitrooxypropanol affected rumen methanogenesis and hydrogen metabolism in beef cattle.** Y. Choi*[1], M. Zhou[1], A. Romero-Pérez[2], K. A. Beauchemin[3], M. Kindermann[4], and L. L. Guan[1,5], [1]*University of Alberta*, *Edmonton, Alberta, Canada*, [2]*National Autonomous University of Mexico*, *Mexico City, Mexico*, [3]*Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre*, *Lethbridge, Alberta, Canada*, [4]*DSM*, *Basel, Switzerland*, [5]*University of British Columbia*, *Vancouver, British Columbia, Canada*.
14:50	32	**Extracellular electron transfer in the rumen ecosystem is stimulated by conductive materials.** A. Ortiz-Chura, M. Popova, and D. P. Morgavi*, *Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores*, *Saint-Genes-Champanelle, France*.
15:10 PM	33	**Emission profiles and responses to inhibitor supplements in beef grazing systems.** S. Denman*, G. Fernandez, and C. McSweeney, *CSIRO Agriculture and Food*, *Brisbane, Australia*.
15:30 PM		**Break**

Poster session
Beckman Institute Room 1005
16:00 PM – 17:00 PM

Social function
Riggs Brewery
17:30 PM – 22:30 PM

Wednesday, April 10

Podium presentations: Session 3
Chair: Isaac Cann, University of Illinois, USA
NCSA Building Room 1122 (NCSA Auditorium)
09:00 – 13:00

09:00	34	***Invited talk:* A census of genomes from the rumen: Charting new frontiers beyond Hungate1000.** R. Seshadri*, *Department of Energy Joint Genome Institute*, *Berkeley, CA, USA*.

09:45 35 **Holistic multidisciplinary analysis of antimicrobial resistance in neonatal calves and dairy farm environments.**
K. Lawther*[1], F. Godoy Santos[1], L. B. Oyama[1], G. Scoley[2], N. Dimonaco[1], A. J. Brown[2], C. J. Creevey[1], S. Morrison[2], and S. A. Huws[1], [1]*Queen's University Belfast*, *Belfast, Antrim, Northern Ireland*, [2]*Agri-Food and Biosciences Institute*, *Hillsborough, Down, Northern Ireland*.

10:05 36 **Peptidase distribution among rumen ciliates: A bioinformatic perspective on lysosomal enzyme profiles.**
Sripoorna Somasundaram*, Ming Yan, and Zhongtang Yu, *The Ohio State University*, *Columbus, OH, USA*.

10:25 **Break**

11:00 37 **Interrogating the diversity and ecological importance of viral dark matter in the rumen ecosystem.**
M. Yan*[1], A. A. Pratama[1], S. Somasundaram[1], Z. Li[2], Y. Jiang[2], M. B. Sullivan[1], and Z. Yu[1], [1]*The Ohio State University*, *Columbus, OH, USA*, [2]*Northwest A&F University*, *Yangling, Shanxi, China*.

11:20 38 **Montana's wild ruminants are protected from a toxic dietary alkaloid by rumen-located fungi.**
S. G. Grace[1], A. Simmons[1], M. Elsahed[2], C. Carr[1], J. L. C. Borgogna[1], B. Bothner[1], L. McNew[1], and C. J. Yeoman*[1], [1]*Montana State University*, *Bozeman, MT, USA*, [2]*Oklahoma State University*, *Stillwater, OK, USA*.

11:40 39 **Using color as an indicator of the effectiveness of buccal swabs as a proxy for direct ruminal sampling of the rumen bacterial community in Holstein dairy cows.**
J. H. Skarlupka*[1,2], M. S. Cox[2,3], A. J. Steinberger[1,2], D. Sbardellati[2,4], J. McClure[5], D. Bickhart[5,6], A. Scheftgen[2], E. Paget[2], C. Skadron[2], N. Attipetty[2], and G. Suen[2], [1]*Microbiology Doctoral Training Program*, *Madison, WI, USA*, [2]*Department of Bacteriology*, *Madison, WI, USA*, [3]*University of Wasington*, *Seattle, WA, USA*, [4]*University of California–Davis*, *Davis, CA, USA*, [5]*USDA Dairy Forage Research Center*, *Madison, WI, USA*, [6]*Hendrix Genetics*, *Boxmeer, the Netherlands*.

12:00 **Lunch, NCSA Atrium**

Podium presentations: Session 4

Chair: Todd R. Callaway, University of Georgia
NCSA Building Room 1122 (NCSA Auditorium)
13:00 – 14:35

13:00 40 **Complexities and simplicities highlighted by systematic evaluation and improvement of partial gene predictions on unassembled reads.**
NJ Dimonaco*[1,2], A. Clare[3], and M. Surette[1], [1]*McMaster University*, *Hamilton, ON, Canada*, [2]*Queen's University Belfast*, *Belfast, Northern Ireland, UK*, [3]*Aberystwyth University*, *Aberystwyth, Wales, UK*.

13:20 41 ***Lessons learnt in microbiome intervention strategies*.**
P. B. Pope*[1,2], T. O. Anderson[2], C. M. Kobel[2], V. T. E. Aho[2], O. Øyås[2], T. R. Hvidsten[2], A. V. P. Leon[2], M. Ø. Arntzen[2], L. H. Hagen[2], S. McIlroy[1], G. W. Tyson[1], and S. L. La Rosa[2], [1]*Centre for Microbiome Research, Queensland University of Technology*, *Brisbane, Australia*, [2]*Norwegian University of Life Sciences*, *Ås, Norway*.

13:40	42	**The use of fast protein in diets of weaned pigs improves total gain.** J. van Leeuwen, M. Bible*, and S. Husballe-Rasmussen, *Hamelt Protein A/S*, *Horsens, Denmark*.
14:00	43	**The effect of a *Bacillus*-based probiotic on the ruminal microbiota and milk measurements in lactating cows.** J. M. Rehberger*[1], M. N. de Jesus[1], J. S. Thompson[1], E. T. McKinley[1], L. J. Spicer[2], and A. H. Smith[1], [1]*Arm & Hammer Animal and Food Production, ScienceHearted Center*, *Waukesha, WI, USA*, [2]*Department of Animal and Food Sciences, Oklahoma State University*, *Stillwater, OK, USA*.
14:20		**Presentation of Russell Awards** Best oral presentations by graduate students and young investigators.
14:35		**Closing remarks and Invitation to CGIF 2026**

POSTER PRESENTATIONS

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

Beckman Institute Room 1005 (with overflow to Room 5602)

44 **Utilizing the COVID-19 pandemic as an opportunity to examine the role of mass gatherings in the emergence of antimicrobial resistance: A wastewater-based surveillance from 2020 to 2022.**
Changzhi Wang*[1], Yevhen Myshkevych[1], Tiannyu Wang[1], Mohammad Monjed[2], and Pei-Ying Hong[1], [1]*King Abdullah University of Science and Technology*, *Thuwal, Makkah, Saudi Arabia*, [2]*Umm Al-Qura University*, *Makkah, Makkah, Saudi Arabia*.

45 **Evaluating agriculture byproducts as potential feed additives for reducing enteric methane emissions.**
P. Romero*, R. Duong, and M. Hess, *University of California Davis*, *Davis, CA, USA*.

46 **Genome analysis of ruminal *Selenomonas* reveals multiple pathways for hydrogen production and utilization.**
G. T. Attwood*, L. Crouzet, P. Soni, and W. J. Kelly, *AgResearch Ltd*, *Palmerston North, New Zealand*.

47 **Prevalence and characteristics of ESBL-producing *Escherichia coli* in clinically healthy pigs: Implications for antibiotic resistance spread in livestock.**
Ruzivia Oliveira[1], Juliana Silva[1], Giarla Silva[1], Jessica Rosa[1], Denise Bazzolli[1], and Hilario Mantovani*[1,2], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*University of Wisconsin–Madison*, *Madison, WI, USA*.

48 **Inhibition of methanogenesis during anaerobic digestion: Effects on short-chain fatty acid production and proportions.**
H. W. Kim*[1], A. Lokhandwala[1], X. Su[2], W. Oh[2], R. D. Cusick[3], P. Liu[4], and R. I. Mackie[1], [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [3]*Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [4]*Shell Exploration and Production Inc., Westhollow Technology Center*, *Houston, TX, USA*.

49 **Assessing the correlations between ruminal methanogenic populations and methane traits: A meta-analysis.**
Arlan Rodrigues[1], Igor Ferreira[2], Alice Assumpcao[3], Fiorella Umana[3], and Hilario Mantovani*[3], [1]*Universidade Federal da Paraiba*, *Areia, PB, Brazil*, [2]*Sao Paulo State University*, *Sao Paulo, Brazil*, [3]*University of Wisconsin–Madison*, *Madison, WI, USA*.

Immunology (including host-microbe interactions)

Beckman Institute Room 1005 (with overflow to Room 5602)

50 **Breed-driven microbiome heterogeneity regulates intestinal stem cell proliferation via *Lactobacillus*-lactate-GPR81 signaling.**
H. Q. Wu*[1,2], X. Li[1,2], C. L. Mu[3], W. L. Fan[1,2], L. Shen[4], and W. Y. 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, Jiangsu, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [3]*Food Informatics, AgResearch, Te Ohu Rangahau Kai*, *Palmerston North, New Zealand*, [4]*Department of Surgery, University of Chicago*, *Chicago, IL, USA*.

51 **Psychological stress-induced changes to gut epithelial transcriptomic profile parallel shifts in the microbiome through a mechanism involving β-adrenergic receptors.**
M. Caetano-Silva*[1], I. Valishev[1], C. Lim[1], A. Shrestha[1], M. Webb[1], C. H. Lin[1], M. Bailey[2], and J. Allen[1], [1]*University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Nationwide Children's Hospital*, *Columbus, OH, USA*.

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

Beckman Institute Room 1005 (with overflow to Room 5602)

52 **Anaerobic fungal carbohydrate-binding modules possess prominent preference to assist the enzymatic hydrolysis of hemicellulose.**
Q. C. Shi[1], D. Y. Wang[1], C. Duan[1], Y. Q. Li[1], Z. Y. Sun[1], W. Y. Zhu[1], Y. F. Cheng*[1], and I. Cann[2], [1]*Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign*, *Champaign, IL, USA*.

53 **Microbiome-mediated colonization resistance against necrotic enteritis.**
Jing Liu, Isabel Tobin, Joy Scaria, and Glenn Zhang*, *Oklahoma State University*, *Stillwater, OK, USA*.

54 **Understanding the host–microbiome interactions involved in liver abscess formation in beef cattle.**
R. J. Gruninger*[1], E. O. O'Hara[1], Y. Wang[2], R. Zaheer[1], N. Chomistek[1], G. O. Ribeiro[3], L. L. Guan[2,4], and T. A. McAllister[1], [1]*Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*, [2]*Faculty of Agriculture, Life and Environmental Sciences, University of Alberta*, *Edmonton, AB, Canada*, [3]*College of Agriculture and Bioresources, University of Saskatchewan*, *Saskatoon, SK, Canada*, [4]*Faculty of Land and Food Systems, University of British Columbia*, *Vancouver, BC, Canada*.

55 **Bioinformatic prediction of potential ncRNAs in *Escherichia coli* O157:H7.**
S. Bialobzyski*[1], M. Zhou[2], T. McAllister[3], and L. Guan[1], [1]*University of British Columbia*, *Vancouver, BC, Canada*, [2]*University of Alberta*, *Edmonton, AB, Canada*, [3]*Agriculture and Agri-Food Canada*, *Lethbridge, AB, Canada*.

56 **Conversion of plant polysaccharides to propionate by anaerobic (gut) *Bacteroidota*.**
S. E. Kurrer*[1], C. Döring[1], A. Poehlein[2], J. M. Schwarzbauer[1], and M. Basen[1], [1]*University of Rostock*, *Rostock, Germany*, [2]*Georg-August-University Göttingen*, *Göttingen, Germany*.

57 **Investigating the distinguished gut bacteria for pregnant and long-lived sows in the early breeding stage and their variation through 4 parities.**
Ziyu Liu*, Tsungcheng Tsai, Charles V. Maxwell, and Jiangchao Zhao, *Department of Animal Science, University of Arkansas*, *Fayetteville, AR, USA*.

58 **Transcriptomic insights into the anti-ruminal *Streptococcus* activity of natural compound betulin.**
M. Adachi*[1], K. Ito[1], R. Yano[1], R. Hiyama[2], K. Seki[2], D. Kondoh[1], M. Hanada[1], T. Nishida[1], and N. Fukuma[1], [1]*Obihiro University of Agriculture and Veterinary Medicine*, *Obihiro, Hokkaido, Japan*, [2]*Forest Research Department, Forest Products Research Institute, Hokkaido Research Organization*, *Asahikawa, Hokkaido, Japan*.

59 **Hydrogenotrophic methanogens facilitate microbial energy harvesting from plant polysaccharides in breed-determined obese pigs.**
Xuan Li*[1,2], Haiqin Wu[1,2], Chunlong Mu[3], Erwin G. Zoetendal[2,4], Ruihua Huang[1,2], and Weiyun 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, Jiangsu, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu, China*, [3]*Food Informatics, AgResearch, Te Ohu Rangahau Kai*, *Palmerston North, New Zealand*, [4]*Laboratory of Microbiology, Wageningen University & Research*, *Wageningen, the Netherlands*.

60 **Fibrolytic function of the horse fecal microbiome: Elderly versus adult healthy individuals.**
M. Baraille*[1,2], M. Buttet[2], V. Milojevic[3], S. Julliand[2], and V. Julliand[1], [1]*Institut Agro Dijon, Univ. Bourgogne Franche–Comté, PAM UMR A 02.102, 21000*, *Dijon, France*, [2]*Lab To Field, 21000*, *Dijon, France*, [3]*Sandgrue Foundation, 8132 Egg, Zürich, Switzerland*.

61 **Defining the post-evisceration bovine gastrointestinal tract microbiome after lairage at a USDA processing facility.**
M. K. Costello*[1], J. C. McClure[2], J. A. Brown[1], H. C. Mantovani[1], and S. C. Ricke[1], [1]*University of Wisconsin–Madison*, *Madison, WI, USA*, [2]*USDA-ARS Dairy Forage Research Center*, *Madison, WI, USA*.

62 **Effect succinate on the metabolic activity of *Selenomonas ruminantium*.**
L. Miyazaki, Y. Suzuki, and S. Koike*, *Hokkaido University*, *Sapporo, Hokkaido, Japan*.

63 **Characterization of a dual steroid 3β-, 17β-oxidoreductase in the gut bacterium *Eggerthella lenta*.**
Briawna Binion* and Jason Ridlon, *University of Illinois Urbana-Champaign*, *Urbana, IL, USA*.

64 **Optimizing the recovery of prokaryote metagenome-assembled genomes (MAGs) from the challenging cow rumen microbiome.**
R. D. Wollenberg*[1], E. Aa. Sørensen[1], P. B. Pope[2,3], C. M. Koble[3], and M. T. Søndergaard[1], [1]*DNASense*, *Aalborg, Denmark*, [2]*Center for Microbiome Research, Queensland University of Technology*, *Woolloongabba, QLD, Australia*, [3]*Norwegian University of Life Sciences*, *Ås, Norway*.

65 **Harnessing endogenous type II-A CRISPR system to achieve genome editing in *Lacticaseibacillus rhamnosus* GG.**
Z. Xie*[1], Y-S. Jin[1,2], and M. Miller[1,2], [1]*Department of Food Science and Human Nutrition, University of Illinois Urbana-Champaign*, *Urbana, IL, USA*, [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign*, *Urbana, IL, USA*.

66 **Dietary effects on large intestine fibrolytic function and mucosal integrity in healthy elderly horses.**
M. Baraille*[1,2], V. Milojevic[3], S. Julliand[2], and V. Julliand[1], [1]*Institut Agro Dijon, Univ. Bourgogne Franche–Comté, PAM UMR A 02.102*, *Dijon, France*, [2]*Lab To Field*, *Dijon, France*, [3]*Sandgrue Foundation, 8132 Egg, Zürich, Switzerland*.

67 **Selection of *Streptococcus equinus* HC5 variants with increased production of bovicin HC5 by adaptive laboratory evolution.**
Rodrigo Dias[1], Yasmin Sabino[2], Katialaine Araujo[1], Juliana Silva[1], and Hilario Mantovani*[1,2], [1]*Universidade Federal de Vicosa*, *Vicosa, MG, Brazil*, [2]*University of Wisconsin–Madison*, *Madison, WI, USA*, [3]*Universidade Federal de Juiz de Fora*, *Juiz de Fora, MG, Brazil*.

68 **Pangenome analysis of *Clostridium scindens*: A bile acid and cortisol metabolizer gut bacterium.**
Francelys Fernandez*[1,2], Kelly Olivos-Caicedo[3], Karthik Anantharaman[4], Steven Daniel[1], João Alves[2], and Jason Ridlon[1,5], [1]*University of Illinois at Urbana-Champaign*, *Urbana, IL, USA*, [2]*Carl R. Woese Institute for Genomic Biology*, *Urbana, IL, USA*, [3]*University of São Paulo*, *São Paulo, Brazil*, [4]*University of Wisconsin–Madison*, *Madison, WI, USA*, [5]*Virginia Commonwealth University School of Medicine*, *Richmond, VA, USA*.

69 **Methanogenesis inhibition stimulates acetogenesis by novel microbes in ruminants.**
Gaofeng Ni*[1], Nicola Walker[2], André Fischer[2], René Stemmler[2], Rhys Grinter[1,3], Mick Watson[4], Emiel Ver Loren van Themaat[2], Maik Kindermann[2], and Chris Greening[1], [1]*Department of Microbiology, Biomedicine Discovery Institute, Monash University*, *Melbroune, Victoria, Australia*, [2]*DSM Nutritional Products, Animal Nutrition and Health*, *Kaiseraugst, Switzerland*, [3]*Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne*, *Melbourne, Victoria, Australia*, [4]*Centre for Digital Innovation, DSM Biotechnology Center*, *Delft, the Netherlands*.

70 **Extra-chromosomal elements encode essential functions in rumen *Butyrivibrio fibrisolvens* cultures.**
W. J. Kelly*[1], N. Palevich[1], F. P. Palevich[2], and G. T. Attwood[1], [1]*AgResearch Ltd., Grasslands Research Centre*, *Palmerston North, New Zealand*, [2]*AgResearch Ltd., Hopkirk Research Institute*, *Palmerston North, New Zealand*.

71 **Lactate utilization in anaerobic rumen bacteria.**
W. J. Kelly* and G. T. Attwood, *AgResearch Ltd., Grasslands Research Centre*, *Palmerston North, New Zealand*.

Nutrition and metabolism of livestock, humans, and companion animals

Beckman Institute Room 1005 (with overflow to Room 5602)

72 **Effect of essential oils blend on growth performance, gut morphology, and meat quality in broilers.**
A. Rahman*, *University of Veterinary and Animal Sciences*, *Lahore, Punjab, Pakistan*.

73 **Detection of ethanol in the rumen and saliva of lactating dairy cows undergoing a feed-induced ruminal acidosis challenge.**
I. Farag[1], F. Yang[1], J. Embree[1], B. Anderson[1], J. Sarturi[2], A. Lago[3], and M. Embree*[1], [1]*Native Microbials*, *San Diego, CA, USA*, [2]*Texas Tech University*, *Lubbock, TX, USA*, [3]*DairyExperts*, *Tulare, CA, USA*.

74 **Induced hindgut acidosis affected ruminal fermentation and gut permeability.**
H. F. Linder*, B. R. Loman, R. C. Fries, S. D. Gutierrez-Nibeyro, E. F. Garrett, and J. C. McCann, *University of Illinois*, *Urbana, IL, USA*.

75 **Effects of biochar, monensin and nitrate in beef cattle diets on in vitro volatile fatty acids profile.**
J. M. C. Souza*[1,2], E. D. Batista[1], and J. C. McCann[1], [1]*Universidade Federal de Lavras*, *Lavras, Minas Gerais, Brazil*, [2]*University of Illinois*, *Urbana-Champaign, IL, USA*.

Prebiotics, probiotics, and DFM development

Beckman Institute Room 1005 (with overflow to Room 5602)

76 **Redirecting 1,2-propanediol from *Salmonella* Dublin to lactobacilli.**
Martin Moran*[1], Anna Widenmann[2], Nilusha Malmuthuge[3], Michael Gänzle[2], and Le Luo Guan[1], [1]*University of British Columbia*, *Vancouver, BC, Canada*, [2]*University of Alberta*, *Edmonton, AB, Canada*, [3]*Agriculture, Agri-Food Canada*, *Lethbridge, AB, Canada*.

77 **Galacto-oligosaccharides regulates intestinal mucosal glycosylation (sialylation) by restoring intestine-microbiota homeostasis.**
Laipeng Xu*[1,2], Xuan Li[1,2], Haiqin Wu[1,2], Chunlong Mu[1,2], and Weiyun 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, Jiangsu province, China*, [2]*National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University*, *Nanjing, Jiangsu province, China*.

78 **Effects on monensin on growth and nitrate/nitrite metabolism of a hypernitrite-metabolizing *Paenibacillus*.**
Robin C. Anderson*[1], Gordon E. Carstens[2], and William E. Pinchak[3], [1]*United States Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit*, *College Station, TX, USA*, [2]*Department of Animal Science, Texas A&M University*, *College Station, TX, USA*, [3]*Texas AgriLife Research*, *Vernon, TX, USA*.

