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Monday, April 20

Special Session: 75th Diamond Jubilee - History of the Gut Function Congress and the Discovery of Interspecies Hydrogen Transfer

Chair: Phil B. Pope, Centre for Microbiome Research - QUT, Australia
NCSA Auditorium - Room 1122
8:45 AM - 2:00 PM

8:45 AM		**Introduction.** Itzhik Mizrahi, *Ben Gurion University of the Negev, Israel.*
9:00 AM	1	**CGIF is 75!: A history of the Congress of Gastrointestinal Function and its predecessor, the Rumen Function Conference.** R. Mackie* and S. Daniel, *Department of Animal Sciences and Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana-Champaign, IL, USA.*
9:45 AM	2	**Interspecies hydrogen transfer: Discovery, expansion, and the future.** J. Ferry*, *Department of Biochemistry and Molecular Biology, Penn State University, State College, PA, USA.*
10:30 AM		**Break** (Foyer outside auditorium).
11:00 AM	3	**The Hungate1000 project: The first large scale rumen microbial genome sequencing project.** W. Kelly*, S. Leahy, and G. Attwood, *AgResearch, Palmerston North, New Zealand.*
11:20 AM	4	**The RUMEN Gateway Project: Unlocking rumen microbial diversity to transform sustainable ruminant production.** F. Santos* and The Rumen Gateway Consortia, *School of Biological Sciences/ Institute for Global Food Security Queen's University Belfast, Belfast, United Kingdom.*
11:40 AM		**Discussion and Synthesis.**
12:00 PM		**Lunch Break** (Foyer outside auditorium).

2026 CGIF Opening Session Invited presentations and Bryant Memorial Lecture

Chair: Itzik Mizrahi, Ben Gurion University of the Negev, Israel
NCSA Auditorium - Room 1122
2:00 PM - 5:00 PM

2:00 PM		**Welcome.** Rod Mackie, Chair, *University of Illinois at Urbana-Champaign, USA.*
2:15 PM	5	**Mapping and engineering the intestinal host-microbiome interface.** H. Wang*, *Department of Systems Biology, Columbia University Irving Medical Center, New York, NY, USA.*
3:00 PM	6	**Evolution of gut microbiota in humans and mice.** A. Moeller*, *Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA.*
3:45 PM		**Break** (Foyer outside auditorium).

4:00 PM 7 **Bryant Memorial Lecture: How cows, microbes, and evolution taught humans to drink milk.**
R. Ley*, *Microbiome Science, Max Planck Institute for Biology Tübingen, Tübingen, Germany.*

5:00 PM **Mixer and Informal Poster Session** (Foyer outside auditorium).

Tuesday, April 21

Podium presentations: Session 1—Advances in Rumen Biology

Chair: Phil B. Pope, Centre for Microbiome Research - QUT, Australia
NCSA Auditorium - Room 1122
9:00 AM - 1:30 PM

9:00 AM 8 **Early-life interventions: Rewiring the rumen microbiome and shaping long-term ruminant health and performance.**
D. Pitta*, *PennVet New Bolton Center, University of Pennsylvania, Kennett Square, PA, USA.*

9:45 AM 9 **Metagenomic dissection of *Asparagopsis*-mediated methane reduction reveals vitamin B12 biosynthesis disruption and functional redundancy in the rumen microbiome.**
K. Lawther[1,2,] N. J. Dimonaco[1], P. Donnelly[1], A. Guinguina[3], S.J. Krizsan[4], and S. A. Huws*[1], [1]*School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, United Kingdom,* [2]*Laboratory of Microbiology, Wageningen University, Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands,* [3]*Production Systems, Natural Resources Institute Finland (LUKE), Production Systems, Natural Resources Institute Finland (LUKE), Jokioinen, Finland,* [4]*Department of Agricultural Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Blæstad, Norway.*

10:05 AM 10 **Smart Underwear: A novel wearable for long-term monitoring of gut microbial gas production via flatus.**
B. Hall*, *Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.*

10:40 AM **Break** (Foyer outside auditorium).

11:00 AM 11 **Metabolic ecology as a framework for understanding and controlling bioactive metabolite production in the gut microbiome.**
S. Light*, *University of Chicago, Biological Science Division, Chicago, IL, USA.*

11:30 AM **Platinum sponsor.**
Dairy Management Inc., Juan Tricarico.

11:50 AM **Business Meeting: CGIF 2026** (open to all registrants).

12:50 PM **Lunch Break** (Foyer outside auditorium).

Podium presentations: Session 2—Advances in Rumen Biology

Chair: Itzik Mizrahi, Ben Gurion University of the Negev, Israel
NCSA Auditorium - Room 1122
1:30 PM - 10:30 PM

1:30 PM 12 **Microbial adaptation to combined feeding strategies alters ecosystem structure and fermentation.**
R. Petri* and C. Benchaar, *Science and Technology Branch Agriculture and Agri-Food Canada/Government of Canada, Ottawa, ON, Canada.*

2:00 PM 13 **Differential rumen responses in neonatal ruminants to volatile fatty acids, glucose, and physical stimulation: Insights into the drivers of early rumen development.**
T. Chen*[1,2], K. Li[1,2], Y. Yin[3], K. Huang[1,2], Q. Hong[2], J. Wang[1,2], Y. Zhang[1,2], Z. Yuan[4], Y. Wang[5], Z. Yu[6], and J. Wang[1,7], [1]*Institution of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*MoE Key Laboratory of Molecular Animal Nutrition, Zhejiang University, Hangzhou, Zhejiang, China,* [3]*Huzhou Academy of Agricultural Sciences, Huzhou, Zhejiang, China,* [4]*Jiaxing Animal Husbandry and Veterinary Station, Jiaxing, Zhejiang, China,* [5]*Kemin (China) Technologies Co. Ltd, Zhuhai, Guangdong, China,* [6]*Department of Animal Sciences and Center of Microbiome Science, The Ohio State University, Columbus, OH, USA,* [7]*Zhejiang Key Laboratory of Nutrition and Breeding for High-quality Animal Products, Zhejiang University, Hangzhou, Zhejiang, China.*

2:20 PM 14 **Host genetic variation in *SPINK5* regulates rumen epithelial barrier function and shapes heritable microbial communities through SCFA-mediated mechanisms in dairy cattle.**
Z. Lai*[1,2], Z. Chen[1], J. Tian[1], Z. Huang[1], J. P. Schoonmaker[2], L. J. R. Nolasco Padilla[2], D. C. St Herzog[2], C. M. Snethen[2], and L. Ferreira De Souza[2], [1]*Nanjing Agricultural University, Nanjing, Jiangsu, China,* [2]*Purdue University, West Lafayette, IN, USA.*

2:40 PM **Platinum sponsor.**
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3:00 PM **Break** (Foyer outside auditorium).

3:30 PM **Poster Session.**

5:30 PM **Social Function.**
Riggs Brewery: Open Bar and Food Truck.

Wednesday, April 22

Podium presentations: Session 3—The human microbiome/host-microbe interactions in health and disease

Chair: Rod I. Mackie, Chair, University of Illinois at Urbana-Champaign, USA
NCSA Auditorium - Room 1122
9:00 AM - 1:00 PM

9:00 AM 15 **Toward microbiome-informed personalized medicine: Rescuing the non-responders.**
S. Gibbons*, *Institute for Systems Biology, Seattle, WA, USA.*

9:45 AM 16 **Niche dimensionality drives microbial community structure.**
K. Srinivasan[1], G. Plata[2], and P. Dixit*[1], [1]*Yale University, New Haven, CT, USA,* [2]*BiomEdit LLC, Fishers, IN, USA.*

10:05 AM 17 **Revealing the pervasive landscape of MGE-host interactions in situ with single-cell genomics.**
M. Yan*[1], J. F. Banfield[1,2], and R. Sachdeva[1], [1]*Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA, USA,* [2]*Department of Microbiology, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia.*

10:25 AM **Break** (Foyer outside auditorium).

11:00 AM **Platinum sponsor.**
Applied Microbiology International (AMI).

11:20 AM 18 ***Bacteroides intestinalis*-driven arabinoxylan fermentation mitigates inflammatory and metabolic dysfunction.**
Z. Zhou, K. L. Nguyen, S. Chen, Y. Wang, M. Li, D. Bianchi, W. Ge, S. Kuo, R. Khorana, A. Hetta, I. Cann, R. Mackie, G. Lau, J. Yang, W. Mei*, *University of Illinois Urbana-Champaign, Urbana, IL, USA.*

11:40 AM 19 **A functional role for a microbial cortisol-metabolizing enzyme in the human gut.**
M. Freiberg*[1,2], J. Ridlon[1,2], T. Wang[1,2], R. Rosa[3], R. Bernardi[3], and J. W. Lee[4], [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [2]*Microbial Metabolic Engineering Theme, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [3]*Physics Department – COSAM Leach Science Center, Auburn AL, USA,* [4]*Sungshin, Seoul, South Korea.*

12:00 PM **Lunch Break** (Foyer outside auditorium).