79 **Native rumen-derived probiotics alter the rumen microbiome and improve production and feed efficiency when fed to lactating dairy cows.**
B. Anderson*[1], C. Marotz[1], I. Farag[1], C. Martino[1], J. Lefler[1], A. Washburne[2], K. Calapa[1], J. Drackley[3], T. Overton[4], M. Vandehaar[5], J. Santos[6], J. Osorio[7], E. Uddin[7], A. Lago[8], M. Embree[1], [1]*Native Microbials*, *San Diego, CA, USA*, [2]*Selva Analytics*, *Bozeman, MT, USA*, [3]*University of Illinois Urbana-Champaign*, *Urbana, IL, USA*, [4]*Cornell University*, *Ithaca, NY, USA*, [5]*Michigan State University*, *East Lansing, MI, USA*, [6]*University of Florida*, *Gainesville, FL, USA*, [7]*South Dakota State University*, *Brookings, SD, USA*, [8]*DairyExperts*, *Tulare, CA, USA*.

80 **The effect of *Bacillus* strain supplementation to sows on maternal microbiome and piglet microbiome.**
Bin Zuo*, Tsungcheng Tsai, Jianmin Chai, Samantha Howe, and Jiangchao Zhao, *University of Arkansas*, *Fayetteville, AR, USA*.

Special Session: Maximizing the impact of next-generation approaches

1 Intertwining plasmids, microbial interactions and adaptations to gut environments.

I. Mizrahi*,
The Department of Life Sciences & The School of Sustainability & Climate Change, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Gut environments, densely populated with microorganisms, serve as fertile grounds for horizontal gene transfer and microbial genome plasticity, with plasmids emerging as potent agents in this evolutionary process. This prompts inquiries into the driving forces behind plasmid dispersal across populations, the intricate factors shaping genetic material flow among them, and their impact on microbial interactions and host adaptation. In my presentation, I will talk about findings from our recent studies that probe these questions, offering deeper insights to these aspects of microbial ecology and evolution.

2 Withdrawn.

3 Microbial microproteins as mediators of microbe-microbe and microbe-host communication and warfare.

A. Bhatt*,
Department of Medicine and Genetics, Stanford University, Stanford, CA, USA.

This presentation will focus on recent developments in the study of microbial microproteins. After the discovery thousands of new families of these microproteins (≤50aa) we developed a method to annotate these microproteins in metagenomes and metatranscriptomes. To better understand the functions of these microproteins, we have been working on methods to investigate their roles in microbe-microbe warfare, quorum sensing, and microbe-host crosstalk. In this talk, I will present progress made in all 3 of these areas. First, I will present our findings on the discovery of new, highly potent antimicrobial peptides derived from microproteins. Second, I will discuss a novel quorum sensing system that we identified in

Enterococcus faecalis. Finally, I will introduce a platform that we have developed for screening microbial agonists and antagonists of human signaling receptors, which could provide new insights into microbe-host interactions. Overall, this presentation will highlight the importance of studying microbial microproteins and their functions, and provide unpublished insights into their potential roles in various microbial processes and host-microbe interactions.

Key Words: microbial microproteins, microbe

2024 Opening Session: Invited presentations

4 The yin and yang of microbiome signatures in early life.

L. Hall*,
University Birmingham, Birmingham, United Kingdom.

This presentation will delve into the intricate dynamics of microbial communities during critical early life stages, exploring the complex interplay between beneficial bacteria, such as *Bifidobacterium*, and potentially harmful species like *Clostridium*. Additionally, the pivotal role these microbial players play in shaping health outcomes during crucial periods of pregnancy and infancy will be covered. Finally, work will be presented that highlights how targeted strategies aimed at restoring perturbed ecosystems offer promising avenues for enhancing early life health outcomes.

Key Words: *Bifidobacterium*, *Clostridium*, microbial

5 Journey through time: Unraveling the evolution of the human gut microbiome.

A. Kostic*,
Harvard Medical School, Boston, MA, USA.

In this talk, we explore the dynamic evolutionary journey of the human gut microbiome, highlighting its intricate relationship with human health and metabolism. The emphasis is on understanding the origins of the human-microbiome symbiosis and the pivotal lessons we can derive from studying ancient and modern human hunter-gatherers. Our research delves into the “ancestral” gut microbiomes, revealing a stark contrast with today’s industrialized human microbiomes. Through comparative genomic analysis of both ancient humans and modern hunter-gatherers, our studies enable a unique perspective on the journey of human gut microbes in their anthropic spaceships across the Earth. A central topic of the talk will be the high fiber content of ancestral diets and the role of fiber-specialized microbes, now exceedingly rare in industrialized populations. These disappearing microbes may be a key to unlocking the nutritional benefits of the ultra-high-fiber hunter-gatherer diets, and may be a potent tool in the war against the epidemic of modern metabolic diseases such as obesity and diabetes. In summary, this talk will weave together the insights gained from our studies on ancestral human microbiomes, the metabolic implications of ultra-high-fiber diets, and the potential of reintroducing ancestral human microbes in a safe and ethical manner.

Bryant Memorial Lecture

6 Cellulosome-producing bacteria from the environment to the rumen and human-gut microbiomes.

Ed Bayer*[1,2],
[1]*The Weizmann Institute of Science, Rehovot, Israel,* [2]*Ben-Gurion University of the Negev, Beersheva, Israel.*

It is an honor to present the Marvin P. Bryant Memorial Lecture. Marv Bryant was a true early pioneer in establishing the field of gut microbiology. He and his colleagues isolated and characterized many of the original anaerobic fiber-degrading and methanogenic microbes that we continue to investigate today. His kind and humble personality prompted his colleagues to call him "the gentle giant of rumen microbiology." My own fascinating life in science has taken me along a winding road through the fields of organic chemistry, enzymology, multi-protein complexes, genetic engineering and synthetic biology, as well as anaerobic, environmental and rumen microbiology. Along the way, these combined disciplines led to my participation in the invention of the remarkable avidin-biotin system, the discovery of the multi-enzyme cellulosome complex, and the invention of designer cellulosome technology. We initially discovered and coined the term "cellulosome" in the anaerobic, thermophilic bacterium, *Clostridium thermocellum*, and later described their broad occurrence in other environmental bacteria. These bacteria can produce over 200 different cellulosomal components, including dozens of different cellulases and other enzymes, and non-enzymatic scaffold in proteins that organize them into a functional complex that efficiently degrades cellulose and other plant cell wall components. Subsequently, we discovered an extensive cellulosome system in the principal, cellulose-degrading *rumen* bacterium, *Ruminococcus flavefaciens*. When the *human*-gut cellulolytic bacterium, *R. champanellensis*, was identified, we pondered whether it too produced a cellulosome and discovered a viable cellulosome system in this species as well. In additional work, we found a cellulosome-like "amylosome" among diverse human and rumen isolates of the keystone starch-degrader, *Ruminococcus bromii*. Further research unearthed a variety of additional ruminococcal species in the gut microbiota of humans and other primate populations that assemble functional cellulosome systems. We have come a long way in our understanding of cellulosomes at different levels, from the structural level, to their combined enzymatic and non-enzymatic interactions and their contributions to microbiomes of ruminants and humans.

Key Words: multi-enzyme complexes, avidin-biotin technology, designer cellulosomes, ruminococci, cellulose degradation

Podium presentations: Session 1

7 Metaproteomics to investigate functional diet-microbiota interactions.

Manuel Kleiner*,
North Carolina State University, Raleigh, NC, USA.

Metaproteomics is the large-scale identification and quantification of proteins from microbial communities and thus provides direct insight into the phenotypes of microorganisms on the molecular level. Initially metaproteomics was mainly used to assess the "expressed" metabolism and physiology of microbial community members. However, we have recently increased the range of questions we can address with metaproteomics by developing new approaches that allow us to quantify species biomass contributions to determine community structure, to determine in situ carbon sources of community members, and the uptake of labeled substrates by community members. I will present 2 of our recent studies that rely on

metaproteomic approaches. In the first study we investigated the effect of different dietary protein sources on the gut microbiota in mice. We found that different sources of dietary protein had major impacts on the composition and function of the gut microbiota and that functional shifts were largely driven by the metabolism of glycan side chains of dietary proteins. Amino acid metabolism also differed significantly between sources of proteins potentially leading to different beneficial or detrimental end products of amino acid degradation. In the second study we used stable isotope fingerprinting with metaproteomics (Protein-SIF) to link different components of the diet to the microbial species in the mouse gut that consume them. We were able to link specific microbial groups to their diet derived substrates. Additionally, we found rapid uptake and re-release of dietary protein by the mouse host into the gut environment leading to rapid changes in isotope signatures of known host-foraging microorganisms. We expect that metaproteomic approaches will rise in their use and application in the next decade as they allow microbiome researchers to address critical questions that are hard to address with other approaches. Additionally, the equipment and tools needed to employ metaproteomics are becoming more widely available and standardized.

Key Words: microbiome, metaproteomics, metagenomics, mass spectrometry, stable isotope

8 Intestinal microbiota consume specific dietary proteins that escape host digestion.

Ayesha Awan*, Alexandria Bartlett, Alfredo Blakeley-Ruiz, Tanner Richie, Casey Theriot, and Manuel Kleiner,
North Carolina State University, Raleigh, NC, USA.

Dietary components, including fiber and dietary protein, interact with the gut microbiota, influencing host health. Studies have shown that the gut microbiota can transform dietary protein into metabolites that affect host health, including beneficial fatty acids or proinflammatory amines. However, which dietary proteins escape host digestion and undergo gut microbial metabolism remains unknown. Metaproteomics directly detects and quantifies dietary proteins in intestinal samples to answer these questions more efficiently than existing techniques. We used metaproteomics to measure the fate of purified dietary proteins from 6 different plant and animal sources, including casein, soy, egg, yeast, pea, and rice, in germ-free and conventionally raised mice. We quantified the impact of the gut microbiota on the degradation of specific dietary proteins by comparing the abundance of dietary proteins between fecal samples of conventional vs. germ-free mice. We found that purified dietary proteins contain a wide range of distinct proteins, ranging from 44 proteins in egg white to 1,476 proteins in yeast. The digestive efficiency of dietary proteins differed significantly in the presence and absence of the gut microbiota. We detected the highest abundance of dietary protein in fecal samples of mice fed the brown rice protein, followed by egg white and casein, suggesting resilience to host and microbial degradation. We further identified dietary proteins resilient to host digestion but consumed by the gut microbiota. These refractory proteins, including Ovomucin in egg and the Kunitz trypsin inhibitor in soy, were enriched in germ-free fecal samples but depleted in fecal samples from mice with a conventional microbiome. We also identified dietary proteins resistant to both host and microbial conversion. Our study highlights substantial differences in dietary protein degradation by the host and the gut microbiota, where some proteins evade host digestion but are accessible to microbial degradation. Additionally, the source of dietary protein drives the amount and type of specific dietary proteins available to the gut microbiota. Identifying dietary protein substrates of gut microbial metabolism is vital for developing nutritional and therapeutic interventions against various intestinal diseases.

Key Words: dietary proteins, gut microbiota, metaproteomics, intestinal health, host digestion

9 Gut microbial production of epitestosterone driving metastatic prostate cancer.

T. Wang*[1,2], S. Ahmad[3], K. Yovani Olivos Caicedo[4], F. Fernández[1,2], B. Binion[1,2], J. W. Lee[5], J. D. Kang[6], S. C. Harris[6], H. R. Gaskins[1,7], J. W. Erdman Jr.[7,8], S. L. Daniel[1], P. B. Hylemon[9], S. E. Ernst[10], A. Cruz-Lebrón[10], and K. S. Sfanos[10], Karthik Anantharaman[11], Joao M. P. Alves[4], Joseph Irudayaraj[3], Jason M. Ridlon[1,2,7,8,9]*,
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It is becoming clear that the gut microbiome should be considered an integral component of the host endocrine system as the microbiota can change the class of hormones and generate derivatives through mechanisms distinct from the host. Prostate cancer is the second most diagnosed cancer among men worldwide where androgens play a role in driving prostate cancer growth. Previously, we reported a pathway (*desAB*) by which *Clostridium scindens* ATCC 35704 converts 11-deoxycortisol to androstenedione serving as the precursor of androgens. Here, we aim to find the microbial enzyme produced by *C. scindens* VPI 12708 that is involved in converting androstenedione to epi-testosterone and investigate the unknown role of epitestosterone in prostate cancer. First, we performed conversion assays of androstenedione to epi-testosterone. To identify the gene of interest, we induced the enzyme expression in vitro and performed RNA-Seq analysis to find the upregulated steroid-inducible reductases. A gene was induced encoding a predicted 27.69 kDa short-chain dehydrogenase/reductase family protein, and was further demonstrated to convert androstenedione to epi-testosterone. We named this gene *desF*. We next examined both sequenced genomes and publicly available metagenomes, obtaining ~300 *C. scindens* genomes and metagenome assembled genomes, and determined the proportion of *C. scindens* strains harboring genes involved in steroid metabolism. To investigate a potential role of epi-testosterone in prostate cancer, both testosterone and epi-testosterone were added (10 n*M*) to the cultures of androgen-sensitive prostate cancer cells, the results observed that epi-testosterone significantly drives prostate cancer cell proliferation with better stimulation than testosterone ($P < 0.001$). Concomitantly, prostate-specific antigen levels with epi-testosterone are increased 25-fold compared with those with testosterone after 96-h incubation. We also present preliminary clinical data relating to *des* gene abundances in metastatic prostate cancer patients responding to androgen-deprivation therapy relative to those who are not. Our novel findings reveal that the gut microbiota could be the potential target for the treatment of prostate cancer by inhibiting the gut microbial production of epitestosterone.

Key Words: gut microbiota, prostate cancer, epitestosterone, androgens, *Clostridium scindens*

10 The healthy microbiome in an unhealthy context: Chronic pouchitis.

S. M. Dall*[1], S. J. Kousgaard[1], O. Thorlacius-Ussing[1,2], and M. Albertsen[1],
[1]*Aalborg University, Aalborg, Denmark,* [2]*Aalborg University Hospital, Aalborg, Denmark.*

Pouchitis is an inflammatory condition affecting patients with an ileal pouch-anal anastomosis, a prevalent complication among those who have

undergone surgical treatment for ulcerative colitis. Treatment options are limited and primarily consist of antibiotic therapy. Treatment failure can ultimately result in the surgical removal of the pouch, necessitating the creation of a stoma. Given the suspected involvement of the gut microbiome in pouchitis pathogenesis, we previously investigated fecal matter transplantation (FMT) from healthy donors as an alternative treatment in a randomized placebo-controlled study. However, no significant clinical effect was observed between the treated and placebo group. We speculated that FMT was ineffective due to significant differences between a healthy pouch microbiome and a typical microbiome, prompting us to investigate and characterize the healthy pouch microbiome. We compared the microbiome of 30 pouchitis patients (SickPouch), 48 people with a healthy pouch (HealthyPouch), and 92 individuals with normal physiology (Normal). Fecal samples were collected at Aalborg University Hospital and subsequently prepared for sequencing with Nanopore 10.4.1 and Illumina NovaSeq 6000. In total, 16912 MAGs (8155 high quality) were generated from 3.8 Tbp of quality filtered Nanopore data. Ordination revealed all groups have distinct microbiomes. Notably, the HealthyPouch microbiome is characterized by a high relative abundance of the genus *Prevotella* compared with both the SickPouch and Normal microbiome. Additionally, the HealthyPouch microbiome shows a significantly higher mean microbial richness (Welch's *t*-test, $P < 0.01$). Future goals will be assessing strains and anti-microbial resistance using the Nanopore data set. In conclusion, FMT treatment from donors with a normal microbiome did not have a significant clinical effect in chronic pouchitis patients. However, the distinct microbiome profiles of the HealthyPouch versus SickPouch versus the Normal microbiome suggest that using FMT from donors with a normal physiology might not be an effective treatment. Tailoring the FMT treatment to use the HealthyPouch microbiome, however, could be a potential alternative and need further research.

Key Words: pouchitis, fecal matter transplantation, nanopore, ulcerative colitis, microbial diversity

11 Exploring the link between socioeconomic environment, microbial diversity and colonization of multi-drug resistant bacteria in the human gut microbiome.

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Persistent social inequalities continue to limit universal access to a healthy environment, directly affecting both life expectancy and quality of life. However, there is limited evidence on how socioeconomic disparities relate to the health of the human gut microbiome and its subsequent impact on health outcomes. To address this gap, we examine associations between neighborhood-level economic hardship, an indicator of overall cumulative risk, gut microbiome composition, and additional cofactors that may be involved in this association, including diet quality and food insecurity. We conducted a cross-sectional analysis of 721 adults living in geographically diverse (urban vs. rural) regions of Wisconsin, USA. We analyzed 16S rRNA gene amplicons from human stool samples using QIIME2 to assess the composition and diversity of the gut microbiota of 721 participants. We then determined correlations with each individual's neighborhood score for socioeconomic status, or Economic Hardship Index (EHI), using simple and zero-inflated negative binomial regression models. Our model of the EHI, with α diversity (inverse Simpson) as the outcome, found a correlation ($\beta = 1.68$, $P = 0.03$) that was partially mediated by food insecurity ($P = 0.02$). In addition, we found differential abundances of known health-associated bacteria, including members of the genera *Bifidobacterium* and *Akkermansia*, and the family *Ruminococcaceae*, between high and low levels of EHI that were robust to correction for multiple comparisons. Finally, we found a higher prevalence ($P = 0.03$) and number ($P = 0.02$) of multidrug-resistant bacteria isolated from individuals with low α diversity and high EHI. Our analyses suggest that living in low socioeconomic communities is associated with lower gut microbial diversity, which could allow increased colonization by multi-drug resistant

microbial pathogens. In summary, our study helps to elucidate the interplay of social and economic factors in shaping microbiome composition and how these changes in community structure and diversity appear to impact microbiome health. With this work, we aim to provide a framework for developing future interventions to mitigate the SES health gap.

Key Words: microbial ecology, environmental effectors, human gut, food security

12 Gut microbiota and bile salt hydrolase activity in heavy and light broiler chickens.

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Body weight is an important indicator of the overall health and production efficiency in broiler chickens. In broiler houses, body weight of chicks is variable despite the same genetics, hatching and feeding practices within a production system. The objective of this study was to investigate the intestinal microbiota and bile salt hydrolase (BSH) activity in light and heavy broiler chickens, which belonged to the 10th and 90th percentile body weight group, respectively. A total of 300 Ross 308 broiler chickens (100 from 3 independent cohorts) were selected and mucosal samples from the jejunum, ileum, and cecum were collected at d 0, 11 and 25 (n = 450). Then, viable cell counts, Illumina Hiseq sequencing, Fluidigm real-time qPCR for 7 targets, as well as BSH activity were analyzed. Results of viable cell counts showed no significant difference between light and heavy groups ($P > 0.05$), but they tended to be higher in the light group in all measured bacterial groups. 16S rRNA amplicon sequencing revealed higher relative abundance of *E. coli-Shigella* (71.3–79.8%) in the baseline sample (d 0), while the most abundant microorganisms at d 25 were *Candidatus arthromitus* (light: 44.5%; heavy: 27.4%) and *Faecalibacterium* (light: 23.7%; heavy: 25.7%) in small intestine and cecum, respectively. qPCR results indicated notable differences in bacterial populations between the light and heavy groups, especially higher total bacteria, *Enterococcus*, and *Clostridium* cluster I in the light group and higher lactic acid bacteria and *Bifidobacteria* in the heavy group at d 25. BSH activity was also higher in the light group than the heavy group (light: 0.48; heavy: 0.26 ΔOD/protein [μg/mL]; $P < 0.0001$), and correlation analysis highlighted associations between BSH activity, body weight, feed intake, body weight gain, and bacterial counts. We postulate that high total bacteria and *Enterococcus* lead to high BSH activity, resulting in low feed intake and body weight gain, ultimately resulting in separation into light and heavy birds. The findings of this study contribute to understanding the relationship between gut microbiota, BSH activity, and host physiology in broiler chickens, with potential implications for poultry production.

Key Words: chickens, heavy, light, gut microbiota, bile salt hydrolase activity

13 Utilizing the COVID-19 pandemic as an opportunity to examine the role of mass gatherings in the emergence of antimicrobial resistance: A wastewater-based surveillance from 2020 to 2022.

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Mass gatherings (MGs), as defined by the World Health Organization (WHO), represent large congregations during short durations, exerting substantial strain on health planning and response capacities. An exemplary MG is the annual Hajj in Makkah and Medinah, with approximately 2 million attendees, and the year-round Umrah with reduced congregation. MGs, especially those involving global pilgrims, pose challenges to antimicrobial resistance (AMR) transmission due to potential carriage of antimicrobial resistance genes (ARG)

and bacteria (ARB). Inadequate wastewater treatment systems further compounds AMR concerns. Amidst the COVID-19 pandemic, Saudi Arabia temporarily closed its borders, impacting Hajj and Umrah attendance. Upon reopening, pilgrim numbers surged, prompting questions about the role of MGs in AMR dissemination. To address these queries, we developed a statistical model predicting ARG importation into Saudi Arabia post-border reopening, utilizing global sewage metagenomes from 2017 to 2019 and socioeconomic factors of each country. Concurrently, we collected raw sewage from Makkah and Medinah, which are more MG-impacted than the control site at KAUST. Omics-based analysis indicated no general AMR increase that correlated with pilgrim numbers. However, specific ARGs, particularly Extended Spectrum Beta-Lactamase/Metallo-Beta-Lactamase (ESBL/MBL), revealed dynamic responses to MGs. New ESBL/MBL genes were identified in raw wastewater and were potentially introduced by foreign pilgrim during post-border reopening. Bacterial isolation confirmed *Shewanella putrefaciens* as carriers of ESBL/MBL, notably PER-7. Genomic analysis further uncovered PER-7 integration into chromosomes, leading to genomic alterations and the acquisition of multiple ARGs by the bacterial host. Dynamic AMR responses during MGs, coupled with genomic insights into integration events and horizontal gene transfer, underscore the complexity of AMR transmission pathways. In conclusion, our study pioneers the detection of AMR dissemination during global travel, emphasizing the need to understand AMR from a One-Health perspective.

This Fire Poster Pitch describes abstract 44 in the poster session.