Podium presentations: Session 4—Animal nutrition

Chair: Katie Lawther, Research Fellow at Queen's University Belfast
NCSA Auditorium - Room 1122
1:00 PM - 2:35 PM

1:00 PM **Platinum sponsor.**
Adisseo.

1:20 PM 20 **Abrupt dietary shifts as a pre-harvest food safety intervention strategy lead to profound shifts in the ruminant gastrointestinal microbiome in feedlot cattle.**
H. G. Perez, A. M. Osorio-Doblado, K. P. Feldmann, M. M. Dycus, C. Burner, S. Locke, J. M. Lourenco, F. L. Fluharty, and T. R. Callaway*, *Department of Animal and Dairy Science, University of Georgia, Athens, GA, USA.*

1:40 PM 21 **Effects of supplementing the homoacetogen *Blautia pseudococcoides* as an alternative hydrogen sink on in vitro rumen fermentation and methanogenesis.**
T. Stoikidou*[1], R. Duong[1], P. Romero[1], and M. Hess[1,2], [1]*University of California Davis, Davis, CA, USA,* [2]*University of California Berkeley, Berkeley, CA, USA.*

2:00 PM 22 **Effect of dietary supplementation of bovine-derived *Bifidobacterium longum* ssp. *longum* on the gut bacterial establishment in colostrum-compromised calves.**
X. Wu*[1], R. Nakandalage[2], P. Griebel[3], K. Rajamanickam[3], N. Malmuthuge[4], and L.L. Guan[1,2], [1]*The University of British Columbia, Vancouver, BC, Canada,* [2]*University*

of Alberta, Edmonton, AB, Canada, [3]*University of Saskatchewan, Saskatoon, SK, Canada,* [4]*University of Calgary, Calgary, AB, Canada.*

2:20 PM **Presentation of Russell Awards.**
Best oral presentations by Graduate students and Young investigators.

2:35 PM **Closing remarks and Invitation to CGIF 2026.**

POSTER PRESENTATIONS

Computational approaches and applications

Foyer outside auditorium
3:30 PM - 5:00 PM

23 **Characterization of cooperative network structure of rumen bacterial communities centered on *Aristaeella* spp. (*Christensenellaceae* R-7 group) and its role in fermentation.**
K. Ito*[1], N. Sogawa[2], and S. Koike[1], [1]*Hokkaido University, Hokkaido, Japan,* [2]*Honda R&D Co., Ltd., Tochigi, Japan.*

24 **Method choice matters—Identifying intestinal fungi and protists from shotgun metagenomic data.**
D. Claypool* and B. Bernabé, *University of Illinois, Chicago, IL, USA.*

Environmental impacts (Including livestock waste, GHG's, and antibiotic resistance)

Foyer outside auditorium
3:30 PM - 5:00 PM

25 **3-Nitropropionic acid induces biphasic microbial restructuring and redirects hydrogen flux during in vitro rumen fermentation.**
M. M. Mulandi*[1], C. Yamaga[1], R. Yano[2], and N. Fukuma[2,3], [1]*Graduate School of Animal and Veterinary Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [2]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [3]*Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.*

26 **The transcriptional plasticity of rumen microbes underlies growth performance and methane output in hay-fed beef cattle.**
A. N. Hall[1], S. R. Talley[2], N. T. Baxter[1], K. Srinivasan[3], J. C. Ellis[1], J. Carter[1], D. Susanti[1], P. Dixit[3], A. Foote[2], D. L. Lalman[2], and G. Plata*[1], [1]*BiomEdit Inc, Greenfield, IN, USA,* [2]*Oklahoma State University, Stillwater, OK, USA,* [3]*Yale University, New Haven, CT, USA.*

27 **Genetic toolkits for rumen bacteria to advance methane control.**
Y. Li*[1], A. Hetta[1], M. Adachi[1], K. M. Pilkington[2], W. Alexander[3], A. Guss[3], W. J. Kelly[2], G. T. Atwood[2], W. W. Metcalf[1,4], R. Mackie[1,5], and I. Cann[1,5], [1]*Carl R Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [2]*Grasslands Research Centre, Bioeconomy Science Institute, Palmerston North, New Zealand,* [3]*Oak Ridge National Laboratory, Oak Ridge, TN, USA,* [4]*Department of Microbiology, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [5]*Department of Animal Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA.*