Key Words: antimicrobial resistance, CO-VID-19, wastewater-based surveillance, mass gatherings

14 Genome analysis of ruminal *Selenomonas* reveals multiple pathways for hydrogen production and utilization.

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Selenomonas are anaerobic, gram-negative, crescent-shaped, motile, rumen bacteria which grow on a wide range of soluble carbohydrates. The genomes of 16 *Selenomonas* strains were sequenced in the Hungate1000 project, which confirmed many of their metabolic capabilities, but also revealed multiple pathways for H_2 production and utilization. Most strains encode multiple hydrogenases, including at least one and up to 3 [FeFe] Group A1, fermentative, H_2-evolving, hydrogenases using reduced ferredoxin as the electron donor, and a [NiFe] Group 1d hydrogenase enabling hydrogenotrophic respiration using fumarate as the terminal electron acceptor. *S. ruminantium* strains HD4, L14, S137, GACV-9, AB3002, KH1T6, and GA192 also encode the fumarate reductase (*frdA*) involved in fumarate respiration. In *S. ruminantium* strains S137, AC2024, C3, WCT3, *S. ruminantium lactilytica* TAM6421 and *Selenomonas* sp. FC4001, their [FeFe] Group A1 hydrogenase was consistently associated with a [NiFe] Group 3c heterodisulfide reductase-linked hydrogenase which bifurcates electrons from H_2 to heterodisulfide and ferredoxin. All but 3 *Selenomonas* encoded the genes required for dissimilatory sulfate reduction, and growth tests of *S. ruminantium* GA192, AB3002 and HD4 and *S. ruminantium lactilytica* PC18 confirmed sulfate reduction while *S. bovis* 8–14–1 was negative. Respiratory membrane-bound nitrate reductase genes *narGHIJ* were found in *S. ruminantium* C3, GACV-9, HD4, L14, WCT3, Z108, S137, *S. ruminantium lactilytica* PC18, TAM6421 and *Selenomonas* sp. FC4001, ND2010, while a periplasmic dissimilatory nitrate reductase *napA* was present in all strains except *S. bovis*, *S. ruminantium* WCT3, and *Selenomonas* sp.AE3005, ND2010. All *Selenomonas* strains apart from *S. bovis* 8–14–1, possess a respiratory nitrite reductase (*nrfA*) which catalyzes the reduction of nitrite to ammonium, and apart from *S. ruminantium* C3, GACV-9, Z108 and *Selenomonas* sp. AE3005, FC4001, encode the N_2-fixing di-nitrogenase reductase *nifHDK* genes even though ruminal N_2 fixation is extremely low. All strains had 2 L-lactate dehydrogenases

(except *S. bovis*) and an alcohol dehydrogenase explaining their production and use of lactate and suggesting ethanol as a possible fermentation product.

This Fire Poster Pitch describes abstract 46 in the poster session.

Key Words: *Selenomonas*, genome, hydrogen

15 Anaerobic fungal carbohydrate-binding modules possess prominent preference to assist the enzymatic hydrolysis of hemicellulose.

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Anaerobic fungi dwelling at the gastrointestinal tract of herbivores robustly deconstruct the plant biomass by diversified carbohydrate active enzymes (CAZymes) acting in concert. As the auxiliary domains of the CAZymes, carbohydrate-binding modules (CBMs) influence the enzymatic hydrolysis of substrates by the recognition and adhesion effects. In this study, the microbial source and distribution of CBMs were investigated. Results showed that bacteria and fungi are the 2 dominant contributors to produce CBMs. Then the architecture analysis of bacterial and fungal CBM proteins was completed through the proteomic data from CAZy database. Data displayed that more than 50% of CBMs from bacteria and fungi are incorporated into their parent CAZymes, respectively, which suggests the crucial role of CBMs in the diverse enzymatic reactions. Moreover, the 68% of fungal CBMs are integrated into the cellulase, hemicellulase, and acetyl xylan esterase. Based on this consequence, the composition module of CAZymes from 36 representative aerobic fungi and 12 anaerobic fungi was furtherly analyzed. Results showed that the anaerobic fungal CBMs are biased to connect with to hemicellulase (e.g., GH10, GH11 and GH43) and acetyl xylan esterase (e.g., CE1 and CE4). Averagely 32% of these anaerobic fungal CBM-fused CAZymes are presented in the carbohydrate active enzyme gene clusters (CGCs). Subsequently, the distribution of CBM-fused CAZymes in the CGCs related to hemicellulose degradation were compared between the present 12 anaerobic fungi and 12 typical fibrolytic bacteria. Results indicate that anaerobic fungi have greater numbers of CBM-fused CGCs available for degrading hemicellulose than that of fibrolytic bacteria. Finally, 3 upregulated CBM-fused CGCs related to the breakdown of arabinoxylan based on the transcriptomic data of anaerobic fungus *Pecoramyces ruminantium* sp. F1 were characterized. These results suggest that anaerobic fungal CBMs harbor unprecedented potential to assist the enzymatic hydrolysis of hemicellulose based on the CGCs level.

This Fire Poster Pitch describes abstract 52 in the poster session.

Key Words: anaerobic fungi, carbohydrate-binding modules, carbohydrate active enzyme gene clusters, hemicellulose degradation

16 Microbiome-mediated colonization resistance against necrotic enteritis.

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Necrotic enteritis (NE), caused by *Clostridium perfringens*, ranks among the most financially devastating diseases in poultry. Unfortunately, there are currently no effective preventive or therapeutic measures available. The intestinal microbiota plays a critical role in maintaining animal health and productivity by resisting the colonization and proliferation of invading pathogens. Inbred chicken lines such as Fayoumi line M5.1 are known to be naturally resistant to NE. To explore whether the microbiomes of NE-resistant chickens offer better protection against NE than those of susceptible chicken lines, we transplanted the cecal microbiota from different chicken breeds to naïve, day-of-hatch Cobb broilers, followed by NE induction and evaluation of the disease outcome. We found that the cecal microbiota of the resistant chicken line provided 95–98% protection from

NE, while approximately 40% of chickens died from severe intestinal lesions without microbiota transplantation. Interestingly, the cecal microbiota from susceptible chicken lines also conferred significant protection of naïve Cobb chickens from NE, although with slightly reduced efficacy. Our findings strongly suggest that the gut microbiota provides strong colonization resistance to NE. To further identify commensal bacteria responsible for NE resistance, we screened a library of cecal bacteria from healthy feral chickens and identified several bacteria with a strong ability to inhibit *C. perfringens*, while also enhancing innate immunity through the induction of host defense peptide synthesis. Notably, oral administration of a selected bacterium to day-of-hatch broilers improved the survival rate to 98% in a chicken model of NE, while 52% of chickens died without intervention. Moreover, the bacterium significantly alleviated the severity of intestinal lesions. Collectively, these results clearly demonstrate the potential of commensal bacteria as probiotics for NE mitigation.

This Fire Poster Pitch describes abstract 53 in the poster session.

Key Words: colonization resistance, necrotic enteritis, microbiome, microbiota transplantation, probiotics

17 Understanding the host–microbiome interactions involved in liver abscess formation in beef cattle.

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Feedlot diets are usually formulated to provide ≤10% dry matter as roughage. Diets low in roughage increase the incidence of metabolic disorders, with 20–35% of cattle in feedlots developing liver abscesses. Severe liver abscesses are linked to reduced feed efficiency costing the Canadian beef industry ≈$61.2 million annually. The processes involved in the development of liver abscesses are not well understood. We present our efforts to examine potential host–rumen microbiome interactions involved in the development of liver abscesses in cattle. To examine the impact abscesses have on liver function, we have employed RNA-seq and found significant alterations in gene expression in abscessed livers. We characterized the rumen microbiome of cattle with and without liver abscesses and did not observe a relationship between the rumen microbial community and the development of a liver abscess. We have also characterized the liver abscess microbiome using a metataxonomic approach and found a low diversity community dominated by *Fusobacterium* spp. and *Bacteroides* spp. Abscess size and severity impacted the richness and composition of the abscess microbiome with large, severe abscesses having more diversity than small abscesses. To further characterize these pathogens we have isolated *Fusobacteria* from liver, abscess and gut samples. Genomic approaches are being used to compare the phylogenomic relationship of *Fusobacteria* isolated from the gut and abscessed liver of cattle. This work provides novel insight into the host-pathogen interactions that lead to the development of liver abscesses, and should provide insight into strategies to reduce antimicrobial use in beef cattle.

This Fire Poster Pitch describes abstract 54 in the poster session.

Key Words: liver abscess, cattle, rumen, *Fusobacterium*

18 Transcriptomic insights into the anti-ruminal *Streptococcus* activity of natural compound betulin.

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Streptococcus is a causative agent of rumen acidosis, a gastrointestinal disorder in ruminal livestock. In vitro studies have shown that betulin, a natural compound contained in the outer bark of birch trees, selectively inhibits the growth of *Streptococcus*, including ruminal *S. equinus* ATCC33317, without negatively impacting rumen fermentation. While these results suggest that betulin could effectively prevent the onset of rumen acidosis, the mechanism by which betulin inhibits ruminal *Streptococcus* remains unknown. To address this, we examined the effect of betulin on the transcriptional activity of *S. equinus* ATCC33317. *S. equinus* ATCC33317 was cultured in MRS medium and inoculated with a solution of betulin (final concentration 300 μg/mL) or equivalent concentration of dimethyl sulfoxide as a control. Triplicate tubes were incubated anaerobically for 12 h at 39°C, and samples were collected for transcriptomic analysis and betulin quantification. We identified the *pur* gene cluster and the *nrdIEF* gene cluster as being downregulated by betulin. The *pur* gene cluster encodes a group of enzymes crucial for purine de novo biosynthesis, which is essential for DNA and RNA biosynthesis. The *nrdIEF* gene cluster encodes for multiple enzymes, including a rate-limiting enzyme for DNA/RNA synthesis. These findings indicate that betulin partially suppressed the transcriptional activity for DNA/RNA synthesis in *S. equinus* ATCC3317. Moreover, gene clusters involved in the metabolism of arabinan were upregulated. Arabinan is catabolized to arabinose, which can be synthesized into phosphoribosyl pyrophosphate, a purine de novo biosynthesis precursor. These genes may be upregulated to mobilize pentose for DNA/RNA synthesis due to the inhibitory effects of betulin. Furthermore, betulin concentration was decreased during the logarithmic growth phase, indicating that betulin was metabolized by *S. equinus* ATCC33317. These findings offer valuable insight into the molecular mechanism by which betulin may inhibit the growth of *Streptococcus* in the rumen, and its potential use as an antimicrobial agent for preventing rumen acidosis in livestock.

This Fire Poster Pitch describes abstract 58 in the poster session.

Key Words: *Streptococcus*, betulin, rumen acidosis, transcriptome, antimicrobial agents

19 Hydrogenotrophic methanogens facilitate microbial energy harvesting from plant polysaccharides in breed-determined obese pigs.

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Gut microbiome has been thought to promote obesity by increasing energy extraction from indigestible plant polysaccharides. H_2 removal by hydrogenotrophic microbes has a crucial impact on the efficiency of polysaccharides fermentation in the gut. However, the relationship between H_2 metabolism and obesity remains far from clear. Employing obese breed Meishan pigs and lean breed Yorkshire pigs, we used metatranscriptomic, metabolomic and in vitro incubation approaches to gain a system-wide understanding regarding how hydrogenotrophic microbes impact host energy balance. Compared with Yorkshire pigs, Meishan pigs exhibited higher fiber digestibility and more SCFA generation in gut. Increased abundance of *Bacteroides* with more abundant arabinoxylan-targeting CAZymes (e.g., GH43) were observed in Meishan pigs compared with Yorkshire pigs. Distinct H_2 uptake pathways were identified between the 2 breed pigs: methanogenesis (e.g., *Methanobrevibacter*) and acetogenesis (e.g., *Blautia*) were significantly enriched in Meishan pigs, whereas sulfate reduction (e.g., *Desulfovibrio*) was enriched

in Yorkshire pigs. In vitro experiment showed the lower H_2 concentration in the incubations of Meishan pigs compared with Yorkshire pigs during fermentation, confirming that the higher numbers of methanogens would accelerate microbial arabinoxylan degradation by efficiently removing H_2 in obese-type pigs. These observations emphasize that H_2 transfer between saccharolytic *Bacteroides* and H_2-utilizing methanogens or acetogens may server as an important mechanism for improving host energy harvesting from indigestible polysaccharides. Our findings also provide novel therapeutic clues for the manipulation of specific hydrogenotrophic species to prevent obesity.

This Fire Poster Pitch describes abstract 59 in the poster session.

Key Words: gut microbiome, obesity, hydrogenotrophic microbes, polysaccharide fermentation

20 Effect of succinate on the metabolic activity of *Selenomonas ruminantium*.

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Ruminant animals utilize plant fiber as an energy source by converting cellulose and hemicellulose to short-chain fatty acids by ruminal fermentation. In the rumen, fiber-degrading bacteria and non-fiber-degrading bacteria cooperatively degrade plant fibers. In the previous study, we have revealed that 2 bacterial species, the fiber-degrading bacterium *Fibrobacter succinogenes* and the non-fiber-degrading bacterium *Selenomonas ruminantium*, cooperatively contribute to fiber degradation. It is known that *S. ruminantium* metabolizes succinate, the primary fermentation product of *F. succinogenes*, and converts it to propionate. In this study, we investigated the alteration of the metabolic activity of *S. ruminantium* in the presence of succinate. *S. ruminantium* S137 was used as the test strain, and xylose, one of the fiber degradation products, was used as the carbon source. The motility of *S. ruminantium* S137 was evaluated based on the colony size formed after 12 h of incubation on a soft agar medium with or without succinate. The growth and gene expression of *S. ruminantium* S137 in the medium with or without succinate were monitored by real-time PCR and RNA-Seq, respectively. The colony size of *S. ruminantium* S137 decreased on the medium containing succinate, while succinate did not affect the growth. RNA-Seq analysis showed that exposure to succinate suppressed the expression of flagellar protein genes, which reduced the motility of *S. ruminantium* S137. On the other hand, adding succinate to the medium increased the gene expression of proteins involved in succinate metabolism and pentose uptake. These results suggest that succinate alters the metabolic activity of *S. ruminantium* by affecting the expression of genes involved in motility and carbohydrate metabolism.

This Fire Poster Pitch describes abstract 61 in the poster session.

Key Words: rumen bacteria, fiber digestion, bacterial interaction, gene expression

21 Characterization of a dual steroid 3β-, 17β-oxidoreductase in the gut bacterium *Eggerthella lenta*.

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As of 2023 there are over 200,000 new cases and over 30,000 deaths caused by prostate cancer, with African American (AA) men having a higher incidence then other ethnicities according to the American Cancer Society. Numerous therapeutic approaches are used to treat prostate cancer such as hormone therapy, radiation therapy, chemotherapy, and chemical castration. However, prostate cancer often becomes metastatic resulting in death with the 5-year survival rate for AA men presenting with castration resistant prostate cancer (CRPC) being ~32%. Androgen production is known to be the main driver of CRPC progression. It generally remains unclear why this progression is taking place after patients have received a combination of these therapeutic methods to stop androgen production and metabolism. *Eggerthella lenta* has a long history as an important bile acid and steroid metabolizing gut microbial species. We

and others have previously published on the extensive oxidation and epimerization of bile acid hydroxyl groups. Previous work demonstrated that *E. lenta* may be involved in the conversion of 17-keto steroids to testosterone. By cloning and expressing genes predicted to encode pyridine nucleotide-dependent oxidoreductases from *E. lenta* DSM 2243, we identified a recombinant enzyme with dual 3β, 17β-hydroxysteroid dehydrogenase activity. We performed kinetic analysis with both 5α-reduced bile acids ("allo"-bile acids) and 5α-reduced steroids. There is a paucity of work at present on gut bacteria that metabolize the 17β-hydroxy group on steroids, so this observation demonstrates that a unique enzyme in the gut microbiome can alter host steroids. These findings are consistent with the possibility that gut or urinary tract bacteria may contribute to CRPC.

This Fire Poster Pitch describes abstract 63 in the poster session.

Key Words: *Eggerthella lenta*, oxidoreductase, 17β-hydroxysteroid dehydrogenase, testosterone

22 Optimizing the recovery of prokaryote metagenome-assembled genomes (MAGs) from the challenging cow rumen microbiome.

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Ruminant animals, including beef and dairy cattle, contribute significantly to methane emissions[1] and, as such, play a pivotal role in the environmental challenges associated with the emission of greenhouse gasses. An in-depth understanding of the ruminant microbiome adds to our knowledge of the intricate connections between the host, feed, gut microbiome, production yield, and methane emissions. Obtaining a detailed genetic blueprint of the microbiome coupled with state-of-the-art taxonomy provides a solid foundation for understanding the metabolic potential and is a prerequisite for downstream analysis such as metatranscriptomics, metaproteomics, and metabolomics. The cow rumen microbiome is particularly challenging due to a high level of *Prevotella* microdiversity, which limits resolution and hinders obtaining a high-quality data set using traditional short-read DNA sequencing and analysis workflows. Here, we demonstrate the use of a state-of-the-art Oxford Nanopore long-read DNA sequencing[2] workflow to generate 24 cow rumen metagenomes and evaluate the use of different metagenomic binning strategies for the recovery of MIMAG[3] high-quality metagenome-assembled genomes (MAGs) from the cow rumen microbiome. [1]Ripple, W. J. et al. Ruminants, climate change and climate policy. *Nat Clim Chang*4, 2–5 (2014). [2]Sereika, M. et al. Oxford Nanopore R10.4 long-read sequencing enables the generation of near-finished bacterial genomes from pure cultures and metagenomes without short-read or reference polishing. *Nat Methods*19, 823–826 (2022). [3]Bowers, R. M. et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nature Biotechnology* vol. 35 725–731 Preprint at https://doi.org/10.1038/nbt.3893 (2017).

This Fire Poster Pitch describes abstract 64 in the poster session.

Key Words: metagenomics, DNA long-read sequencing, rumen microbiomics, MAG binning

23 Methanogenesis inhibition stimulates acetogenesis by novel microbes in ruminants.

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Rumen microbiota enable ruminants to convert carbohydrate-rich feed into valuable proteins, but methane production by microbes accounts for over 5% of global greenhouse gas emissions and a loss of gross energy content from the feedstock by up to 12%. Methanogenesis inhibitors such as the 3-nitrooxypropanol (3-NOP), a potent inhibitor of the key enzyme methyl-coenzyme M reductase (MCR), decrease methane emissions and potentially increase productivity in ruminants when added to the feed in milligram amounts. However, we lack a species-resolved understanding of the collective microbiota responses to inhibitors, including the fate of the hydrogen gas typically consumed by methanogens and whether it is consumed by alternative pathways such as acetogenesis. Here, we conducted a comprehensive analysis of microbiota responses to 3-NOP administration across 3 large-scale field trials, pairing host performance, emissions, and nutritional profiles integrated with metagenome and metatranscriptome data and over 10 thousand rumen microbial genomes. Across all 3 trials, 3-NOP inhibits phylogenetically diverse methanogens in a dose-dependent manner, as supported by inhibitor docking studies. 3-NOP caused changes in the levels and expression of other hydrogen-producing microbes, with effects varying between studies and diets. In the trial with the highest methanogenesis inhibition (90%), there was a strong stimulation of acetogenesis. Novel uncultivated microbial lineages are predicted to become the dominant acetogens under these conditions. Collectively, these findings suggest interventions should be prioritized that simultaneously inhibit methanogenesis and stimulate acetogenesis, providing a route to reduce greenhouse gas emissions while increasing animal production.

This Fire Poster Pitch describes abstract 69 in the poster session.

Key Words: enteric methane, hydrogen, 3-nitro-oxypropanol, intervention, livestock

24 Extra-chromosomal elements encode essential functions in rumen *Butyrivibrio fibrisolvens* cultures.

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Rumen butyrivibrios are among the few bacteria shown to possess 2 distinct respiratory enzyme complexes, Rnf and Ech, which couple with 2 ATP synthases in separate chemiosmotic circuits and impact energy conservation in these organisms. In finished genomes from *B. hungatei*, *B. proteoclasticus* and *P. xylanivorans* these genes are all located on the main chromosome. However, each species also has one or more large plasmids that may contain essential genes but have plasmid-like replication machinery. In the closed sequence of the *B. fibrisolvens* type strain (D1, DSM 3071) the genes for the Ech hydrogenase (*echABCDEF*) and the associated hydrogenase maturation proteins (*hypA* and *hypCDEF*) are clustered adjacent to the replication origin of a 243 kb plasmid. Ten other sequenced *B. fibrisolvens* strains isolated in Argentina, Australia, New Zealand, and the USA belong to a different species group (Genome Taxonomy Database (GTDB): *B. fibrisolvens*_C) from the type strain, but these all showed a similar gene organization. In 2 strains with finished genomes (INBov1 and ASCUSDY19) the *ech* and maturation genes were found on replicons of 267 and 337 kb. In all strains this same secondary replicon also encodes a large cell wall-associated pectin methylesterase (PME) and numerous genes for glycosyl transferases and other components for synthesis of extracellular polysaccharides. Transcriptome analysis of cocultures between the methanol-producing *B. fibrisolvens* D1 and the methanol-utilizing rumen methanogen *Methanosphaera* sp. ISO3-F5 indicated that the plasmid-encoded PME is responsible for methanol production by D1. When the draft genomes from other rumen *Lachnospiraceae* isolates were screened 5 *Lachnospira* isolates, belonging to 3 GTDB

species groups, also encoded clustered *ech* and hydrogenase maturation genes and a PME gene located close to the replication origin of a putative plasmid. It remains to be established if the copy number of these secondary replicons differs from that of the larger main chromosome and if having these genes on a smaller replicon influences hydrogen or methanol production by these organisms.

This Fire Poster Pitch describes abstract 70 in the poster session.

Key Words: *Butyrivibrio*, plasmid, hydrogenase, pectin methyl esterase, methanol

25 Lactate utilization in anaerobic rumen bacteria.

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Lactate production is widespread among rumen microbes with approximately half of the microbial genomes from the Hungate1000 collection encoding genes for lactate production. Despite this, lactate does not usually accumulate in the rumen because of the presence of cross-feeding bacteria that convert lactate to other VFAs. The small rumen lactate pool belies its potential importance to ruminal metabolism and there is evidence from studies in cattle and sheep that lactate production and utilization underpin low methane (CH_4) emission phenotypes. The genetic basis for lactate utilization is not fully understood in rumen bacteria and few lactate-utilizing cultures have been identified. Competition for lactate between different lactate utilizers in the rumen has not been explored in detail. To investigate endogenous lactate metabolism in the rumen and its linkage with reduced CH_4 emissions we screened the genomes of bacteria from the Hungate1000 collection for genes encoding NAD-independent lactate dehydrogenases (iLDHs), lactate permease and lactate racemase. Lactate utilization gene clusters containing a GntR family transcriptional regulator, D-iLDH, electron transfer flavoprotein subunits, lactate permease and lactate racemase genes were found in acetogenic bacteria from the *Eubacteriaceae* and *Peptostreptococcaceae* families and also in *Lachnospiraceae* bacterium FE2018 and in members of the family *Clostridiaceae*. These bacteria are all predicted to convert lactate to butyrate. Bacteria belonging to the class Negativicutes are regarded as the main ruminal lactate utilizers but show different gene arrangements. *Anaerovibrio* and *Selenomonas* convert lactate to propionate via the succinate pathway although *Selenomonas* isolates are diverse and vary in their ability to both produce and utilize lactate. Rumen strains of *Megasphaera elsdenii* can metabolize lactate to butyrate and/or propionate. Genes predicted to be involved in butyrate production by *Megasphaera* have been identified in transcriptomic studies and include lactate permease, lactate racemase and D-iLDH genes. Propionate is produced via the acrylate pathway which is encoded by a cluster of 7 genes. Genes for the acrylate pathway are also found in rumen bacteria from the *Fusobacteriaceae* and *Lachnospiraceae* families.

This Fire Poster Pitch describes abstract 71 in the poster session.

Key Words: lactate, methane, butyrate, propionate, *Megasphaera*

26 Effects on monensin on growth and nitrate/nitrite metabolism of a hypernitrite-metabolizing *Paenibacillus*.

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Paenibacillus strain, 79-R4, isolated from the rumen of a nonlactating Jersey cow grazing bermudagrass pasture, was selected via consecutive culture in media supplemented with increasing concentrations of nitrite to acquire a hypernitrite-metabolizing phenotype. The hypernitrite-metabolizing strain has been patented as a probiotic to mitigate methane

emissions from ruminants fed supplemental nitrate to mitigate rumen methane emissions. This study was conducted to test the susceptibility of the hypernitrite-metabolizing strain to monensin, an antibiotic commonly fed in US cattle production systems. Growth curves during pure culture of the hypernitrite-metabolizing strain in anaerobic Brain Heart Infusion broth exhibited moderate sensitivity to monensin when tested at a dose expected to be encountered in practice, 0.0083 mg/mL culture fluid, with growth initiation occurring after a 4 h lag phase in monensin-treated cultures which was twice that observed with non-treated controls. Maximum optical densities (600 nm) were 50% lower in monensin-treated cultures (0.27) than controls (0.51). However, mean specific growth rates of control and monensin-treated cultures were similar (0.32 and 0.30/h, respectively). Amounts of nitrate catabolized after 24 h incubation did not differ between controls and monensin-treated cultures (7.15 versus 7.45 µmol nitrate/mL, respectively) which was about 87% the amount of nitrate measured at 0 time (8.45 µmol/mL). Residual nitrate was 1.31 µmol/mL or less in control and monensin-treated cultures. Considering that nitrate catabolized is reflective of the amounts of nitrite produced, the amount of nitrite catabolized was calculated as the difference between the amounts of nitrate reduced minus the amounts of nitrite measured. Accordingly, the amounts of nitrite catabolized were nearly 76 to 92% the amount of nitrate reduced, and amounts of residual nitrite measured at the end of the 24 h incubation were 1.50 µmol nitrite/mL in controls and monensin-treated cultures. Results from the present work revealed that growth rates and metabolic nitrate and nitrite-catabolizing activity of the hypernitrite-metabolizing strain were modestly affected by monensin concentrations expected to be encountered in US production systems and thereby may be compatible with US production practices.

This Fire Poster Pitch describes abstract 78 in the poster session.

Key Words: *Paenibacillus*, probiotic, methane, ruminants

27 Native rumen-derived probiotics alter the rumen microbiome and improve production and feed efficiency when fed to lactating dairy cows.

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Advances in next generation sequencing have revealed that a dairy cow's efficiency of milk production may be better predicted by the rumen microbiome than bovine genes. This makes targeted microbiome manipulation a potential pathway to improve animal production efficiency. To test this hypothesis, we performed 16S rRNA and ITS gene amplicon sequencing on over 750 rumen fluid samples from lactating dairy cows across 2 independent experiments, with diet challenges that acutely induced a drop in ECM production and feed efficiency. To identify organisms associated with efficient, high producing animals, we used Louvain heuristics to partition microbial sequences and metadata into communities optimized for modularity. We ranked the organisms most associated with high production and efficiency and found that the highest ranked organisms had a high degree of connectivity, highlighting their potential to influence microbiome composition. From this ranked list, we were able to cultivate 4 representative isolates from rumen fluid (*Clostridium beijerinckii*, *Pichia kudriavzevii*, *Ruminococcus bovis* and *Butyrivibrio fibrisolvens*) that were further developed into a shelf-stable, live-microbe supplement (LMS). Daily administration of LMS in vivo, through animal feed, was tested across 6 independent trial sites. A meta-analysis using a Random-

Effects model demonstrated that LMS conferred a significant improvement in feed efficiency (+0.05 pts, *P* = 0.0003). The factors driving increased feed efficiency correlated with the stage of lactation at the start of treatment, wherein animals that began earlier in lactation showed improved energy-corrected milk production. 16S amplicon sequencing of rumen fluid samples collected during the trials showed a relative reduction in microbial diversity. This aligns with prior research, which has also shown that the rumen microbiome of more feed-efficient dairy cows is dominated by specific functional groups of microbes. Differential abundance analysis using phylofactorization suggested that LMS enriched for bacterial species encoding CAZyme families GH13 and GH43 which specialize in cellulose and hemicellulose degradation. Future studies are needed to understand how microbial changes translate to the observed physiological changes.

This Fire Poster Pitch describes abstract 79 in the poster session.

Key Words: probiotic, rumen, dairy, milk production

Podium presentations: Session 2

28 Holo-omic network analysis reveals bistability in the rumen microbiome.

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The holobiont concept emphasizes analyzing the host and its microbiome as a whole. In this context, "holo-omics" presents quantitative methods that can be used to investigate host-microbiome data and facilitate predictions on biological interactions. Here, we deeply sampled 24 beef cattle (2 breeds, 30% variation in CH4) and their microbiomes across rumen contents, rumen epithelium, and liver tissues. From all samples several molecular layers (genomic, transcriptomic, proteomic, metabolomic) were generated to create a high-resolution data set to investigate metabolism that spanned across the host-microbiome axis. To reconstruct metagenome-assembled genomes (MAGs) that could detect strain-level variation we applied long-read Oxford Nanopore Technology (ONT) using accurate r10.4 flowcells. Together with these ONT-MAGs and collaborator-sourced eukaryotic genomes, we compiled a comprehensive rumen genome database that served as a foundation for extracting, mapping and investigating downstream biomacromolecules. A series of multi-omic analyses established that there appears to be a ubiquitous binary type of strain composition that was defined across 2 distinct groups of animals and is enriched with intermediate fermentative bacteria and methanogens. Interestingly, our investigations to date have shown that neither strain-defined animal group is correlated to animal breed nor any measured key performance indices. Finally, by overlaying modules of co-expressed biomacromolecules across the host-microbiome axis and several molecular layers, we can show that this strain-defined "microbiome split" has a host response in the rumen wall and are subsequently investigating if this signal can also be picked up in the liver.

Key Words: rumen metabolism, holo-omics, multi-omics, network analysis

29 Exploring the influence of plant fiber on gut microbiome diversity.

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The relationship between gut microbe diversity and dietary habits, particularly high-fiber intake, is widely recognized. However, the precise mechanisms underlying this connection and their consistency across diverse gut ecosystems remain enigmatic. Through meticulous examination of community composition, genetic content, and expression patterns, we aim to elucidate the intricate interplay between diet and microbiome dynamics. Our findings suggest a correlation between heightened diversity and phenomena such as niche differentiation and collaborative behaviors among microbial populations, as evidenced by the distribution of genetic pathways. This association transcends various mammalian species and is evident within individual animals across their lifespan. Moreover, we identify specific pathways driving the selection process within microbial communities, ultimately contributing to enhanced community richness. By framing the genetic interactions of microbes within the context of diet, we envision the potential to engineer synthetic microbiomes tailored to desired phenotypic outcomes based on microbial genetic capabilities.

30 Unveiling rumen microbial responses to seaweed supplementation for sustainable livestock nutrition and methane mitigation.

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Seaweed is increasingly recognized as a valuable biomass source for various applications, including biofuel, human consumption and animal feed. Incorporating specific types of seaweed into livestock feed has gained traction in the recent years due to its proven methane mitigating potential. This “blue” biomass additionally holds promise for increased food security when used as a nutritional source for livestock in areas or periods where access to land-based crops is limited, e.g., during drought. Feeding seaweed to cattle is not a novel strategy, and farmers in coastal areas has historically supplemented livestock feed with, e.g., kelp during poor harvest years. However, seaweed-based nutritional supplementation remains relatively unexplored, and the effects on rumen microbiome function and host performance are largely unknown. In this context, our primary objective is to explore the rumen microbial response to seaweed supplementations in the feed. We present findings from diverse in vivo seaweed feeding trials conducted with cattle and lambs, aiming to unveil the potential of nutritional manipulation for methane inhibition (using *Asparagopsis taxiformis*) and feed utilization (using *Saccharina latissima*). Through multilayered meta-omics analysis of rumen microbiomes, we identify functionally important microbial populations through recovery of metagenome-assembled genomes (MAGs) and their expressed protein. The meta-omics data are connected to comprehensive host-related metadata, encompassing enteric gas production, rumen fermentation products, as well as livestock production metrics. Our findings include mechanism for ruminal decomposition of seaweed-derived polymers and shed lights into often overlooked aspects of rumen microbiota such as micro-eukaryotes (i.e., protozoa), providing greater insights into rumen microbiome populations and their functional roles in methane metabolism and feed conversion.

Key Words: rumen microbiome, seaweed, livestock feed, meta-omics

31 Metagenome analysis revealed supplementation with 3-nitrooxypropanol affected rumen methanogenesis and hydrogen metabolism in beef cattle.

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Low carbon agriculture is a global goal for all countries, especially those with a large number of ruminants. Methane (CH_4) is the main greenhouse gas produced by ruminants and is the second most abundant anthropogenic greenhouse gas after carbon dioxide. Ruminant enteric CH_4 production is influenced by the dynamic flow of molecular hydrogen, generated during rumen fermentation and consumed by methanogenic, acetogenic, and respiratory microbes. It has been shown that 3-nitrooxypropanol (3-NOP) reduces enteric CH_4 production in ruminants. It specifically inhibits methyl-coenzyme M reductase (*mcr*), a central enzyme that catalyzes the final step in methanogenesis, resulting in CH_4 production. We conducted metagenome analysis to investigate the impact of short and long-term dietary supplementation of 3-NOP to beef heifers on their rumen microbiome. Briefly, in the short term study, 3-NOP was supplemented to beef heifers at 4 dose levels (0, 0.5, 1.4, and 2.8 g/d) for 28 d, while in the long term study, 3-NOP was supplemented at doses of 0 and 2 g/d for 146 d. Metagenomic analysis revealed that the abundance of the *mcr* gene, measured in counts per million (cpm), tended to be lower in the short term ($P = 0.056$), while it was significantly lower in the long term ($P < 0.05$) compared with those without 3-NOP supplementation. Both studies revealed that the abundance of *mtd* gene which encodes Methylenetetrahydromethanopterin dehydrogenase, another key enzyme in the methanogenesis pathway, was also lowered ($P < 0.05$). Furthermore, the abundance of gene encoding NiFe methanogenic hydrogenase was reduced after 3-NOP supplementation ($P < 0.05$). The studies also showed the abundance of *Methanobrevibacter gottschalkii*, *Methanobrevibacter sp. YE315*, and *Isotricha prostoma* (a protozoan species) were reduced ($P < 0.05$) after 3-NOP supplementation. The observed changes in the pathways studied, including the significant reduction of the *mcr* gene, corresponded with decreased enteric CH_4 production, indicating that 3-NOP not only effectively lowered methanogenesis but also significantly impacted the abundance of methanogens and protozoa. These findings will be the basis for further studies on the relation of reduced CH_4 production, rumen metabolism, and microbial interactions.

Key Words: 3-nitrooxypropanol, metagenome, beef, methane, hydrogen

32 Extracellular electron transfer in the rumen ecosystem is stimulated by conductive materials.

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Hydrogenogenic and hydrogenotrophic (mainly methanogens) rumen microbes establish trophic collaboration via interspecies electron (e^-) transfer mediated by soluble e^- shuttles. However, the presence in the rumen of shuttle-free e^- transfer mechanisms found in other not-gut methanogenic environments is not known. To reveal such mechanisms, we hypothesized that conductive materials (CM) would enrich for microbes linked to the extracellular e^- transfer (EET) and result in increased methane (CH4) production. Consecutive batch culture and in sacco rumen incubations were used to explore this hypothesis. Ten series of 72 h CBC incubations consisting on CM treatments graphene (GPH), magnetite (MGN), or control (CTL without CM) in culture media containing solid or soluble substrates were performed in triplicate. From incubation #3, cultures stabilized and, compared with CTL, CH4 production increased by 40% ($P < 0.001$) in GPH and MGN, regardless of substrate. Major microbial changes were observed in the soluble substrate, where CM enriched for microbes that were poorly or not detected in the CTL and the initial inoculum. *Methanomicrobium mobile* and *Prevotella heparinolytica* increased 3-fold in MGN; while *Desulfovibrio_desulfuricans* increased 4-fold in GPH. Both CM increased *Treponema_D bryantii*, *Clostridium_G cochlearium*, *Succinivibrio*

hippie_B and *Synergistes jonesii* bacteria by >2-fold. Genome-centric analysis, performed on GPH and MGN at incubation #10, showed that cytochrome and pili proteins, which may be involved in direct e^- transfer, were detected in enriched bacterial species. Adherent bacteria recovered from membranes containing CM incubated in sacco in the rumen of 4 sheep confirmed the enrichment of *Treponema_D bryantii* and *Prevotella*. Increased CH4 production and the presence of genes associated with EET in enriched species suggest that this mechanism occurs in the rumen microbial ecosystem.

Key Words: electron transfer, rumen, metagenomic, methane

33 Emission profiles and responses to inhibitor supplements in beef grazing systems.

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Recent studies have shown large reductions (50%–90%) in methane with the use of 3-NOP in feedlot cattle (Almeida et al., 2023). However, the methane mitigating potential and the impact on productivity have not been evaluated in beef cattle under the varying seasonal conditions of northern Australian extensive grazing systems. The aim of the trial was to evaluate the effectiveness of 3 dose levels of inhibitor incorporated in pellets supplemented to heifers grazing tropical pasture in northern Australia compared with the known efficacy in chamber trials. A total of 64 heifers (*Bos indicus*) were allocated to one of the treatment groups: Control; low dose – ~1.25 mg 3-NOP/kg body weight (BW); mid dose – ~2.5 mg 3-NOP/kg BW; high dose – ~5 mg 3-NOP/kg BW. Animals grazed tropical pasture at Lansdown Research station (QLD) during 9 mo (wet and dry seasons). Frequency and timing of supplements was recorded as animals entered autodrafters to gain access to the supplement feeders. Estimates of methane and hydrogen were collected in paddock using 4 Greenfeed Emission Monitors (C-Lock Inc., Rapid City, SD, USA). Animals were sampled for changes in rumen function and monitored for live weight gain across the trial to evaluate the impact to productivity traits. Under grazing conditions there was a significant methane reduction observed with 3-NOP doses, ranging between 4% and 21% for methane released and 2% to 20% for methane intensity (g/kg live weight). Conversely, H_2 expelled by treated animals showed a significant increase when methane was inhibited with no significant treatment effect observed on live body, indicating no negative impact on productivity. Changes in volatile fatty acids were significant increases in propionate, butyrate and branch-chain fatty acids and a decrease in acetate at mid and high doses of 3-NOP along with higher levels of formic acid. Seasonal variation in pasture quality was observed to increase the frequency of visits to the supplement feeder in the dry and reduce the herd variance in methane emissions for each treatment. On poorer-quality pastures the daily emission profile for methane release was consistent across the day compared with a biphasic pattern on better-quality pastures. The methane abatement and hydrogen increase in the current trial were similar to the results obtained when the same doses were supplemented to cattle fed tropical forage in control feeding conditions, which validates the use of this compound under grazing conditions. No detrimental effects were observed on the animals, and rumen fermentation and microbial patterns were typical of methanogenesis inhibition by 3-NOP. Further research needs to be carried out to study new supplement delivery methods and technologies to redirect the hydrogen excess available when methane is inhibited.

Key Words: methane, grazing behavior, methane intensity, rumen

Podium presentations: Session 3

34 *Invited talk:* A census of genomes from the rumen: Charting new frontiers beyond Hungate1000.

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Gaining a complete understanding of rumen microbiota is pivotal for enhancing ruminant livestock productivity, curbing methane emissions, and advancing biofuel development. Previously, the Hungate1000 study unveiled 410 bacterial and archaeal isolates accompanied by their genomes, encompassing every cultivated rumen-associated family. Subsequently, rumen metagenomes and metagenome-assembled genomes (MAGs) flooded sequence databases and illuminated hidden uncultivated taxa. Here, we assess the extent of diversity represented by these cultivated and uncultivated genomes by conducting a comprehensive census of the rumen through a metagenomic lens. We also explore comparisons of isolates versus MAGs as an avenue to glean new insights and support renewed cultivation efforts targeting rumen microbes.

Key Words: methane emissions, MAGs, rumen microbes

35 Holistic multidisciplinary analysis of antimicrobial resistance in neonatal calves and dairy farm environments.

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The neonatal phase is critical for calves, marked by heightened susceptibility to diseases and health challenges. Despite its significance, our understanding of antimicrobial resistance (AMR) in neonatal calves and their associated environments is limited. Here we perform AMR surveillance within 10 dairy farms. These were surveyed for >90 parameters including hygiene practices and antibiotic usage. Both culture-based and culture-independent techniques including shotgun metagenomic sequencing were used to characterize the resistomes present in calf feces and calf housing environments including feed equipment, calf feed and milk fed to calves. Samples were tested in vitro for phenotypic AMR against 7 antibiotic classes: penicillins, macrolides, phenicols, aminoglycosides, tetracyclines, synthetic and polymyxins. Resistant bacteria were isolated and underwent 16S rDNA and genome sequencing, and multidrug resistance (MDR) testing. The study revealed high AMR abundance and diversity in calf houses, with metagenomics revealing high resistance gene abundances within fecal samples, while resistant bacterial cfu/mL reached 9.57×10^8. Phenotypic resistance to 6 antibiotic classes was detected on all farms, with the highest resistance against neomycin and trimethoprim. Of the 84 anaerobic AMR isolates 16S sequenced, 66 isolates (78.6%) were MDR, while 36.0% of the 75 aerobic isolates were MDR. Our multidisciplinary study in dairy farms reveals a highly diverse resistome in neonatal calves and their housing environments, emphasizing the urgent need for targeted interventions to address this growing threat. The prevalence of MDR isolates underscores the complexity of the challenge and highlights the importance of understanding and therefore mitigating AMR in dairy farming.

Key Words: antimicrobial resistance, calves, microbiome, ruminants, calf housing

36 Peptidase distribution among rumen ciliates: A bioinformatic perspective on lysosomal enzyme profiles.

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The rumen microbiome, comprising bacteria, archaea, fungi, ciliates, and viruses, significantly influences feed digestion, ruminant nutrition, and

animal productivity. Despite their biodiversity and significant role in rumen functions, rumen ciliates remain understudied. As predators, they regulate the populations and metabolism of rumen microbes. Rumen ciliates uniquely engulf, disrupt their prey, and degrade microbial protein, leading to the wasteful intraruminal protein recycling and low dietary nitrogen efficiency. Lysosomal enzymes, particularly peptidases, are mainly responsible for microbial protein degradation. Some studies have demonstrated that inhibition of peptidases reduces ciliate growth and ammonia production *invitro*. However, only few lysosomal enzymes have been identified. To address this knowledge gap, we delved into the peptidases encoded by the genomes of rumen ciliates, spanning 23 strains across 10 species within 6 genera, including entodiniomorphs and holotrichs. Using various bioinformatic tools, we profiled all the peptidases across the ciliates and elucidated their phylogenetic relationships. All the catalytic types of peptidases were found in all the ciliate species, with cysteine peptidases being the most predominant (30–60% of all peptidases), followed by metallo (11–50%) and serine (11–30%). The strains of *Dasytricha ruminantium* appeared to have a lower predominance of cysteine peptidases (30–38%) than the other ciliates (46–61%). Besides, some protease families are consistently predominant in all the ciliates. These include C01 (papain), C02 (calpain), and C19 (ubiquitin-specific protease) of cysteine; M20 (carboxypeptidase) of metallo, and S09 (prolyl oligopeptidase) of serine peptidases. Subcellular locations of the peptidase were predicted based on signal peptides and transmembrane domains. Lysosomal enzymes were observed to be abundant in all the ciliate species. Species of *Entodinium*, the most predominant genus of rumen protozoa, appeared to possess more lysosomal peptidases compared with others, in line with their high bacterivorous activities. This study sheds new light on lysosomal peptidases in ciliates, offering insights into targeted inhibition of lysosomal peptidases to improve dietary nitrogen assimilation in ruminants.