28 **Inhibition of methanogenesis with biochar in combination with nitrate in continuous culture of ruminal microbes.**
A. Kolganova*[1], J. Firkins[1], R. Lal[1], B. Wenner[2], M. Minnema[3], K. E. Mitchell[4], and N. Pickard[1], [1]*The Ohio State University, Columbus, OH, USA,* [2]*Feedworks USA, Cincinnati, OH, USA,* [3]*Biochar Solutions, White City, OR, USA,* [4]*Elanco, Indianapolis, IN, USA.*

29 **Isolation of rumen acetogens and methanogens from bovine and ovine rumen contents.**
K. Rajasekaran[1], L. Crouzet[1], M. Tavendale[1], D. Gagic[2], A. Sutherland-Smith[2], R. Chanyi*[1], P. Janssen[1], W. Kelly[1], and G. Attwood[1], [1]*Bioeconomy Science Institute, Palmerston North,*

Manawatu-Whanganui, New Zealand, [2]School of Food Technology and Natural Sciences, Massey University, Palmerston North, Manawatu-Whanganui, New Zealand.

Immunology (Including host-microbe interactions)

Foyer outside auditorium
3:30 PM - 5:00 PM

30 **Fermented food associated aromatic amino acid microbial metabolites regulate macrophage inflammatory tone and function.**
E. Eck*, C. Lim, M. Kasparek, B. McCusker, M. Miller, and J. Allen, *University of Illinois Urbana-Champaign, Urbana-Champaign, IL, USA.*

31 **Identification of arabinoxylan-responsive bacteria in the gut microbiota via D-amino acid metabolism probe technology.**
Y. He*, *Zhejiang University, Hangzhou City, Zhejiang Province, China.*

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

Foyer outside auditorium
3:30 PM - 5:00 PM

32 **Vitamin B12-mediated regulation of BtuB receptor-dependent bacteriophage L6jm infection of *Salmonella enterica* serovar Choleraesuis.**
X. Y. Yang*[1,2], W. Y. Zhu[1,2], and Y. Lin[1,2], [1]*National Center for International Research on Animal Gut Nutrition, Nanjing, Jiangsu, China,* [2]*College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu, China.*

33 **Cross-platform and cross-database comparative analysis of 16S rRNA sequencing analysis for high-resolution profiling of rumen bacterial communities.**
H. Kim*[1], M. Zhou[1], L. Lin[1], W. Zhu[2], T. McAllister[3], and L. Guan[1], [1]*The University of British Columbia, Vancouver, BC, Canada,* [2]*Anhui Agricultural University, Hefei, China,* [3]*Agriculture and Agri-Food Canada, Lethbridge, AB, Canada.*

34 **Analysis of rumen microbial composition of Scottish Blackface sheep consuming pasture- or concentrate-based diets using culture based and culture-independent metagenomic techniques.**
G. Karakaya*[1], S. Mills[1], F. Campion[2], N. Claffey[2], V. O'Flatherty[1], and S. Waters[1], [1]*School of Biological and Chemical Sciences and Ryan Institute, University of Galway, Galway, Ireland,* [2]*Teagasc, Animal and Bioscience Research Department, Animal & Grassland Research Centre, Mellow's Campus, Athenry, Galway, Ireland.*

35 **Community context reshapes microbial proteomes and reduces functional overlap.**
S. Morais*[1], M. Mazor[1], I. Amit[1], P. Gerth[2], A. Trautwein-Schult[2], S. Maaß[2], I. Grinshpan[1], Y. Shelly[1], L. Levin[1], D. Becher[2], and I. Mizrahi[1], [1]*Ben Gurion University, Israel,* [2]*University of Greisfwald, Germany.*

37 **UHPLC-QTOF-IMS metabolomics-based phytochemical characterization of high-altitude Himalayan green leafy crops and determination of gastro-intestinal digestibility of antioxidants.**
S. Gupta*[1,2] and V. Srivatsan[1,2], [1]*CSIR – Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India,* [2]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India.*

38 **Diet-influenced hydrogen accumulation, VFA shifts, and methane responses to 3-nitrooxypropanol in a dual flow continuous culture rumen fermentation system.**
N.-K. Kim*[1], J. A. Hartman[1], J. C. McCann[1], and R. I. Mackie[1,2], [1]*Department of Animal*

Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA, [2]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA.