Key Words: feed digestion, lysosomal peptidase, protease family, rumen protozoa

37 Interrogating the diversity and ecological importance of viral dark matter in the rumen ecosystem.

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The rumen hosts a diverse ecosystem comprising multiple kingdoms, including bacteria, archaea, fungi, protozoa, and viruses. Together, the rumen microbes digest and ferment otherwise indigestible feed, supplying the majority of energy and metabolizable nitrogen to ruminants. While extensive research has highlighted the significant roles of rumen bacteria, archaea, fungi, and protozoa, the understanding of rumen viruses remains limited. Here, we mine 975 rumen metagenomes from diverse ruminants, both domesticated and wild, across 5 continents to create the first rumen virome database. From the curated database, we assign the taxonomy to the identified viruses and predict their probable hosts. Then, we identify the auxiliary metabolism genes (AMGs) and antimicrobial resistance genes in the viral genomes and experimentally validate the functionality of AMGs that are of great ecological and nutritional importance. Lastly, we delve into the interaction between rumen viruses and microbes and their associations with critical animal production traits in 9 independent studies. This study leads to the identification of around 400,000 virus species, most of which are unique and divergent when compared with previously identified viruses. Rumen viruses are predicted to infect key rumen microbes, including those involved in fiber digestion, microbial N synthesis, and methane producers. Genomic analyses uncover rumen viruses whose genomes encode unique AMGs directly augmenting and modulating microbial metabolism. Additionally, some viral genomes carry antimicrobial resistance genes, underscoring the potential to mediate antimicrobial resistance transmission. Lastly, we observed that rumen viruses respond to dietary changes and correlate with certain animal production traits, including feed efficiency, lactation performance, and methane emissions. Variations in rumen viral profiles

largely mirror shifts in the microbiome structure, but alterations in the biochemical environment could instigate changes in the viral lifecycle, which can subsequently affect the rumen microbiome structure. Taken together, our results showed that rumen viruses can regulate multiple aspects of the rumen microbial ecosystem, affecting microbial populations, evolution, and physiology with the potential to influence animal performance traits.

Key Words: rumen microbiome, microbe-virus interaction, virome

38 Montana's wild ruminants are protected from a toxic dietary alkaloid by rumen-located fungi.

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Montana is home to 8 species of wild or semi-wild ruminant animals, including pronghorn antelope, mule and white-tailed deer, elk, moose, bighorn sheep, mountain goat, and bison. Due to their range and habitat, wild ruminants are likely to encounter toxic plant species, including larkspur. Larkspur grows abundantly in the mountainous western region of North America and presents a serious toxicity danger to rangeland cattle killing 5–15% of range cattle each year. Over a 2-year period, ruminal gut samples were collected from almost 160 wild ruminants in Montana for comparative molecular analyses of microbiome and diet and tested in vitro for their ability to degrade methyllycaconitine (MLA), the primary toxin of larkspur. MLA was found to be variably degraded by wild rumen samples up to 81% over a 48h period. MLA-degrading activity appeared to correlate with known larkspur distribution across the state and with the ruminal retention of a subset of plants, including many known to be rich in alkaloids. The MLA-degrading activity was traced to the non-bacterial fraction of the microbiome and one fungal isolate from wild sheep was found that was able to degrade >70% of MLA over the same duration.

Key Words: wildlife, rumen, fungi, plant toxin, delphinium

39 Using color as an indicator of the effectiveness of buccal swabs as a proxy for direct ruminal sampling of the rumen bacterial community in Holstein dairy cows.

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Increasing milk production efficiency is vital to cutting costs and reducing greenhouse gas emissions from farms. The rumen microbiome is essential for nutrient provisioning in dairy cows and has been shown to significantly impact milk production efficiency. However, direct ruminal sampling is time- and labor-intensive. A rapid method of capturing the ruminal microbial community will allow producers and researchers to characterize the structure of a herd's microbial community more effectively. The usage of buccal swabbing has been considered as a proxy for direct ruminal sampling, due to the cow's regurgitation of rumen contents to chew to aid in further digestion (chewing the cud). Previous work from our lab identified timing as an important factor in determining the effectiveness of buccal swabs as a proxy. We hypothesized that the color of the collected buccal swab (Light, Medium and Dark) is directly correlated to the structure of the microbial community, where a darker swab is more similar to the rumen community compared with a lighter swab. To test this, we used an Illumina MiSeq to amplify the V4 region of bacterial DNA collected

from 12 rumen solid, 13 rumen liquid, and 303 buccal swab samples collected from a farm in southern Wisconsin, USA. We compared the microbiotas between different color swabs and the ruminal microbiotas. Our results indicate no difference in α diversity between the dark swabs and rumen samples, and a significant difference between the light swabs and rumen samples, and the light swabs and dark swabs ($P < 0.05$). A SIMPER with Kruskal-Wallace analysis identified amplicon sequence variants (ASVs) that drove the differences between the communities. We identified bacteria commonly associated with the oral cavity as being the largest drivers of differences between the light swabs and the rumen samples ($P < 0.05$; *Pasteurellaceae*, *Neisseriaceae*, *Rothia*, *Bibersteinia*). This research suggests we can use the color of collected buccal swabs to quickly determine the ability of the swab to characterize the rumen bacterial community. These results also open the door to future work that would remove oral-associated microbes from the data set to further improve the effectiveness of buccal swabbing.

Key Words: buccal swabbing, rumen ecology, dairy industry

Podium presentations: Session 4

40 Complexities and simplicities highlighted by systematic evaluation and improvement of partial gene predictions on unassembled reads.

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Genome annotation is a difficult computational challenge that is often reliant on the observation of previously discovered genes, both putative and predicted. Statistical analysis conducted on these genes and their host genomes is used to build representative models for describing their characteristics. Additionally, the numerous challenges associated with genome assembly, whether for cultured isolates or environmental DNA, introduce a host of additional complexities, particularly when dealing with metagenomic samples. As sequencing depth and costs have caught up and even surpassed computational capabilities, it is now common for large metagenomic assembly projects to not effectively incorporate large proportions, often up to half, of their read collection. However, while tools to study unassembled reads have been somewhat successful in studying function and taxonomy, they most often rely on alignments of the entire read to a precomputed database. This does not allow for the investigation of genes without database similarities or for the future reconstruction of the full gene product. Predicting gene content directly from unassembled reads can help overcome several variables such as assembly error and reduce computational complexity. Therefore, we provide a full evaluation framework for the prediction of genes, both fragmented and whole, directly from reads. We demonstrate that the additional insights provided by this framework for annotation correctness means that previous tools need to be comprehensively re-evaluated. Furthermore, we developed read annotation approaches, one utilizing a Convolutional Neural Network and another purposefully built using naive assumptions, and found that their performance was similar or sometimes improved over contemporary state-of-the-art methods.

Key Words: assembly-free, annotation, benchmark, alignment-free, metagenomic

41 Lessons learnt in microbiome intervention strategies.

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As humanity expands pressure is being applied to food production systems (FPS) to develop nutritious, efficient and sustainable practices, such as optimizing health benefits from food, improving feed conversion and animal welfare and mitigating harmful byproducts such as greenhouse gases (GHG). One promising route to FPS advancement is using deeper understanding of the intimate genetic and physiological connection between animals and their microbiota to devise microbiome-based interventions that control key phenotypes of interest. However, first we must unlock critical and poorly understood microbiota and their biological pathways that control digestion as well as identify exploitable interactions that exist within the complexity of gut microbiomes. Our research seeks to combine high-resolution genome-guided meta-omics technologies with enzymology, bacteriology, bioinformatics and phenotyping of relevant digestive eco-systems from human and production animals (pigs, fish and ruminants). Herein we highlight how such an integrated approach can be used to visualize the stimulatory effects that distinctive dietary fibers have upon known model microorganisms within a complex endogenous microbiome. We further reveal the metabolic influence of uncharacterized bacterial and eukaryotic populations that are surprisingly conserved across diverse dietary conditions and host species. Importantly, as we develop with the technological advancements, we are actively "retrofitting" our analyses to include high-throughput cultivation strategies to bring keystone microbes into the lab for their first time, with the long-term objective being: to understand, monitor and ultimately manipulate host-microbiome interactions.

42 The use of fast protein in diets of weaned pigs improves total gain.

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The faster a protein can enter the bloodstream, the more of it might be used for protein synthesis. The current study aimed to evaluate whether differences in protein digestion speed, so-called protein kinetics, measured in vitro were indicative for growth performance of weaned pigs. For this study, in vitro protein kinetics were measured for 3 protein sources: soybean meal (SBM), a soy protein concentrate (SPC) and enzyme treated soybean meal (ESBM) using the pH-Stat method. The pH-stat method quantifies progress of protein hydrolysis by indirect measurement of released acid during hydrolysis. In vitro digestion speed was highest for ESBM followed by SBM and then SPC (160, 50, and 40 µL/mole, respectively). Subsequently 4 antibiotic-free diets were formulated containing either LOW (17%) or HIGH (20%) levels of CP using SBM and SPC (SLOW) or SBM and ESBM (FAST) combination. All diets were supplemented with synthetic amino acids to meet the requirements. A total of 256 weaned intact male piglets (YxL; age 28 d at 7.3 kg) were allocated based on weight with 16 replicates per treatments in a 28-d 2×2 factorial design study. Data were analyzed by two-way ANOVA. There were no interactions found so only protein kinetics results will be discussed. Feed intake did not differ for FAST or SLOW for d 0–14 ($P > 0.05$). There was a numerical difference in FCR between FAST and SLOW (1.26 vs. 1.30, d 0–14 and 1.31 vs. 1.32 for d 0–28, respectively; $P > 0.05$). Total weight gain was positively influenced by digestion speed with higher gains for FAST 11.1 ± 2.0 kg compared with SLOW 9.8 ± 2.1 kg, respectively *(*$P < 0.05$). Day-28 BW was higher for pigs fed FAST diets compared with SLOW (18.5 ± 3.0 vs. 17.0 ± 2.6 kg; $P < 0.05$). The results of this study indicate differences in in vitro protein kinetics can be measured in in vivo performance of weaned pigs resulting in improved growth in pigs on fast protein diets. This indicates that faster protein is better utilized than slow protein. To prove this, the nitrogen excretion data from this trial will be analyzed to confirm that pigs on fast protein indeed have a higher protein efficiency and lower nitrogen excretion. The use of protein kinetics in piglet diet allows nutritionists to optimize the protein utilization and reduce (environmental) waste of nitrogen.

Key Words: piglets, protein kinetics, gain, protein utilization

43 The effect of a *Bacillus*-based probiotic on the ruminal microbiota and milk measurements in lactating cows.

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To determine the effect of a *Bacillus*-based probiotic on lactating cows, 28 animals were split into 2 groups with half receiving 2.0x10^9 cfu/g of a 2 strain *Bacillus* based direct-fed microbial (DFM) for 25 wk. There were no differences in parity or days in milk between the groups. Dry matter intake (DMI) was measured daily during wk 1–4 and 19–25, and body weight was measured weekly. Milk samples were taken weekly from both milkings within a day and analyzed for percentage milk fat, protein, lactose, and milk urea nitrogen. Rumen fluid was collected at 13 and 25 wk of treatment. DNA was extracted from rumen fluid samples and the proportions of *Ruminococcus albus*, *Ruminococcus flavefaciens*, as well as *Fibrobacter succinogenes* groups I, II, and IV relative to total bacteria, determined by qPCR. The composition of the ruminal bacteria microbiota was determined by amplicon sequencing the V4 region of the 16S rRNA gene. Beta diversity was compared using Bray-Curtis dissimilarity and Jaccard similarity coefficient. Differences in feed intake, body weight, milk measures, and qPCR results between groups were determined by a repeated measures 2x2 factorial ANOVA. Alpha and β diversity were compared by Kruskal-Wallis test, and pairwise PERMANOVA, respectively. The percentage of variation and taxa associated with groups was determined by constrained linear ordination. The results showed that *Bacillus* supplementation reduced DMI during wk 1 through 4 ($P < 0.05$). Additionally, milk fat percentage and milk urea nitrogen were increased in the treated group throughout the trial ($P < 0.05$). The relative abundance of *R. albus* and *F. succinogenes* group I was increased in the rumen of treated cows ($P < 0.05$). Rumen microbiota were different ($P < 0.05$) between treatments both as a main effect and within time points. Treatment had a larger impact explaining 8.3% of the variation, while time accounted for 5.7%. Three genera of the family Ruminococcaceae were more abundant in treated cows, whereas 2 genera of the class Negativicutes were more abundant in control cows. Overall, supplementation with *Bacillus* DFM altered the rumen microbiota and increased milk nutrients while decreasing DMI.

Key Words: microbiome, probiotics, lactation

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

44 Utilizing the COVID-19 pandemic as an opportunity to examine the role of mass gatherings in the emergence of antimicrobial resistance: A wastewater-based surveillance from 2020 to 2022.

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Mass gatherings (MGs), as defined by the World Health Organization (WHO), represent large congregations during short durations, exerting substantial strain on health planning and response capacities. An exemplary MG is the annual Hajj in Makkah and Medinah, with approximately 2 million attendees, and the year-round Umrah with reduced congregation. MGs, especially those involving global pilgrims, pose challenges to antimicrobial resistance (AMR) transmission due to potential carriage of antimicrobial resistance genes (ARG) and bacteria (ARB). Inadequate wastewater treatment systems further compounds AMR concerns. Amidst the COVID-19 pandemic, Saudi Arabia temporarily closed its borders, impacting Hajj and Umrah attendance. Upon reopening, pilgrim numbers surged, prompting questions about the role of MGs in AMR dissemination. To address these queries, we developed a statistical model predicting ARG importation into Saudi Arabia post-border reopening, utilizing global sewage metagenomes from 2017 to 2019 and socioeconomic factors of each country. Concurrently, we collected raw sewage from Makkah and Medinah which are more MG-impacted than the control site at KAUST. Omics-based analysis indicated no general AMR increase that correlated with pilgrim numbers. However, specific ARGs, particularly Extended Spectrum Beta-Lactamase/Metallo-Beta-Lactamase (ESBL/MBL), revealed dynamic responses to MGs. New ESBL/MBL genes were identified in raw wastewater and were potentially introduced by foreign pilgrim during post-border reopening. Bacterial isolation confirmed *Shewanella putrefaciens* as carriers of ESBL/MBL, notably PER-7. Genomic analysis further uncovered PER-7 integration into chromosomes, leading to genomic alterations and the acquisition of multiple ARGs by the bacterial host. Dynamic AMR responses during MGs, coupled with genomic insights into integration events and horizontal gene transfer, underscore the complexity of AMR transmission pathways. In conclusion, our study pioneers the detection of AMR dissemination during global travel, emphasizing the need to understand AMR from a One-Health perspective.

Key Words: antimicrobial resistance, COVID-19, wastewater-based surveillance, mass gatherings

45 Evaluating agriculture byproducts as potential feed additives for reducing enteric methane emissions.

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Livestock production contributes to a significant amount of greenhouse gas emissions. Enteric methane (CH_4), produced during the anaerobic breakdown of feedstuff in the rumen, alone accounts for 28% of total anthropogenic CH_4 emissions. In addition to the negative environmental impact, the released CH_4 also represents a loss of 2–12% gross energy intake to the animal, which explains the growing interest in advanced and sustainable strategies to reduce CH_4 production from ruminants. Numerous feed additives, including the red macroalgae *Asparagopsis taxiformis*, have shown to reduce enteric CH_4 production. However, large scale utilization of feed additives involves numerous several challenges, including geographic and seasonal availability of feed additives that are

potent inhibitors of methanogenesis. In this context, byproducts from local and regional plant-based agriculture compounds have gained special interest. Agriculture byproducts that are generated from the cultivation and processing of a wide variety of crops such as hulls and meals from the nut industry, pomace from fruits and vegetable processing, and even whole crops that are deemed unsellable by the producers have been considered as viable options as potential feed additives. Despite being unsuitable for human consumption, these agricultural byproducts still contain a high level of energy that can be utilized by ruminants as a food source and may contain bioactive compounds such as tannins and saponins that can alter rumen microbes and rumen fermentation. Here we present first results from a project during which we evaluate the potential of agricultural byproducts to reduce CH_4 emissions from enteric fermentation. We tested these locally sourced agricultural byproducts and compared their methanogenesis inhibition potential to that of *A. taxiformis* over 24 h of in vitro rumen fermentation. Byproducts tested until today did not significantly inhibit CH_4 production, but we anticipate that results from this work will be the foundation to further test other byproducts generated from agriculture.

Key Words: byproducts, feed additives, methane, rumen, mitigation

46 Genome analysis of ruminal *Selenomonas* reveals multiple pathways for hydrogen production and utilization.

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Selenomonas are anaerobic, gram-negative, crescent-shaped, motile, rumen bacteria which grow on a wide range of soluble carbohydrates. The genomes of 16 *Selenomonas* strains were sequenced in the Hungate1000 project, which confirmed many of their metabolic capabilities, but also revealed multiple pathways for H_2 production and utilization. Most strains encode multiple hydrogenases, including at least one and up to 3 [FeFe] Group A1, fermentative, H_2-evolving, hydrogenases using reduced ferredoxin as the electron donor, and a [NiFe] Group 1d hydrogenase enabling hydrogenotrophic respiration using fumarate as the terminal electron acceptor. *S. ruminantium* strains HD4, L14, S137, GACV-9, AB3002, KH1T6, and GA192 also encode the fumarate reductase (*frdA*) involved in fumarate respiration. In *S. ruminantium* strains S137, AC2024, C3, WCT3, *S. ruminantium lactilytica* TAM6421 and *Selenomonas* sp. FC4001, their [FeFe] Group A1 hydrogenase was consistently associated with a [NiFe] Group 3c heterodisulfide reductase-linked hydrogenase which bifurcates electrons from H_2 to heterodisulfide and ferredoxin. All but 3 *Selenomonas* encoded the genes required for dissimilatory sulfate reduction, and growth tests of *S. ruminantium* GA192, AB3002 and HD4 and *S. ruminantium lactilytica* PC18 confirmed sulfate reduction while *S. bovis* 8–14–1 was negative. Respiratory membrane-bound nitrate reductase genes *narGHIJ* were found in *S. ruminantium* C3, GACV-9, HD4, L14, WCT3, Z108, S137, *S. ruminantium lactilytica* PC18, TAM6421 and *Selenomonas* sp. FC4001, ND2010, while a periplasmic dissimilatory nitrate reductase *napA* was present in all strains except *S. bovis*, *S. ruminantium* WCT3, and *Selenomonas* sp.AE3005, ND2010. All *Selenomonas* strains apart from *S. bovis* 8–14–1, possess a respiratory nitrite reductase (*nrfA*) which catalyzes the reduction of nitrite to ammonium, and apart from *S. ruminantium* C3, GACV-9, Z108 and *Selenomonas* sp. AE3005, FC4001, encode the N_2-fixing di-nitrogenase reductase *nifHDK* genes even though ruminal N_2 fixation is extremely low. All strains had 2 L-lactate dehydrogenases (except *S. bovis*) and an alcohol dehydrogenase explaining their production and use of lactate and suggesting ethanol as a possible fermentation product.

Key Words: *Selenomonas*, genome, hydrogen

47 Prevalence and characteristics of ESBL-producing *Escherichia coli* in clinically healthy pigs: Implications for antibiotic resistance spread in livestock.

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This study aimed to compare and characterize the resistance profile and the presence of ESBL-related genes in *E. coli* isolated from healthy finishing pigs fed with or without antibiotics in their diets. A total of 27 ceftiofur-resistant *E. coli* isolates were obtained from 96 healthy pigs. The antibiotic resistance profile was tested, and all 27 isolates were classified as multidrug resistant (MDR). A high proportion of isolates were resistant to cephalosporins, ampicillin, ciprofloxacin, and tetracyclines. The ESBL production was observed in 85% of isolates by double-disc synergy test. The MDR-*E. coli* isolates harbored ESBL genes, such as $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, and $bla_{CTX\text{-}M\text{-}8,25}$. In addition, other ARGs were also detected, such as *sul2*, *ant(3")-I*, *tetA*, and *mcr-1*. The mobilization of the $bla_{CTX\text{-}M}$ gene was confirmed for 9 *E. coli* isolates by conjugation assays. The presence of $bla_{CTX\text{-}M}$ on mobile genetic elements in these isolates was demonstrated by Southern blot hybridization, and the resistance to cephalosporins was confirmed in the transconjugants. Our results indicate the prevalence of CTX-M-producing *E. coli* strains harboring mobile genetic elements in the normal microbiota of healthy pigs. These findings highlight the significance of ESBL genes as a global health concern in livestock and the potential spread of resistance to other members of the gastrointestinal tract microbiota.

Key Words: antimicrobial resistance, swine, PCR, CTX-M-type extended-spectrum β-lactamases, conjugative plasmids

48 Inhibition of methanogenesis during anaerobic digestion: Effects on short-chain fatty acid production and proportions.