39 **Life without an enolase does not disadvantage *Butyrivibrio* and *Pseudobutyrivibrio* species growth on glucose.**

K. Reilly*[1,3], N. Palevich[1], P. H. Janssen[1], W. J. Kelly[1], S. C. Leahy[2], S. E. Morales[4], G. M. Cook[3], and G. T. Attwood[1], [1]*Rumen Microbiology, Bioeconomy Science Institute, Agresearch Group, Palmerston North, New Zealand,* [2]*AgEmmissions Centre, Palmerston North, New Zealand,* [3]*Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand,* [4]*MPG Ranch, Montana, USA.*

40 **Establishing a working culture collection of anaerobic rumen bacteria and methanogenic archaea for the development of genetic toolkits.**

S. A. Sanguedolce*[1], K. M. Pilkington[2], W. J. Kelly[2], G. T. Attwood[2], W. W. Metcalf[1], I. Cann[1], and R. I. Mackie[1], [1]*University of Illinois Urbana-Champaign, Urbana, IL, USA,* [2]*New Zealand Institute for Bioeconomy Science, Palmerston North, New Zealand.*

41 **Transcriptional analysis of arabinan utilization and acetate production via heterolactic fermentation in ruminal *Streptococcus*.**

M. Adachi*[1,2], A. J. Scheftgen[1], R. Hiyama[3], K. Seki[3], R. Yano[4], and N. Fukuma[4,5], [1]*Graduate School of Animal and Veterinary Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan,* [2]*Current address: Carl R. Woese Institute for Genomic Biology, Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [3]*Forest Research Department, Forest Products Research Institute, Hokkaido Research Organization, Asahikawa, Japan,* [4]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan,* [5]*Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.*

42 **Microbial correlates of fecal lactate concentration and evaluation of the genomic potential for lactate utilization in a *Schwartzia*-related MAG from Japanese draft horses.**

R. Yano*[1], A. J. Scheftgen[2], and N. Fukuma[1,3], [1]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [2]*Graduate School of Animal and Veterinary Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [3]*Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.*

43 **Comparative fecal microbiome analysis of Japanese Dosanko and Japanese draft horses reveals functional and resistome differences.**

A. J. Scheftgen*[1], R. Yano[2], and N. Fukuma[2,3], [1]*Graduate School of Animal and Veterinary Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [2]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [3]*Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.*

44 **Isolation and partial characterization of a cellulolytic consortium derived from the rumen of a grazing cow.**

F. Moralejo[1], S. Cravero[2], O. Ontañon[2], R. Castaño Zubieta[1], M. Popova[3], D. Morgavi[3], and M. Cerón-Cucchi*[1], [1]*Instituto de Patobiología/IPVet, CICVyA, Instituto Nacional de Tecnología Agropecuaria UEDD INTA-CONICET, Hurlingham, Buenos Aires, Argentina,* [2]*Instituto de Agrobiotecnología y Biología Molecular (IABIMO), CICVyA, Instituto Nacional de Tecnología Agropecuaria UEDD INTA-CONICET, Hurlingham, Buenos Aires, Argentina,* [3]*Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement INRAE, Saint-Genès-Champanelle, France.*

45 **Metagenomic profiling using long reads PacBio sequencing revealed key metabolic functions of rumen microbiome in beef cattle across seasonal grazing.**
Y. Choi*[1], S. W. Na[2], M. Zhou[1], H. Kim[1], Y. Chen[2,3], E. W. Bork[2], C. Fitzsimmons[2,3], and L. L. Guan[1,2], [1]*The University of British Columbia, Vancouver, British Columbia, Canada,* [2]*University of Alberta, Edmonton, Alberta, Canada,* [3]*Lacombe Research and Development Centre, Lacombe, Alberta, Canada.*

46 **Diet quality and microbial tryptophan metabolism in colorectal cancer risk.**
A. Chhetri*[1], A. Moffitt[1], B. Binion[2], H. Dai[3], D. Bhaskaran[5], C. Greening[4], C. Welsh[4], H. R. Gaskins[2], K. Anantharaman[5], E. Mutlu[3], J. Ridlon[2], L. Tussing-Humphreys[3], and P. Wolf[1], [1]*Purdue University, West Lafayete, IN, USA,* [2]*University of Illinois, Urbana-Champaign, Urbana, IL, USA,* [3]*University of Illinois Chicago, Chicago, IL, USA,* [4]*Monash University, Melbourne, Australia,* [5]*University of Wisconsin–Madison, Madison, WI, USA.*

47 **Improving rapid identification of rumen bacteria: Development and evaluation of a rumen specific MALDI-TOF MS database.**
J. Pickup*[1], T. Stoikidou[1], Z. Zhang[1], G. Karakaya[2], E. Fuertes[1], F. Godoy Santos[1], and S. Huws[1], [1]*Queen's University Belfast, Belfast, Northen Ireland, United Kingdom,* [2]*University of Galway, Galway, Ireland.*

48 **The effects of tail docking status on fecal microbiome adaptation to pasture in Polyplay ewe lambs.**
J. M. Woods[1], M. K. Costello*[1,3], S. A. Bowdridge[2], H. C. Mantovani[1], and S. J. J. Adcock[1], [1]*University of Wisconsin–Madison, Madison, WI, USA,* [2]*West Virginia University, Morgantown, WV, USA,* [3]*Oak Ridge Institute for Science Education, Oak Ridge, TN, USA.*

49 **Enterosignature dynamics of healthy and diarrheic dairy calves during the preweaning period.**
A. Rodrigues*, G. Alam, G. Larsen, N. Sheybani, J. Laporta, and H. Mantovani, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, USA.*

50 **Carbon-responsive phase variation drives adaptive regulation in *Bacteroides thetaiotaomicron*.**
H. Choi*[1], R. Chanin[1], Y. Kiguchi[1], and A. Bhatt[1,2], [1]*Division of Hematology, Department of Medicine, Stanford University, Stanford, CA, USA,* [2]*Department of Genetics, Stanford University, Stanford, CA, USA.*

51 **A functionally selected *Acinetobacter* sp. phosphoethanolamine transferase gene from the goose fecal microbiome confers colistin resistance in *Escherichia coli*.**
E. Bernate[4], Y. Shi[1], E. Franck[5], and T. Crofts*[1,2], [1]*Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [3]*Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [4]*College of Veterinary Medicine, University of Florida, Gainesville, FL, USA,* [5]*Department of Biomedical Sciences, Florida State University, Tallahassee, FL, USA.*

52 **Capsule-mediated sensitivity to antipsychotic drugs reveals a new vulnerability in *Escherichia coli*.**
M. O. Gill*[1], J. A. Cook[1], K. X. Jiang[2], A. Ambat[2,3], H. Shi[2,3], A. Natarajan[4], R. B. Chanin[4], K. C. Huang[2,3], and A. S. Bhatt[1,4], [1]*Department of Genetics, Stanford University, Stanford, CA, USA,* [2]*Department of Microbiology & Immunology, Stanford University, Stanford, CA, USA,* [3]*Department of Bioengineering, Stanford University, Stanford, CA, USA,* [4]*Department of Medicine, Division of Hematology, Stanford University, Stanford, CA, USA.*

53 **Longitudinal gut microbiome restructuring associates with pain burden following kidney transplantation.**
L. Raasch*[1], S. Alvernaz[1], E. Lee[1], S. Green[2,3], G. Chlipala[4], M. Mainschein Cline[4], M. Samra[5], H. DeVon[6,7], L. Tussing-Humphreys[8], C. Park[9], M. Spaggiari[10], L. Gallon[11],

A. Doorenbos[11], B. Peñalver Bernabé[1,12], M. Lockwood[9], [1]*Department of Biomedical Engineering, University of Illinois Chicago, Chicago, IL, USA,* [2]*Core Laboratory Services and Genomics and Microbiome Core Facility, Rush University Medical Center, Chicago, IL, USA,* [3]*Department of Internal Medicine, Rush University Medical Center, Chicago, IL, USA,* [4]*Research Informatics Core, Chicago, IL, USA,* [5]*Department of Medicine, Edward Hines Jr. VA Transplant Center, Loyola University Medical Center, Chicago, IL, USA,* [6]*Community Health Research, University of California Los Angeles School of Nursing, Los Angeles, CA, USA,* [7]*University of California San Diego, San Diego, CA, USA,* [8]*Department of Kinesiology and Nutrition, University of Illinois Chicago, College of Applied Health Sciences, Chicago, IL, USA,* [9]*Department of Population Health Nursing Science, University of Illinois Chicago, College of Nursing, Chicago, IL, USA,* [10]*University of Illinois Chicago, College of Medicine/ Surgery, Chicago, IL, USA,* [11]*Department of Biobehavioral Health Science, University of Illinois Chicago, College of Nursing, Chicago, IL, USA,* [12]*Center of Bioinformatics and Computational Biology, Chicago, IL, USA.*