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Anaerobic digestion is a classic example of a resource recovery process that generates methane-containing biogas. However, CH_4 is a cheap and abundant commodity and thus alternate higher-value compounds are desirable in addition to eliminating CH_4 production. In this research, we focused on inhibiting methanogenesis during anaerobic digestion and its effects on short-chain fatty acid production and proportions. Custom-designed CSTR bioreactors were employed, inoculated with manure from beef cattle (10% of inoculum), and operated at 40°C with a 20-d retention time. These bioreactors continuously received a glucose-based medium (10 and 20 g/L of glucose), yielding a mix of acids including lactate (average 7.14 g/L[rm1]), acetate (1.13 g/L), butyrate (0.59 g/L), and propionate (0.32 g/L) and some ethanol (maximum 19.6 g/L). The predominant lactate production at low pH is consistent with fermentation by lactic acid bacteria and *Streptococcus bovis.* In general, when comparing glucose 20 g/L with 10 g/L conditions, several differences were observed. There was a higher total acid concentration (average 10.31 g/L vs. 8.18 g/L); the production of butyrate, propionate, and acetate increased; and ethanol production was also higher (average 5.06 g/L vs. 3.54 g/L). When the pH was adjusted to above pH 6.5, ethanol production was not observed. Our bioreactor studies

demonstrated that maintaining low pH (around pH 4.0) effectively prevented methane formation by inhibiting methanogenesis. Our future research will focus on investigating the microbial community composition and activity to refine our understanding and the overall carboxylate production process when methanogenesis is inhibited especially at higher and more neutral pH.

Key Words: anaerobic digestion, cow manure, carboxylation, ethanol, bioreactor

49 Assessing the correlations between ruminal methanogenic populations and methane traits: A meta-analysis.

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In this study, the objective was to investigate the relationship between the abundance of ruminal methanogenic populations and methane production in ruminant animals. We analyzed 25 studies published between 2011 and 2023, involving cattle, buffaloes, and sheep, with data from 30 experiments. The selection of articles was based on a systematic review, prioritizing methane production and total population of rumen archaea. Preliminary results revealed that there is no significant correlation between the population of methanogenic archaea and the methane traits in the rumen of cattle (R = −0.056, P = 0.68) and sheep (R = 0.057, P = 0.85). However, a robust correlation was observed in buffaloes (R = 0.86, P < 0.01). Furthermore, significant correlations were found between ruminal methane traits and concentration of ruminal acetate in buffaloes (R = 0.65, P = 0.003) and sheep (R = 0.55, P = 0.04). However, in cattle, this correlation was only evident when diets were supplemented with industrial by-products (R = 0.72, P = 0.046) and plant products (R = 0.69, P = 0.038). Other analyzes indicated correlations between the archaeal population and ruminal acetate in buffaloes (R = 0.54, P = 0.017) and sheep (R = 0.64, P = 0.015), being significant mainly in cattle supplemented with solid-state fermentation products (R = 0.92, P = 0.01). Moreover, ruminal methane traits were not correlated with ruminal propionate in buffaloes (R = −0.038, P = 0.88) and sheep (R = 0.23, P = 0.43), and in cattle this correlation was only detected when animals were supplemented with plant products (R = −0.94, P < 0.01). Similarly, no correlation was observed between ruminal propionate concentration and the archaeal population in the rumen of buffaloes (R = 0.043, P = 0.86) and sheep (R = 0.14, P = 0.63), while in cattle supplemented with chemical additives (R = 0.65, P = 0.043) and solid-state fermentation products (R = −0.88, P = 0.021), a significant correlation was observed. These findings point to a variable influence of archaea on methane production and ruminal fermentation processes, with dietary conditions playing a crucial role in modulating their effects. Understanding specific contributions of ruminal methanogens to enteric methane emissions can lead to targeted approaches to improve the environmental sustainability of ruminant production.

Key Words: qPCR, enteric fermentation, ruminant, methanogens

Immunology (including host-microbe interactions)

50 Breed-driven microbiome heterogeneity regulates intestinal stem cell proliferation via *Lactobacillus*-lactate-GPR81 signaling.

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Genetically lean and obese individuals have distinct intestinal microbiota and function. However, the underlying mechanisms of the microbiome heterogeneity and its regulation on epithelial function such as intestinal stem cell fate remain unclear. Employing pigs of genetically distinct breeds (obese Meishan and lean Yorkshire), we identified the transcriptome-wide difference in microbial ecology of small intestine that characterized by enrichment of active *Lactobacillus* species, notably the predominant *L. amylovorus*, and lactate metabolism network in obese breeds. The *Lactobacillus*-dominant heterogeneity was paralleled with epithelial functionality difference as reflected by more proliferative intestinal stem cells and activated Wnt/β-catenin signaling. Studies using in-house developed porcine jejunal organoids proved that live *L. amylovorus* and its metabolite lactate promoted intestinal organoids growth. Further investigation has demonstrated that lactate activated Wnt/β-catenin signaling in a GPR81-dependent manner to promote intestinal stem cell-mediated epithelial proliferation. However, heat-killed *L. amylovorus* failed to cause these changes. These results demonstrate that *Lactobacillus* species are important determinants linking to phenotype variation and critical regulators affecting intestinal stem cell function in the small intestine.

Key Words: microbiome, *Lactobacillus*, small intestine, intestinal stem cells, Wnt/β-catenin signaling

51 Psychological stress-induced changes to gut epithelial transcriptomic profile parallel shifts in the microbiome through a mechanism involving β-adrenergic receptors.

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Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract with complex and not fully understood etiology. Although the genetic basis of IBD has been extensively studied, less is known about the underlying mechanisms of environmental factors, such as psychological stress, that contribute to its development. Interactions between gut microbiota and abnormal immune responses have been reported to play a crucial role, but the mechanisms remain unclear. Here, we investigated how psychological stress modifies gut epithelial-microbiome interactions at the mucosal interface and the underlying signaling pathways. Adult male C57BL/6 mice (n = 6/group) were subjected to social disruption stress (SDR) for 6 consecutive days after receiving daily i.p. injections of pharmacological antagonists to inhibit α2-adrenergic receptor (AR; Idazoxan), β-AR (Propranolol), glucocorticoid receptor (Mifepristone), and CRH1 receptor (Antalarmin). Colonic cells were sorted by MACS into CD45−/EpCAM+ (intestinal epithelial cells; IECs) and CD45+ (intraepithelial lymphocytes; IELs). IECs were analyzed by targeted gene expression Fluidigm, and IELs by untargeted RNA-Seq. 16s rRNA sequencing for microbiome analysis was performed in colonic contents. Stress led to significant changes in the transcriptomic profile of IECs, particularly upregulation of ROS/RNS signaling. Blockade of β-AR prevented these changes, whereas other stress-hormone blockades did not, suggesting a key role for β-AR in mediating stress effects. Interestingly, IECs do not express β-AR, indicating a potential extrinsic regulation. The transcriptomic profile of IELs dramatically changed with stress, while it returned to the baseline profile upon stress + β-AR blockade. Additionally, the host-microbiome interactions were affected by stress, where β-AR blockade rescued the stress-induced decrease in α diversity and relative abundance of stress-sensitive bacterial taxa. These findings suggest that psychological stress disrupts the epithelial physiology and the key gut immune cells, which

express β-AR, directly interface with IECs and the gut microbiota. Our long-term goal is to determine how stress-induced modification of IECs, IELs, and gut microbiota leads to mucosal disruption and IBD predisposition.

Key Words: intestinal epithelial cells, intraepithelial lymphocytes, psychological stress, microbiome shift

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

52 Anaerobic fungal carbohydrate-binding modules possess prominent preference to assist the enzymatic hydrolysis of hemicellulose.

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Anaerobic fungi dwelling at the gastrointestinal tract of herbivores robustly deconstruct the plant biomass by diversified carbohydrate active enzymes (CAZymes) acting in concert. As the auxiliary domains of the CAZymes, carbohydrate-binding modules (CBMs) influence the enzymatic hydrolysis of substrates by the recognition and adhesion effects. In this study, the microbial source and distribution of CBMs were investigated. Results showed that bacteria and fungi are the 2 dominant contributors to produce CBMs. Then the architecture analysis of bacterial and fungal CBM proteins was completed through the proteomic data from CAZy database. Data displayed that more than 50% of CBMs from bacteria and fungi are incorporated into their parent CAZymes, respectively, which suggests the crucial role of CBMs in the diverse enzymatic reactions. Moreover, the 68% of fungal CBMs are integrated into the cellulase, hemicellulase, and acetyl xylan esterase. Based on this consequence, the composition module of CAZymes from 36 representative aerobic fungi and 12 anaerobic fungi was furtherly analyzed. Results showed that the anaerobic fungal CBMs are biased to connect with to hemicellulase (e.g., GH10, GH11 and GH43) and acetyl xylan esterase (e.g., CE1 and CE4). Averagely 32% of these anaerobic fungal CBM-fused CAZymes are presented in the carbohydrate active enzyme gene clusters (CGCs). Subsequently, the distribution of CBM-fused CAZymes in the CGCs related to hemicellulose degradation were compared between the present 12 anaerobic fungi and 12 typical fibrolytic bacteria. Results indicate that anaerobic fungi have greater numbers of CBM-fused CGCs available for degrading hemicellulose than that of fibrolytic bacteria. Finally, 3 upregulated CBM-fused CGCs related to the breakdown of arabinoxylan based on the transcriptomic data of anaerobic fungus *Pecoramyces ruminantium* sp. F1 were characterized. These results suggest that anaerobic fungal CBMs harbor unprecedented potential to assist the enzymatic hydrolysis of hemicellulose based on the CGCs level.

Key Words: anaerobic fungi, carbohydrate-binding modules, carbohydrate active enzyme gene clusters, hemicellulose degradation

53 Microbiome-mediated colonization resistance against necrotic enteritis.

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Necrotic enteritis (NE), caused by *Clostridium perfringens*, ranks among the most financially devastating diseases in poultry. Unfortunately, there are currently no effective preventive or therapeutic measures available. The intestinal

microbiota plays a critical role in maintaining animal health and productivity by resisting the colonization and proliferation of invading pathogens. Inbred chicken lines such as Fayoumi line M5.1 are known to be naturally resistant to NE. To explore whether the microbiomes of NE-resistant chickens offer better protection against NE than those of susceptible chicken lines, we transplanted the cecal microbiota from different chicken breeds to naïve, day-of-hatch Cobb broilers, followed by NE induction and evaluation of the disease outcome. We found that the cecal microbiota of the resistant chicken line provided 95–98% protection from NE, while approximately 40% of chickens died from severe intestinal lesions without microbiota transplantation. Interestingly, the cecal microbiota from susceptible chicken lines also conferred significant protection of naïve Cobb chickens from NE, although with slightly reduced efficacy. Our findings strongly suggest that the gut microbiota provides strong colonization resistance to NE. To further identify commensal bacteria responsible for NE resistance, we screened a library of cecal bacteria from healthy feral chickens and identified several bacteria with a strong ability to inhibit *C. perfringens*, while also enhancing innate immunity through the induction of host defense peptide synthesis. Notably, oral administration of a selected bacterium to day-of-hatch broilers improved the survival rate to 98% in a chicken model of NE, while 52% of chickens died without intervention. Moreover, the bacterium significantly alleviated the severity of intestinal lesions. Collectively, these results clearly demonstrate the potential of commensal bacteria as probiotics for NE mitigation.

Key Words: colonization resistance, necrotic enteritis, microbiome, microbiota transplantation, probiotics

54 Understanding the host–microbiome interactions involved in liver abscess formation in beef cattle.

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Feedlot diets are usually formulated to provide ≤10% dry matter as roughage. Diets low in roughage increase the incidence of metabolic disorders, with 20–35% of cattle in feedlots developing liver abscesses. Severe liver abscesses are linked to reduced feed efficiency costing the Canadian beef industry ≈$61.2 million annually. The processes involved in the development of liver abscesses are not well understood. We present our efforts to examine potential host–rumen microbiome interactions involved in the development of liver abscesses in cattle. To examine the impact abscesses have on liver function, we have employed RNA-seq and found significant alterations in gene expression in abscessed livers. We characterized the rumen microbiome of cattle with and without liver abscesses and did not observe a relationship between the rumen microbial community and the development of a liver abscess. We have also characterized the liver abscess microbiome using a metataxonomic approach and found a low diversity community dominated by *Fusobacterium* spp. and *Bacteroides* spp. Abscess size and severity impacted the richness and composition of the abscess microbiome with large, severe abscesses having more diversity than small abscesses. To further characterize these pathogens we have isolated *Fusobacteria* from liver, abscess and gut samples. Genomic approaches are being used to compare the phylogenomic relationship of *Fusobacteria* isolated from the gut and abscessed liver of cattle. This work provides novel insight into the host-pathogen interactions that lead to the development of liver abscesses, and should provide insight into strategies to reduce antimicrobial use in beef cattle.

Key Words: liver abscess, cattle, rumen, *Fusobacterium*

55 Bioinformatic prediction of potential ncRNAs in *Escherichia coli* O157:H7.

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Escherichia coli O157:H7 (STEC) infection remains one of the most frequently reported foodborne illnesses in humans due to its production of Shiga-toxins, adhesion proteins, and plasmid-derived virulence factors. Cattle are a definitive source of STEC, and the prevention of STEC shedding in cattle and/or reduction of STEC virulence is an important control strategy. It is estimated that a decrease of 50% of STEC colonization in cattle could reduce infections in humans by 80%. Non-coding RNAs (ncRNA) are naturally expressed regulatory elements in microbes and are species specific, raising the possibility that they could be used for a targeted RNA interference approach of virulence factors and/or essential survival genes in STEC. Therefore, the objective of this study was to identify ncRNAs in the STEC genome that could potentially reduce the prevalence of virulent STEC in cattle. The whole genome of *E. coli* O157:H7 (NC_0002695) was acquired from the NCBI database and subjected to ncRNA prediction using RNAspace. A total of 872 ncRNAs were predicted. Repeated hits were combined, and transfer RNAs and ribosomal RNAs were removed, leaving 199 hits to be further analyzed. The remaining 199 ncRNA candidates were visualized on SnapGene against the NCBI annotated *E. coli* O157:H7 genome (NC_0002695). A total of 83 ncRNAs were filtered out based on coding sequence overlap and strand direction, leaving a total of 116 predicted ncRNAs for further investigation. The predicted ncRNAs ranged from 30 nt to 409 nt in size; 112 were regulatory ncRNAs (<300 nt), and only 4 were housekeeping ncRNAs (>300 nt), with the majority classed as short ncRNAs (<200 nt). Additionally, 30 ncRNAs were predicted to have antisense activity to known coding genes (partial or full overlap), while the majority of the predicted ncRNAs were intergenic (n = 86). Further research is ongoing to confirm whether these sequences are unique to *E. coli* O157:H7, and whether they can be expressed in vivo.

Key Words: cattle, *Escherichia coli*, foodborne illness, non-coding RNA, rumen

56 Conversion of plant polysaccharides to propionate by anaerobic (gut) *Bacteroidota*.

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Bacteria of the phylum *Bacteroidota* are abundant members of the gut microbiome and are well-known for their ability to degrade a variety of polysaccharides [1]. Besides, they also produce organic acids such as propionate, which is an important precursor chemical in industry [2]. Although the degradation of dietary fibers by *Bacteroidota* has been extensively studied with regard to host health, their biotechnological potential has not yet been investigated. In this study, we aim to identify strains that degrade plant polymers and convert them to organic acids with regard to sustainable propionate production. New isolates have been obtained using selective conditions for polymer using *Bacteroidota*. We compared the ability of 13 isolates and 14 type strains to produce organic acids with xylan, pectin, starch, inulin or cellobiose as substrate. *B. graminisolvens* and isolate *D. gadei* BGG-A1 were the only strains which formed propionate as the main product, but only BGG-A1 was able to use all tested substrates. To test the isolate's fermentation characteristics, it was grown in a pH-controlled batch culture with a high xylan concentration (36 g/L), where a shift in product composition was observed: Now lactate was the main product (106 m*M*), followed by propionate (43 m*M*). Subsequently, we checked the genome of BGG-A1 for the presence of known key polysaccharide-degrading enzymes. This was done by homology search with the PUL-database entries for the *D. gadei* type strain. A polysaccharide utilization locus (PUL) containing a GH10 xylanase could be found in both strains, whereas a GH13 amylase is not present in the

type strain according to PUL-database. This was surprising, since BGG-A1 shows good growth on starch and a GH13 is crucial in *Bacteroides* sp. starch utilization [3]. In conclusion, we screened 27 *Bacteroidota* strains for their conversion of different substrates to organic acids, where isolate BGG-A1 turned out to be the most promising candidate with regard to propionate production. In the future, we aim to characterize the function of selected GH enzymes from BGG-A1 as well as to apply genetic tools to reduce side products during the fermentation process. [1] Glowacki and Martens, 2021, *J. Bacteriol.* [2] Gonzalez-Garcia et al., 2017, *Fermentation.* [3] Brown et al., 2023, *Cell Mol. Life Sci.*

Key Words: *Bacteroidota*, polysaccharides, propionate

57 Investigating the distinguished gut bacteria for pregnant and long-lived sows in the early breeding stage and their variation through 4 parities.

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Sows may be in non-estrus or return to estrus during the breeding stage, which is influenced by genetic, nutritional and environmental factors. The estrus status determines the recyclability and reproductive performance of breeding sows. Meanwhile, microbes seem to be related to reproduction of sows. However, whether and which bacteria are related to the pregnancy status of sows remain vague. To investigate the microbial differences between pregnant sows or not in the early breeding stages, 48 sows in 4 parities were followed. According to the different status of sows, they were divided into 4 groups: non-estrus (NE); return to estrus (RE); fewer than 4 pregnancies (L4P); 4 pregnancies (U4P). The fecal swabs were collected, and DNA were extracted for 16S sequencing via Illumina MiSeq platform. In the first day in breeding stage of first parity, the observed ASVs index was the highest in the L4P group compared with U4P and NE&RE group. To observe the distinguished bacteria in each group, LEfSe analysis was used to identify the ASV99, ASV234 and ASV32 of total 8 bacteria in U4P group. ASV20 and more than 10 bacteria were enriched in NE&RE group while ASV17 and more than 10 bacteria in L4P group. Among these enriched ASVs in different groups, the correlation network showed that enriched ASVs in U4P showed a positive correlation with NE&RE group but less connection with L4P. Additionally, L4P distinguished ASVs demonstrated negative correlations with NE&RE. Following these biomarkers bacteria for different estrus of sows in U4P group through 4 parties, ASV99 (*Arcanobacterium urinimassiliense*) seemed to be the “passenger” bacteria, which only showed the high relative abundance in the first parity. ASV32 (*Peptoniphilus grossensis*) and ASV59 (*Hoylesella timonensis*) seemed to be “resident” bacteria, which maintained a certain abundance through 4 parties. Biomarkers for L4P such as ASV122 (*Treponema porcinum*) showed low but variable abundance in U4P through 4 parties while biomarkers for NE&RE including ASV315 (*Corynebacterium testudinoris*) and several other bacteria showed persistently low abundance.

Key Words: sows, bacteria, pregnancy, longevity

58 Transcriptomic insights into the anti-ruminal *Streptococcus* activity of natural compound betulin.

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Streptococcus is a causative agent of rumen acidosis, a gastrointestinal disorder in ruminal livestock. In vitro studies have shown that betulin, a natural compound contained in the outer bark of birch trees, selectively inhibits the growth of *Streptococcus*, including ruminal *S. equinus* ATCC33317, without negatively impacting rumen fermentation. While these results suggest that betulin could effectively prevent the onset of rumen acidosis, the mechanism by which betulin inhibits ruminal *Streptococcus* remains

unknown. To address this, we examined the effect of betulin on the transcriptional activity of *S. equinus* ATCC33317. *S. equinus* ATCC33317 was cultured in MRS medium and inoculated with a solution of betulin (final concentration 300 μg/mL) or equivalent concentration of dimethyl sulfoxide as a control. Triplicate tubes were incubated anaerobically for 12 h at 39°C, and samples were collected for transcriptomic analysis and betulin quantification. We identified the *pur* gene cluster and the *nrdIEF* gene cluster as being downregulated by betulin. The *pur* gene cluster encodes a group of enzymes crucial for purine de novo biosynthesis, which is essential for DNA and RNA biosynthesis. The *nrdIEF* gene cluster encodes for multiple enzymes, including a rate-limiting enzyme for DNA/RNA synthesis. These findings indicate that betulin partially suppressed the transcriptional activity for DNA/RNA synthesis in *S. equinus* ATCC3317. Moreover, gene clusters involved in the metabolism of arabinan were upregulated. Arabinan is catabolized to arabinose, which can be synthesized into phosphoribosyl pyrophosphate, a purine de novo biosynthesis precursor. These genes may be upregulated to mobilize pentose for DNA/RNA synthesis due to the inhibitory effects of betulin. Furthermore, betulin concentration was decreased during the logarithmic growth phase, indicating that betulin was metabolized by *S. equinus* ATCC33317. These findings offer valuable insight into the molecular mechanism by which betulin may inhibit the growth of *Streptococcus* in the rumen, and its potential use as an antimicrobial agent for preventing rumen acidosis in livestock.

Key Words: *Streptococcus*, betulin, rumen acidosis, transcriptome, antimicrobial agents

59 Hydrogenotrophic methanogens facilitate microbial energy harvesting from plant polysaccharides in breed-determined obese pigs.

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Gut microbiome has been thought to promote obesity by increasing energy extraction from indigestible plant polysaccharides. H_2 removal by hydrogenotrophic microbes has a crucial impact on the efficiency of polysaccharides fermentation in the gut. However, the relationship between H_2 metabolism and obesity remains far from clear. Employing obese breed Meishan pigs and lean breed Yorkshire pigs, we used metatranscriptomic, metabolomic and in vitro incubation approaches to gain a system-wide understanding regarding how hydrogenotrophic microbes impact host energy balance. Compared with Yorkshire pigs, Meishan pigs exhibited higher fiber digestibility and more SCFA generation in gut. Increased abundance of *Bacteroides* with more abundant arabinoxylan-targeting CAZymes (e.g., GH43) were observed in Meishan pigs compared with Yorkshire pigs. Distinct H_2 uptake pathways were identified between the 2 breed pigs: methanogenesis (e.g., *Methanobrevibacter*) and acetogenesis (e.g., *Blautia*) were significantly enriched in Meishan pigs, whereas sulfate reduction (e.g., *Desulfovibrio*) was enriched in Yorkshire pigs. In vitro experiment showed the lower H_2 concentration in the incubations of Meishan pigs compared with Yorkshire pigs during fermentation, confirming that the higher numbers of methanogens would accelerate microbial arabinoxylan degradation by efficiently removing H_2 in obese-type pigs. These observations emphasize that H_2 transfer between saccharolytic *Bacteroides* and H_2-utilizing methanogens or acetogens may server as an important mechanism for improving host energy harvesting from indigestible polysaccharides. Our findings also provide novel therapeutic clues

for the manipulation of specific hydrogenotrophic species to prevent obesity.