54 **Enrichment of a novel methanogen from the bovine rumen.**
S. R. Khan*[1], G. Cronan[2], R. I. Mackie[1], and W. W. Metcalf[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL USA,* [2]*Abbott Nutrition, Columbus, OH, USA.*

55 **Sensing and regulation of the utilization of dietary polysaccharides in *Bacteroides* spp.**
M. A. Alhawsawi[1,2], A. M. Hetta*[2], J. Akresi[3], D. M. Bianchi[4], J. J. Cavalcante[2], G. V. Pereira[2], K. A. Boateng[2], Y. Li[2], M. Adachi[2], J. Chen[2], W. Me[1,5], N. M. Koropatkin[6], Y. Yin[3], R. I. Mackie[2,7], I. Cann[2,7], [1]*Division of Nutritional Sciences, University of Illinois, Urbana, IL, USA,* [2]*Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL, USA,* [3]*Department of Food Sciences and Technology, University of Nebraska–Lincoln, Lincoln, NE, USA,* [4]*National Center for Supercomputing Applications, University of Illinois, Urbana, IL, USA,* [5]*Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, Urbana, IL, USA,* [6]*Department of Microbiology & Immunology, University of Michigan Medical School, Ann Arbor, MI, USA,* [7]*Department of Animal Science, University of Illinois, Urbana, IL, USA.*

77 **Persistent auxiliary microbiome of early colonizers shapes the developing rumen ecosystem.**
O. Furman*, *Mizrahi Lab, Ben-Gurion University of the Negev, Beer Sheva, Israel.*

Nutrition and metabolism of livestock, humans, and companion animals

Foyer outside auditorium
3:30 PM - 5:00 PM

56 ***In vitro* evaluation of feed additive combinations for methane emission mitigation.**
I. R. R. Castro*[1], B. Hiley[1], O. C. Carballo[2], G. Scoley[2], and S. Huws[1], [1]*Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, Northern Ireland, United Kingdom,* [2]*Agri-Food and Biosciences Institute, Large Park, Hillsborough, Northern Ireland, United Kingdom.*

57 **Dietary transition from high-forage to finishing ration reshapes the ecology of *Neocallimastigomycota* in the rumen.**
R. J. Gruninger*, T. Rogelio Ramos, N. Chomistek, and S. A. Terry, *Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.*

58 **Genomic insights into tryptophan metabolism in lactic acid bacteria of bovine origin.**
Y. Kim*, H. Kim, M. Moran, and L. L. Guan, *The University of British Columbia, Vancouver, BC, Canada.*

59 **Ultra-processed foods link to gut microbial sulfite metabolism genes.**
S. Harvey*[1], A. Chhetri[1], E. Pfeifer[1], A. Hamm[2], D. K. Baskaran[4], C. Welsh[5], J. Ridlon[3], K. Anantharaman[4], C. Greening[5], E. Mutlu[2], V. Oddo[2], L. Tussing-Humphreys[2], H. R. Gaskins[3], Q. Wang[3], P. Wolf[1], [1]*Purdue University, West Lafayette, IN, USA,* [2]*University of Illinois at Chicago, Chicago, IL, USA,* [3]*University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [4]*University of Wisconsin–Madison, Madison, WI, USA,* [5]*Monash University, Melbourne, Australia.*

60 **Discriminating peptides from ammonia and branched-chain volatile fatty acids on nutrient digestibility, bacterial protein synthesis, and bacterial communities in continuous culture.**
A. R. Sanders[1,2], B. A. Wenner[3], C. Lee[4], M. T. Socha[5], D. H. Kleinschmit[5], N. Pickard[1], S. Somasundaram[1], Z. Yu[1], and J. L. Firkins*[1], [1]*The Ohio State University, Columbus, OH, USA,* [2]*Novus International Inc, Chesterfield, MO, USA,* [3]*Feedworks USA, Cincinnati, OH, USA,* [4]*The Ohio State University, Wooster, OH, USA,* [5]*Zinpro Corporation, Eden Prairie, MN, USA.*

61 **Dietary modulation of rumen microbial structure and functional potential influences stress physiology and performance in beef bulls.**
P. Donnelly*[1], N. Rutherford[2], N. Dimonaco[1], F. Lively[2], F. Godoy Santos[1], S. Huws[1], and G. Arnott[1], [1]*Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, United Kingdom,* [2]*Agri-Food and Biosciences Institute, Livestock Production Sciences Branch, Agri-Food and Biosciences Institute, Hillsborough, United Kingdom.*