Key Words: gut microbiome, obesity, hydrogenotrophic microbes, polysaccharide fermentation

60 Fibrolytic function of the horse fecal microbiome: Elderly versus adult healthy individuals.

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Fiber digestion results from the vital fibrolytic function carried by the large intestine microbiota. A preliminary study in horses suggests that changes in fiber digestion occur during aging. Concomitantly, a reduction in α-diversity, and a reorganization of fecal bacterial communities are reported. Given the limited data on horse health in this study, it is questionable whether these changes are due to aging alone. To identify shifts that may lead to age-related pathologies, it is essential to define microbiome parameters in elderly horses that have undergone healthy aging. This study aimed to compare the fibrolytic function of the fecal microbiome between healthy horses aged over 20 years (elderly) and those aged under 10 years (adult). After a 3-week habituation period during which the horses were fed a high-fiber diet, we collected their feces. We determined the α- and β-diversity, the type of bacterial communities (16S rRNA gene sequencing), the concentrations of cellulolytic bacteria (cultural approach) and fibrolytic enzymes (colorimetric assay). We also inoculated feces in bottles containing culture medium and fibrous substrate to evaluate gas production and dry matter disappearance over 72 h. The fecal bacterial communities were equally rich (observed ASVs: $P = 0.752$) and diverse (Shannon index: $P = 0.809$) in both groups, but each group had its own structure (PERMANOVA based on Jaccard distance: $P < 0.022$). Elderly horses had a higher abundance of some fibrolytic taxa than adults did (*Fibrobacter:* $P < 0.001$; *Rikenellaceae RC9 gut group:* $P < 0.001$), but concentrations of cellulolytic bacteria ($P = 0.785$) and fibrolytic enzymes (carboxymethylcellulase: $P = 0.164$; xylanase: $P = 0.518$) did not differ between groups. After 72 h of incubation, in vitro gas production ($P < 0.001$) and dry matter disappearance ($P = 0.017$) were greater in the elderly than in the adults. This study confirms that the fecal bacterial communities rearrange during aging, even in elderly horses that had been confirmed to be in good health. Surprisingly, the microbial fibrolytic function appears to increase in healthy elderly compared with healthy adult individuals. The ability to efficiently digest fibers appears to be a hallmark of healthy aging, possibly compensating for the inevitable age-related changes.

Key Words: fecal bacterial communities, fiber breakdown capacity, healthy senior equine, functional assessment

61 Defining the post-evisceration bovine gastrointestinal tract microbiome after lairage at a USDA processing facility.

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In the United States, cattle routinely undergo feed withdrawal between 8 and 24 h before harvest, which can alter the gastrointestinal tract (GIT) microbiome. This study aimed to characterize the GIT microbiome after evisceration, with the length of lairage ranging from 8 to 18 h. Samples were collected in 8 locations across the GIT—rumen solids and liquids, abomasum, duodenum, jejunum, ileum, cecum, and large intestines—from harvested cattle at the USDA Meat Plant at the University of Wisconsin–Madison. Samples were collected after evisceration, and the GIT locations were defined by visual histological identification. After harvest, DNA was extracted with the Qiagen DNeasy Blood & Tissue Kit and amplified at the V4 region of the 16S rRNA gene. Amplicons were then normalized, quantified, and pooled before being loaded onto an Illumina MiSeq.

The paired-end sequences were exported from BaseSpace and analyzed with Qiime2–2023.9 and R Studio. There were significant differences in Kruskal-Wallis evenness ($P < 0.05$) between foregut (rumen solids and liquids) and hindgut (cecum and large intestines), with a higher taxonomic richness in the hindgut locations. Beta diversity metrics were also significant across locations, represented by Bray-Curtis and Weighted Unifrac ($P < 0.001$). Predominant taxa in the foregut include *Prevotella ruminicola* and *Methanobrevibacter* spp., while in the hindgut *Bacteroides* spp. and *Clostridia* spp. were relevant. Interestingly, archaeal populations across the GIT locations had a low relative abundance, with the most dominant genera in all locations being *Methanobrevibacter.* These results provide insight into the bovine GIT microbiome during slaughter. However, further studies are needed to evaluate how the microbial communities shift after feed withdrawal and their influences on meat quality.

Key Words: lairage, harvest, bovine, microbiome, evisceration

62 Effect of succinate on the metabolic activity of *Selenomonas ruminantium*.

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Ruminant animals utilize plant fiber as an energy source by converting cellulose and hemicellulose to short-chain fatty acids by ruminal fermentation. In the rumen, fiber-degrading bacteria and non-fiber-degrading bacteria cooperatively degrade plant fibers. In the previous study, we have revealed that 2 bacterial species, the fiber-degrading bacterium *Fibrobacter succinogenes* and the non-fiber-degrading bacterium *Selenomonas ruminantium*, cooperatively contribute to fiber degradation. It is known that *S. ruminantium* metabolizes succinate, the primary fermentation product of *F. succinogenes*, and converts it to propionate. In this study, we investigated the alteration of the metabolic activity of *S. ruminantium* in the presence of succinate. *S. ruminantium* S137 was used as the test strain, and xylose, one of the fiber degradation products, was used as the carbon source. The motility of *S. ruminantium* S137 was evaluated based on the colony size formed after 12 h of incubation on a soft agar medium with or without succinate. The growth and gene expression of *S. ruminantium* S137 in the medium with or without succinate were monitored by real-time PCR and RNA-Seq, respectively. The colony size of *S. ruminantium* S137 decreased on the medium containing succinate, while succinate did not affect the growth. RNA-Seq analysis showed that exposure to succinate suppressed the expression of flagellar protein genes, which reduced the motility of *S. ruminantium* S137. On the other hand, adding succinate to the medium increased the gene expression of proteins involved in succinate metabolism and pentose uptake. These results suggest that succinate alters the metabolic activity of *S. ruminantium* by affecting the expression of genes involved in motility and carbohydrate metabolism.

Key Words: rumen bacteria, fiber digestion, bacterial interaction, gene expression

63 Characterization of a dual steroid 3β-, 17β-oxidoreductase in the gut bacterium *Eggerthella lenta*.

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As of 2023 there are over 200,000 new cases and over 30,000 deaths caused by prostate cancer, with African American (AA) men having a higher incidence then other ethnicities according to the American Cancer Society. Numerous therapeutic approaches are used to treat prostate cancer such as hormone therapy, radiation therapy, chemotherapy, and chemical castration. However, prostate cancer often becomes metastatic resulting in death with the 5-year survival rate for AA men presenting with castration resistant prostate cancer (CRPC) being ~32%. Androgen production is known to be the main driver of CRPC progression. It generally remains unclear why this progression is taking place after patients have received a combination of these therapeutic methods to stop androgen production and metabolism. *Eggerthella lenta* has a long history as an important bile acid and

steroid metabolizing gut microbial species. We and others have previously published on the extensive oxidation and epimerization of bile acid hydroxyl groups. Previous work demonstrated that *E. lenta* may be involved in the conversion of 17-keto steroids to testosterone. By cloning and expressing genes predicted to encode pyridine nucleotide-dependent oxidoreductases from *E. lenta* DSM 2243, we identified a recombinant enzyme with dual 3β, 17β-hydroxysteroid dehydrogenase activity. We performed kinetic analysis with both 5α-reduced bile acids ("allo"-bile acids) and 5α-reduced steroids. There is a paucity of work at present on gut bacteria that metabolize the 17β-hydroxy group on steroids, so this observation demonstrates that a unique enzyme in the gut microbiome can alter host steroids. These findings are consistent with the possibility that gut or urinary tract bacteria may contribute to CRPC.

Key Words: *Eggerthella lenta*, oxidoreductase, 17β-hydroxysteroid dehydrogenase, testosterone

64 Optimizing the recovery of prokaryote metagenome-assembled genomes (MAGs) from the challenging cow rumen microbiome.

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Ruminant animals, including beef and dairy cattle, contribute significantly to methane emissions[1] and, as such, play a pivotal role in the environmental challenges associated with the emission of greenhouse gasses. An in-depth understanding of the ruminant microbiome adds to our knowledge of the intricate connections between the host, feed, gut microbiome, production yield, and methane emissions. Obtaining a detailed genetic blueprint of the microbiome coupled with state-of-the-art taxonomy provides a solid foundation for understanding the metabolic potential and is a prerequisite for downstream analysis such as metatranscriptomics, metaproteomics, and metabolomics. The cow rumen microbiome is particularly challenging due to a high level of *Prevotella* microdiversity, which limits resolution and hinders obtaining a high-quality data set using traditional short-read DNA sequencing and analysis workflows. Here, we demonstrate the use of a state-of-the-art Oxford Nanopore long-read DNA sequencing[2] workflow to generate 24 cow rumen metagenomes and evaluate the use of different metagenomic binning strategies for the recovery of MIMAG[3] high-quality metagenome-assembled genomes (MAGs) from the cow rumen microbiome. [1]Ripple, W. J. et al. Ruminants, climate change and climate policy. *Nat Clim Chang*4, 2–5 (2014). [2]Sereika, M. et al. Oxford Nanopore R10.4 long-read sequencing enables the generation of near-finished bacterial genomes from pure cultures and metagenomes without short-read or reference polishing. *Nat Methods*19, 823–826 (2022). [3]Bowers, R. M. et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nature Biotechnology* vol. 35 725–731 Preprint at https://doi.org/10.1038/nbt.3893 (2017).

Key Words: metagenomics, DNA long-read sequencing, rumen microbiomics, MAG binning

65 Harnessing endogenous type II-A CRISPR system to achieve genome editing in *Lacticaseibacillus rhamnosus* GG.

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Lacticaseibacillus rhamnosus GG (LGG) is a widely applied probiotic used by the food and pharmaceutical industries. However, the genetic bases of its beneficial properties are mostly uncertain because of the lack of effective genetic manipulation tools. CRISPR-Cas systems are now widely used for genome editing and transcriptional regulation in diverse organisms. The type II system is the most abundant CRISPR-

Cas system found in lactobacilli. In this study, we characterized the type II-A CRISPR system in LGG and successfully established an endogenous CRISPR-Cas9 genome-editing system. Through computer prediction and subsequent confirmation via plasmid interference assays, we identified the CRISPR array and the protospacer adjacent motif (PAM) as 5′-NGAAA-3′. With a PAM and customized single guide RNA (sgRNA) expression cassette, the native CRISPR-Cas9 system was reprogrammed to achieve efficient chromosomal targeting, which was lethal and resulted in the killing of 100% of the cells. This system has been successfully utilized to achieve gene deletion in the fucose catabolic pathway gene, *fucI*, with 100% efficiency. Consequently, the *fucI* knockout strain displayed an inability to utilize L-fucose as the sole carbon source. In the future, this endogenous CRISPR system will be employed for further applications, including more gene deletions, insertions, and single nucleotide substitutions in LGG.

Key Words: CRISPR-Cas9, lactobacilli, genome editing

66 Dietary effects on large intestine fibrolytic function and mucosal integrity in healthy elderly horses.

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Fibrolytic function is correlated with the production of short-chain fatty acids (SCFAs), which help maintain the large intestine (LI) mucosal integrity. A preliminary study reports that LI mucosal integrity is reduced in elderly compared with younger healthy horses. Diet can influence the fibrolytic function, but the consequences for the LI mucosa in elderly horses are unknown. This study aimed to determine the effect of 3 diets on fibrolytic function and LI mucosa integrity in healthy elderly horses. Nine individuals (27 ± 3 years old, 480 ± 76 kg BW) participated in a Latin square design with 3 experimental periods of 21 d each separated by 21-d washout periods. Three iso-energy diets were tested: control (C; 18.8 g DM hay/kg BW), high digestible fiber (HF; 12.3 g DM hay+5 g DM alfalfa+beet pulp mix/kg BW), and starch (S: 12.3 g DM hay+3 g DM barley/kg BW). Feces and blood were collected at the end of each experimental period. In feces, we determined α- and β-diversity, type of bacterial communities (16S rRNA gene sequencing), fibrolytic enzymes (colorimetric assay) and SCFAs (GC) concentrations. In blood, we measured lipopolysaccharides (LPS; HPLC-MS) and acetate (colorimetric assay) concentrations. Fecal bacterial richness (observed ASVs: P = 0.793), diversity (Shannon index: P = 0.874; PERMANOVA based on Bray-Curtis distance: P = 0.964), and relative abundance of fibrolytic taxa did not differ between diets. With the S diet, the microbiota was enriched in the amylolytic family *Succinivibrionaceae* (P < 0.001). Fecal concentrations of fibrolytic enzymes (carboxymethylcellulase: P = 0.941; xylanase: P = 0.788) and total SCFAs (P = 0.180) did not differ between diets. Blood LPS concentration, an indicator of LI mucosal integrity, was higher on the S than on the HF diet (3-OH C18: P = 0.021). Moreover, blood acetate concentration, an indicator of nutrient absorption, was lower on the S than on the C (P = 0.011) and HF (P = 0.008) diets. Although parameters measuring fibrolytic function remained unchanged between diets, the S diet accentuated mucosal permeability and reduced nutrient absorption over a 21-d period. Over a longer period, this could lead to digestive problems and loss of condition in elderly individuals. To limit this, a daily diet based on highly digestible fiber could be recommended.

Key Words: large intestinal permeability, nutrient absorption, microbial fiber degradation

67 Selection of *Streptococcus equinus* HC5 variants with increased production of bovicin HC5 by adaptive laboratory evolution.

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Bovicin HC5 is a bacteriocin synthesized by *Streptococcus equinus* HC5, isolated from rumen, which is capable of inhibiting the growth of various pathogenic and food spoilage bacteria. Achieving a high yield of production and efficient peptide recovery are the main challenges enabling the industrial use of bovicin HC5. In this project, *S. equinus* HC5 was subjected to different incubation temperatures to assess the effects of thermal stress on the growth of this bacterium. Subsequently, adaptive laboratory evolution (ALE) by thermal stress was used to select variants of *S. equinus* HC5 with higher production of bovicin HC5. The results showed that the optimal temperature for *S. equinus* HC5 growth is 42°C and that temperatures above 49°C completely inhibit bacterial growth. The ALE experiment was maintained for 400 generations (100 d) at temperatures of 47°C and 48°C. Eight variants with different phenotypes were selected, and of these, 2 showed increased production of bovicin HC5 by 26% and 140% ($P < 0.05$). The HC5 40048 variant, which had the highest bacteriocin production, showed increased expression of the gene encoding the precursor peptide (bvcA; $P < 0.05$). Furthermore, it presented greater resistance to thermal stress, presenting a higher specific growth rate ($\mu = 1.33 \pm 0.02\ h^{-1}$) and biomass formation ($OD_{600nm} = 4.03 \pm 0.06$) at 48°C than the wild-type strain ($\mu = 0.98 \pm 0.04\ h^{-1}$ and $OD_{600nm} = 1.96 \pm 0.12$; $P < 0.05$). Furthermore, the selected variants showed changes in the cell envelope, with an increase in the concentration of saturated fatty acids and a decrease in the negative residual charge assessed by Zeta potential ($P < 0.05$), which could explain the changes observed in bovicin HC5 recovery. Genomic analyses identified 90 variants in the genomes of adapted cultures, most resulting from single nucleotide polymorphisms (SNPs). Mutations of moderate and high impact (n = 29) occurred in genes encoding protein modifications, such as glycosyltransferases, transcriptional regulators, and proteins involved in cell transport, including a lantibiotic transporter permease. The results presented in this study indicate that ALE applying thermal stress is an effective strategy for selecting bacterial variants with higher production of cell-bound bacteriocins.

Key Words: bacteriocin, comparative genomics, continuous culture, thermal stress

68 Pangenome analysis of *Clostridium scindens*: A bile acid and cortisol metabolizer gut bacterium.

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Clostridium scindens (Csci) is one of few species found responsible for bile acid (BA) dehydroxylation and oxidoreduction, and recently, cortisol conversion into androgens. Excess production of secondary BAs is mechanistically associated with cancers in the GI tract. Csci strains ATCC 35704 (35704) and VPI 12708 (12708) are models to study BA-inducible (*bai*) operons and steroid-17,20-desmolase activity, where each strain activity seems to differ. Thus, Csci genomic architecture may be key to fully understanding cortisol and BA metabolism by microbiome and address possible therapeutic targets. This way, a computational screening in Csci strains was performed to identify common and unique representative genomic and genetic repertoire. A preliminary proteomic profiling of 35704 and 12708 strains with eggNOG suggested that 12708 harbors more genes related to metabolism than 35704. To understand variability among strains an additional total of 32 Csci strains genomes were included. Roary results identified 12,720 gene families, where 1,630 are in the core genome, ~39% related to metabolic processes. Was observed that each genome addition increases the total number of gene families but stabilizes the core genome,

common in open pangenome profiles. Roary and ANI analyses were able to separate the 34 strains into 2 groups of 15 and 19 strains, that differ by ~5%, and an identity ≥ 98% within each group, featured by a pangenome with 428 and 832 coding genes for group 1 (with 35704) and 2 (with 12708) respectively, suggesting 2 distinct microbial species or at least an ongoing speciation process. About 23% of 12708 group pangenome genes are related to carbohydrates and energy metabolism, whereas 37% of 35704 group genes feature coenzymes and inorganic ions metabolism. Alignments for *des* and *bai* genes shows conservation of all *bai* genes known (*baiABCDEFGHIJK*) across 12708 group, but only 2 of 19 genomes harboring the *desABC* operon. In contrast, 35704 group shows more presence of *desABC* genes (10 of 15 strains), but lack of *baiJK* operon. This analysis provided an insight of genomic content and variability among Csci strains denoting clear metabolic differences and the need of understanding the effect of strain variation on host physiology and microbe-microbe interactions.

Key Words: *Clostridium scindens*, pangenome, bile acids, bai, steroid desmolase

69 Methanogenesis inhibition stimulates acetogenesis by novel microbes in ruminants.

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Rumen microbiota enable ruminants to convert carbohydrate-rich feed into valuable proteins, but methane production by microbes accounts for over 5% of global greenhouse gas emissions and a loss of gross energy content from the feedstock by up to 12%. Methanogenesis inhibitors such as the 3-nitrooxypropanol (3-NOP), a potent inhibitor of the key enzyme methyl-coenzyme M reductase (MCR), decrease methane emissions and potentially increase productivity in ruminants when added to the feed in milligram amounts. However, we lack a species-resolved understanding of the collective microbiota responses to inhibitors, including the fate of the hydrogen gas typically consumed by methanogens and whether it is consumed by alternative pathways such as acetogenesis. Here, we conducted a comprehensive analysis of microbiota responses to 3-NOP administration across 3 large-scale field trials, pairing host performance, emissions, and nutritional profiles integrated with metagenome and metatranscriptome data and more than 10,000 rumen microbial genomes. Across all 3 trials, 3-NOP inhibits phylogenetically diverse methanogens in a dose-dependent manner, as supported by inhibitor docking studies. 3-NOP caused changes in the levels and expression of other hydrogen-producing microbes, with effects varying between studies and diets. In the trial with the highest methanogenesis inhibition (90%), there was a strong stimulation of acetogenesis. Novel uncultivated microbial lineages are predicted to become the dominant acetogens under these conditions. Collectively, these findings suggest interventions should be prioritized that simultaneously inhibit methanogenesis and stimulate acetogenesis, providing a route to reduce greenhouse gas emissions while increasing animal production.

Key Words: enteric methane, hydrogen, 3-nitrooxypropanol, intervention, livestock

70 Extra-chromosomal elements encode essential functions in rumen *Butyrivibrio fibrisolvens* cultures.

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Rumen butyrivibrios are among the few bacteria shown to possess 2 distinct respiratory enzyme complexes, Rnf and Ech, which couple with 2 ATP synthases in separate chemiosmotic circuits and impact energy conservation in these organisms. In finished genomes from *B. hungatei*, *B. proteoclasticus* and *P. xylanivorans* these genes are all located on the main chromosome. However, each species also has one or more large plasmids that may contain essential genes but have plasmid-like replication machinery. In the closed sequence of the *B. fibrisolvens* type strain (D1, DSM 3071) the genes for the Ech hydrogenase (*echABCDEF*) and the associated hydrogenase maturation proteins (*hypA* and *hypCDEF*) are clustered adjacent to the replication origin of a 243 kb plasmid. Ten other sequenced *B. fibrisolvens* strains isolated in Argentina, Australia, New Zealand, and the USA belong to a different species group (Genome Taxonomy Database (GTDB): *B. fibrisolvens*_C) from the type strain, but these all showed a similar gene organization. In 2 strains with finished genomes (INBov1 and ASCUSDY19) the *ech* and maturation genes were found on replicons of 267 and 337 kb. In all strains this same secondary replicon also encodes a large cell wall-associated pectin methylesterase (PME) and numerous genes for glycosyl transferases and other components for synthesis of extracellular polysaccharides. Transcriptome analysis of cocultures between the methanol-producing *B. fibrisolvens* D1 and the methanol-utilizing rumen methanogen *Methanosphaera* sp. ISO3-F5 indicated that the plasmid-encoded PME is responsible for methanol production by D1. When the draft genomes from other rumen *Lachnospiraceae* isolates were screened 5 *Lachnospira* isolates, belonging to 3 GTDB species groups, also encoded clustered *ech* and hydrogenase maturation genes and a PME gene located close to the replication origin of a putative plasmid. It remains to be established if the copy number of these secondary replicons differs from that of the larger main chromosome and if having these genes on a smaller replicon influences hydrogen or methanol production by these organisms.

Key Words: *Butyrivibrio*, plasmid, hydrogenase, pectin methyl esterase, methanol

71 Lactate utilization in anaerobic rumen bacteria.

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Lactate production is widespread among rumen microbes with approximately half of the microbial genomes from the Hungate1000 collection encoding genes for lactate production. Despite this, lactate does not usually accumulate in the rumen because of the presence of cross-feeding bacteria that convert lactate to other VFAs. The small rumen lactate pool belies its potential importance to ruminal metabolism and there is evidence from studies in cattle and sheep that lactate production and utilization underpin low methane (CH_4) emission phenotypes. The genetic basis for lactate utilization is not fully understood in rumen bacteria and few lactate-utilizing cultures have been identified. Competition for lactate between different lactate utilizers in the rumen has not been explored in detail. To investigate endogenous lactate metabolism in the rumen and its linkage with reduced CH_4 emissions we screened the genomes of bacteria from the Hungate1000 collection for genes encoding NAD-independent lactate dehydrogenases (iLDHs), lactate permease and lactate racemase. Lactate utilization gene clusters containing a GntR family transcriptional regulator, D-iLDH, electron transfer flavoprotein subunits, lactate permease and lactate racemase genes were found in acetogenic bacteria from the *Eubacteriaceae* and *Peptostreptococcaceae* families and also in *Lachnospiraceae* bacterium FE2018 and in members of the family *Clostridiaceae*. These bacteria are all predicted to convert lactate to butyrate. Bacteria belonging to the class Negativicutes are regarded as the main ruminal lactate utilizers but show different gene arrangements. *Anaerovibrio* and *Selenomonas* convert lactate to propionate via the succinate pathway although *Selenomonas* isolates are diverse and vary in their ability to both produce and utilize lactate. Rumen strains of *Megasphaera elsdenii* can metabolize lactate to butyrate and/or propionate. Genes predicted to be involved in butyrate production by *Megasphaera* have been identified in transcriptomic studies and include

lactate permease, lactate racemase and D-iLDH genes. Propionate is produced via the acrylate pathway, which is encoded by a cluster of 7 genes. Genes for the acrylate pathway are also found in rumen bacteria from the *Fusobacteriaceae* and *Lachnospiraceae* families.

Key Words: lactate, methane, butyrate, propionate, *Megasphaera*

Nutrition and metabolism of livestock, humans, and companion animals

72 Effect of essential oils blend on growth performance, gut morphology, and meat quality in broilers.

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The poultry sector is the most organized and well-developed sector in the world. For a long while, the poultry industry has been using antibiotics as a feed additive to promote growth of the birds. However, antibiotics have been controversial because of severe issues regarding antibiotic residues and antimicrobial resistance. So, it is time to find efficient alternatives. For this purpose, study was conducted on 600 day-old broiler chicks which were divided into 5 treatment groups; each treatment group further comprised 3 replicates. Group A was provided a basal diet having antibiotics and considered as a positive control. Group B was given with basal diet without antibiotics and considered as a negative control. Groups C, D, and E were offered an essential oil blend by spray method at dose rates of 0.12 mL/kg, 0.25 mL/kg, and 0.50 mL/kg of feed, respectively. Duration of the trial was 35 d. Results showed that the body weight gain and feed conversion ratio (FCR) exhibited significant improvement but insignificant in the case of feed consumption. Bodyweight and FCR were better in group C, followed by D. Carcass characteristics such as eviscerated weight and giblet weight were also improved in group C, while dressed weight showed insignificant results. Treatment groups have no effect on antibody titer against ND at the end of the trial. In gut morphology, significantly higher villus height was observed in group D, but villus width and crypt depth remained unaffected. In conclusion, essential oils have enhanced performance, carcass parameters, gut health, meat quality, and decreased total bacterial count.

Key Words: feed additives, essential oils, antibiotics, antimicrobial resistance

73 Detection of ethanol in the rumen and saliva of lactating dairy cows undergoing a feed-induced ruminal acidosis challenge.

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The effects of ruminal microbial activity on the fermentation of substrates and subsequent production of metabolites has been the focus of research endeavors aiming to increase cattle nutrient utilization and mitigate digestive disturbances. Ruminal acidosis, in particular, receives a lot of attention due to its prevalence and financial impact on modern commercial cattle production. It is traditionally defined by a decrease of ruminal pH due to excessive accumulation of volatile fatty acids (VFAs). Under low-pH conditions, ruminal microbes may take advantage of solventogenesis pathways (production of acetone, butanol, and ethanol) as an electron sink. Here, we present a small pilot study in lactating dairy cows that investigated the production of solvents during a feed-induced ruminal acidosis challenge. Three mid-lactation Holstein dairy cows sourced from a commercial farm were acclimated to the facility and subjected to a 10-d acidosis challenge (2 d covariate, 6 d challenge, 2 d recovery). The basal diet was representative of a commercial California TMR ration (48% hay&silage, 52% grain). The challenge diet reduced forage components

(40% hay&silage, 44% grain) and added wheat flour (8%) and steam-rolled barley (8%). Dry matter intake, milk yield, and milk components were measured daily. Saliva was sampled 3 times per day throughout the experiment as a high-throughput proxy measurement of rumen metabolites, and rumen fluid and pH were collected once during the covariate, twice during the induction, and once during the recovery periods. Baseline measurements confirmed low concentrations (<0.01 m*M*) of ethanol in the saliva and rumen fluid of cows, likely arising from the silage. Throughout the challenge period, rumen pH dropped to as low as 4.9, and spikes of ethanol up to 30 m*M* were detected. Clear temporal changes were identified, with the highest concentrations observed 5 h post-feeding. One cow exhibited signs of bathypnea. 16S amplicon sequencing of the rumen microbiome suggests that an accumulation of CO_2 may be occurring before the production of solvents. Additional research is currently underway to better understand and identify the conditions and thresholds that trigger solventogenesis in the rumen, as well as the implications of solvent accumulation on the physiology of the host.

Key Words: rumen, acidosis, ethanol, dairy

74 Induced hindgut acidosis affected ruminal fermentation and gut permeability.

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Leaky gut may be caused by acidotic conditions in the hindgut and lead to systemic inflammation and negative effects on the gastrointestinal tract. The objective was to determine the effects of induced hindgut acidosis in sheep on cecal pH, ruminal fermentation, and gut permeability. Eleven ruminally and cecally cannulated ewes (49 ± 4 kg) were assigned to one of 2 treatments: control (CON) or induced hindgut acidosis (HGA). To induce hindgut acidosis, 3 g wheat starch/kg BW per 24 h was continuously infused via the cecal cannula for 4 d. Control ewes received a constant infusion of deionized water. Chromium EDTA was dosed once daily via the cecal cannula as a marker of gut permeability. Rumen fluid was collected for pH, volatile fatty acids (VFA), and ammonia analysis. Cecal and fecal samples were used to determine pH. Rumen fluid was collected on d 4 for an ex vivo fermentation. Flasks were incubated for 24 h to determine pH, VFA, ammonia and in vitro dry matter digestibility. Tissue samples from the ileum, cecum, and colon were mounted in Ussing chambers to measure transepithelial electrical resistance (TEER). There was a treatment × time effect (P = 0.05) for cecal pH with HGA ewes having lesser cecal pH after d 1. By d 4, cecal pH had dropped to 5.07 for HGA ewes compared with CON ewes which remained above 6.40 throughout the experiment. A treatment × time interaction was also observed (P < 0.01) for fecal pH and followed the same trend as cecal pH. Rumen pH was not affected (P = 0.87) by the interaction of treatment and time. However, treatment affected (P < 0.01) rumen pH as HGA ewes had a lesser rumen pH than CON ewes. Control ewes had lesser ruminal VFA and ammonia concentrations than HGA ewes (P < 0.01). Urinary Cr recovery was not affected by the interaction of treatment and time, or treatment (P ≥ 0.13). Treatment did not affect (P ≥ 0.60) in vitro dry matter disappearance or ex vivo pH. There were no effects (P ≥ 0.11) of treatment, time, or their interaction on ex vivo ammonia or total VFA concentration. In cecal tissue, TEER tended (P = 0.09) to be greater in CON ewes than HGA ewes. In contrast, TEER was not different (P ≥ 0.83) in ileal or colonic tissues between treatments. Induced hindgut acidosis in sheep altered rumen fermentation and tended to increase ex vivo measures of cecal permeability, but did not affect ex vivo rumen fermentation.

Key Words: leaky gut

75 Effects of biochar, monensin and nitrate in beef cattle diets on in vitro volatile fatty acids profile.

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The study aimed to determine the effects of biochar with monensin and nitrate in beef cattle diets under 2 dietary conditions: high forage (Experiment I) and high concentrate (Experiment II) on in vitro volatile fatty acid (VFA) profile. Exp. I utilized a substrate of 80% low-quality hay and 20% concentrate, while Exp. II utilized a substrate composed of 20% corn silage and 80% concentrate. Treatments included: control (no additive); biochar (40 g/kg DM for Exp. I and 20 g/kg DM for Exp. II); monensin (30 mg/kg DM); and nitrate (20 g/kg DM). Both studies were a randomized block design, with treatments as fixed factors and incubation run as a random effect. Each run had 4 flasks (150 mL) per treatment. Each flask contained 500 mg substrate and 50 mL of inoculum (1 buffer: 3 ruminal fluid). After 96 h (Exp. I) and 48 h (Exp. II) of incubation, samples were collected for VFA analysis. In Exp. I, additives affected the total VFA ($P < 0.01$) where additives increased total VFA production compared with the control but had no effect in Exp. II ($P = 0.24$). Treatment effects were observed in individual VFAs. ($P > 0.10$). Monensin led to the least acetate concentration ($P = 0.05$) and greatest propionate concentration ($P < 0.05$). Monensin also yielded the least acetate:propionate ratio, while biochar and nitrate had the greatest A:P ratio ($P < 0.001$). Additives increased butyrate and valerate concentration ($P < 0.05$) and tended to increase isovalerate concentration ($P = 0.10$) compared with the control. In Exp. II, nitrate had the least acetate concentration ($P = 0.02$) compared with control and biochar. Biochar and control had lesser propionate concentrations compared with monensin and nitrate ($P < 0.01$). Consequently, biochar and control had the greatest acetate:propionate ratios. For isobutyrate concentration, control and nitrate had the greatest value ($P = 0.03$). Nitrate also exhibited the greatest valerate concentration ($P = 0.02$). Additives increased isovalerate concentration ($P = 0.02$) compared with the control. Biochar, monensin and nitrate increases VFA total production in high-forage diets, while both monensin and nitrate improve VFA profile in high-concentrate diets.

Key Words: additives, ruminants, sustainability

Prebiotics, probiotics, and DFM development

76 Redirecting 1,2-propanediol from *Salmonella* Dublin to lactobacilli.

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Salmonella enterica serovar Dublin (*S*. Dublin) is a cattle-adapted pathogen transmitted through the fecal-oral route and is associated with high calf mortality, lifetime shedding by cows, and zoonosis. Prevention strategies are lacking; therefore, novel interventions, such as probiotics, can be alternatives to prevent *S*. Dublin colonization and infection in calves. Some lactobacilli have probiotic properties and reduce pathogen gut colonization by acetate, propionate, and antimicrobial production and by competing for nutrients. Gut bacteria that encode the *pdu* operon can utilize 1,2-propanediol (1,2-PD) as a nutrient. Consumption of 1,2-PD by *S. enterica* is proposed to increase virulence, while utilization by *Limosilactobacillus reuteri* promotes acetate and propionate production. Hence, we aimed to determine if *S.* Dublin and calf-derived lactobacilli encode the *pdu* operon and to identify calf-derived lactobacilli that can compete with *S.* Dublin for 1,2-PD. Fourteen lactobacilli isolated from the healthy 2-d-old calf hindgut, and one *S.* Dublin isolate was subjected to whole-genome sequencing using Illumina MiSeq (300

pair end). Among these, we identified the *pdu* operon in *S.* Dublin and 2 *Lm. reuteri* strains (L8 and L13), suggesting competition for 1,2-PD in the calf gut. L8, L13, and a *pduCDE*-negative strain (control) were grown in either de Man, Rogosa, and Sharpe (MRS) or 50% mMRS with or without 1,2-PD, and supernatant metabolite concentration and growth were measured using gas chromatography and spectrophotometry, respectively. Triplicate data were analyzed using Kruskal Wallis non-parametric test in R. The supplementation of 1,2-PD enhanced the final population density and propionate production in L8 and L13, and acetate in L13 ($P < 0.05$). In all conditions, 1,3-propanediol was the dominant metabolite detected in L8 and L13 cultures, indicating synthesis of a unique antimicrobial, reuterin, another benefit of the *Lm. reuteri pdu* operon. Propanediol utilization by L8 and L13 may promote competition with *S.* Dublin in the gut by reducing 1,2-PD availability, increasing *Lm. reuteri* growth, acetate, and propionate, and enabling reuterin synthesis. Future studies should assess the role of *Lm. reuteri* on *S.* Dublin growth and virulence in the presence of 1,2-PD to identify *S.* Dublin mitigation approaches using probiotics.

Key Words: *Salmonella* Dublin, lactobacilli, calves, probiotics

77 Galacto-oligosaccharides regulates intestinal mucosal glycosylation (sialylation) by restoring intestine-microbiota homeostasis.

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Mucus sialylation determines intestinal host-commensal homeostasis. Galacto-oligosaccharides (GOS) play an important role in regulating intestinal microbiota and intestinal mucosal barrier. Whether GOS has a regulatory effect on mucosal sialylation remains unclear. In this study, 30 3 weeks old SD rats were evenly divided into CON group, ABX (antibiotic) group, and ABX+GOS group. After one week of adaptation, rats in the CON group received physiological saline, rats in the ABX group received antibiotic solution, and rats in the ABX+GOS group received a combination of antibiotics and GOS solution for a duration of 2 weeks. The results showed that antibiotics significantly increased intestinal damage as well as the expression levels of inflammatory factors and TLR4/MyD88/NF-κB signaling pathways in intestinal mucosa. Supplementing GOS could repair antibiotic-induced intestinal mucosal damage and reduce the expression levels of inflammatory factors and TLR4/MyD88/NF-κB signaling pathways. Meanwhile, through lectin staining and qPCR, we found that antibiotics significantly increased the level of α 2,6 sialylation and the expression levels of α 2,6 sialyltransferase (ST6GALNACs) in the intestinal mucosa, and significantly decreased the level of α 2,3 sialylation and the expression level of α 2,3 sialyltransferase (ST3GALs). Supplementing GOS restored the levels of sialylation and sialyltransferase expression in the intestinal mucosa. In addition, we found that antibiotics significantly increased the relative abundance of *Escherichia_Shigella* and the content of LPS in the intestine, and significantly decreased the relative abundance of *Lactobacillus*. Supplementing GOS significantly reduced the relative abundance of *Escherichia_Shigella* and the content of LPS in the intestine, and significantly increased the relative abundance of *Lactobacillus*. In summary, antibiotics may affect sialylation of the intestinal mucosa by disrupting the homeostasis of the gut microbiota. GOS may regulate the sialylation level by regulating intestinal microbiota and inhibiting the activation of the TLR4/MyD88/NF-κB signaling pathway in the intestinal mucosa.

Key Words: prebiotic, intestinal microbiota, *Lactobacillus*, O-glycan, sialylation

78 Effects on monensin on growth and nitrate/nitrite metabolism of a hypernitrite-metabolizing *Paenibacillus*.

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Paenibacillus strain, 79-R4, isolated from the rumen of a nonlactating Jersey cow grazing bermudagrass pasture, was selected via consecutive culture in media supplemented with increasing concentrations of nitrite to acquire a hypernitrite-metabolizing phenotype. The hypernitrite-metabolizing strain has been patented as a probiotic to mitigate methane emissions from ruminants fed supplemental nitrate to mitigate rumen methane emissions. This study was conducted to test the susceptibility of the hypernitrite-metabolizing strain to monensin, an antibiotic commonly fed in US cattle production systems. Growth curves during pure culture of the hypernitrite-metabolizing strain in anaerobic Brain Heart Infusion broth exhibited moderate sensitivity to monensin when tested at a dose expected to be encountered in practice, 0.0083 mg/mL culture fluid, with growth initiation occurring after a 4 h lag phase in monensin-treated cultures which was twice that observed with non-treated controls. Maximum optical densities (600 nm) were 50% lower in monensin-treated cultures (0.27) than controls (0.51). However, mean specific growth rates of control and monensin-treated cultures were similar (0.32 and 0.30/h, respectively). Amounts of nitrate catabolized after 24 h incubation did not differ between controls and monensin-treated cultures (7.15 versus 7.45 µmol nitrate/mL, respectively) which was about 87% the amount of nitrate measured at 0 time (8.45 µmol/mL). Residual nitrate was 1.31 µmol/mL or less in control and monensin-treated cultures. Considering that nitrate catabolized is reflective of the amounts of nitrite produced, the amount of nitrite catabolized was calculated as the difference between the amounts of nitrate reduced minus the amounts of nitrite measured. Accordingly, the amounts of nitrite catabolized were nearly 76 to 92% the amount of nitrate reduced, and amounts of residual nitrite measured at the end of the 24 h incubation were 1.50 µmol nitrite/mL in controls and monensin-treated cultures. Results from the present work revealed that growth rates and metabolic nitrate and nitrite-catabolizing activity of the hypernitrite-metabolizing strain were modestly affected by monensin concentrations expected to be encountered in US production systems and thereby may be compatible with US production practices.

Key Words: *Paenibacillus*, probiotic, methane, ruminants

79 Native rumen-derived probiotics alter the rumen microbiome and improve production and feed efficiency when fed to lactating dairy cows.

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Advances in next generation sequencing have revealed that a dairy cow's efficiency of milk production may be better predicted by the rumen microbiome than bovine genes. This makes targeted microbiome manipulation a potential pathway to improve animal production efficiency. To test this hypothesis, we performed 16S rRNA and ITS gene amplicon sequencing on over 750 rumen fluid samples from lactating dairy cows across 2 independent experiments, with diet challenges that acutely induced a drop in ECM production and feed efficiency. To identify organisms associated with efficient, high

producing animals, we used Louvain heuristics to partition microbial sequences and metadata into communities optimized for modularity. We ranked the organisms most associated with high production and efficiency and found that the highest ranked organisms had a high degree of connectivity, highlighting their potential to influence microbiome composition. From this ranked list, we were able to cultivate 4 representative isolates from rumen fluid (*Clostridium beijerinckii*, *Pichia kudriavzevii*, *Ruminococcus bovis*, and *Butyrivibrio fibrisolvens*) that were further developed into a shelf-stable, live-microbe supplement (LMS). Daily administration of LMS in vivo, through animal feed, was tested across 6 independent trial sites. A meta-analysis using a Random-Effects model demonstrated that LMS conferred a significant improvement in feed efficiency (+0.05 pts, P = 0.0003). The factors driving increased feed efficiency correlated with the stage of lactation at the start of treatment, wherein animals that began earlier in lactation showed improved energy-corrected milk production. 16S amplicon sequencing of rumen fluid samples collected during the trials showed a relative reduction in microbial diversity. This aligns with prior research, which has also shown that the rumen microbiome of more feed-efficient dairy cows is dominated by specific functional groups of microbes. Differential abundance analysis using phylofactorization suggested that LMS enriched for bacterial species encoding CAZyme families GH13 and GH43, which specialize in cellulose and hemicellulose degradation. Future studies are needed to understand how microbial changes translate to the observed physiological changes.

Key Words: probiotic, rumen, dairy, milk production

80 The effect of *Bacillus* strain supplementation to sows on maternal microbiome and piglet microbiome.

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The objective was to investigate the impact of *Bacillus subtilis* and *Bacillus licheniformis* supplementation to sows during the late gestation and lactation on maternal milk and fecal microbiome, and piglet oral, nasal, and fecal microbiome. Twenty-four pregnant sows were blocked by parity and randomly assigned to the control (Con) group and *Bacillus* group. From 24 d before farrowing throughout the lactation period, sows were fed with a control diet, or the control diet supplemented with *Bacillus*. Swabs were collected from these different body sites for microbiome analysis. Beta diversity analysis showed the microbial structure is significantly different between sow fecal and milk microbiome (P < 0.05). Moreover, A shift in milk microbial community structure was observed during lactation (P < 0.05). The most dominant phyla are *Firmicutes* and *Bacteroidetes* in sow fecal microbiome, but *Firmicutes* and *Proteobacteria* in milk microbiome. Based on LefSe analysis, ASVs belonging to *Methanobacteriaceae* increased in the milk microbiome from the *Bacillus* group on d 14 in lactation, while ASVs of *Treponema* decreased in the fecal microbiome from the *Bacillus* group on d 14 in lactation. As for piglets, maternal *Bacillus* treatment accelerated the maturation of fecal microbiome based on β diversity. *Lactobacillus* ASVs increased in the *Bacillus* group piglet fecal microbiome. Venn diagram showed 5 core ASVs in piglet fecal microbiome, 27 core ASVs in nasal microbiome, and 14 core microbiomes in oral microbiome. ASV4_*Megasphaera* was present in all 3 body sites in piglet microbiome. Network analysis showed that *Bacillus* treatment enhanced the relationship among the different body sites. Source tracker analysis indicated that nasal microbiome and fecal microbiome were more similar to each other than other sources. Overall, this study showed potential benefits of *Bacillus* supplementation to sows during late gestion and lactation.

Key Words: *Bacillus*, sow, piglet, microbiome

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