62 **Gut *Akkermansia muciniphila* attenuates obesity via modulating bile acid metabolism.**
J. Liu* and X. Wang, *College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

63 **Lower-gut delivery of brassica-derived isothiocyanates links vegetable intake to GLP-1, appetite, and metabolic health.**
F. Yang*, M. Black, and M. J. Miller, *University of Illinois Urbana-Champaign, Urbana, IL, USA.*

64 **Association of *Undaria pinnatifida* rhizoid chemical composition with ruminal methane mitigation.**
C. Yamaga*[1], M. M. Mulandi[1], R. Yano[2], and N. Fukuma[2,3], [1]*Graduate School of Animal and Veterinary Sciences and Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [2]*Department of Life and Food Sciences, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan,* [3]*Research Center for Global Agromedicine, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.*

65 **Investigating the source of liver abscess pathogens in the gastrointestinal tract of ruminants.**
P. E. Berkmeyer*[1], H. F. Linder[1], T. G. Nagaraja[2], and J. C. McCann[1], [1]*University of Illinois at Urbana-Champaign, Champaign, IL, USA,* [2]*College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA.*

Prebiotics, probiotics, and DFM development

Foyer outside auditorium
3:30 PM - 5:00 PM

66 **A *Bacillus*-based direct-fed microbial (DFM) mixture remodels the gut microbiome to augment respiratory health of *Salmonella* infected pigs.**
T. Putman*[1], A. M. Abdel-Hamid[2,3], E. Galbraith[4], P. Schimmel[2], H. Kim[1], T. Yasuma[5,6],

M. A. B. Alhawsawi[2,7], K. Boateng[2], J. Holmes[9], M. Duersteler[4], C. N. D'Alessandro-Gabazza[2,5], H. Fujimoto[11], T. Kobayashi[10,11], K. K. O. Walden[9], G. Rendon[9], [1]*Department of Animal Science, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [2]*Carl R. Woese Institute for Genomic Biology (Microbiome Metabolic Engineering), University of Illinois Urbana-Champaign, Urbana, IL, USA,* [3]*Department of Botany and Microbiology, Faculty of Science, Minia University, El-Minia, Egypt,* [4]*Microbial Discovery Group, Oak Creek, WI, USA,* [5]*Department of Immunology, Mie University Faculty and Graduate School of Medicine, Tsu, Mie, Japan,* [6]*Department of Diabetes and Endocrinology, Mie University Faculty and Graduate School of Medicine, Tsu, Mie, Japan,* [7]*Division of Nutritional Sciences, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [8]*Clinical Nutrition Department, College of Applied Medical Sciences, University of Hafr Al Batin, Hafr Al Batin, Saudi Arabia,* [9]*Roy J. Carver Biotechnology Center, the University of Illinois Urbana-Champaign, Urbana, IL, USA,* [10]*Microbiome Research Center, Mie University, Tsu, Mie, Japan,* [11]*Department of Pulmonary and Critical Care Medicine, Mie University Faculty and Graduate School of Medicine, Tsu, Mie, Japan,* [12]*Department of Pathobiology, College of Veterinary Medicine, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [13]*Center for East Asian and Pacific Studies, University of Illinois Urbana-Champaign, Urbana, IL, USA,* [14]*Department of Microbiology, University of Illinois Urbana-Champaign, Urbana, IL, USA.*

67 **Rumen-native microbe supplementation to consistently improve dairy cow productivity.**

B. Anderson*[1], K. A. Calapa[1], J. K. Drackley[2], T. R. Overton[3], M. J. Vandehaar[4], J. E. P. Santos[5], J. S. Osorio[6], A. Lago[7], G. Martinez Boggio[8], H. Mantovani[8], J. Laporta[8], and M. Embree[1], [1]*Native Microbials, San Diego, CA, USA,* [2]*University of Illinois Urbana-Champaign, Urbana, IL, USA,* [3]*Cornell University, Ithaca, NY, USA,* [4]*Michigan State University, East Lansing, MI, USA,* [5]*University of Florida, Gainesville, FL, USA,* [6]*Virginia Polytechnic Institute, Blacksburg, VA, USA,* [7]*DairyExperts, Tulare, CA, USA,* [8]*University of Wisconsin–Madison, Madison, WI, USA.*

68 **Resistant starch enhances intestinal barrier function by modulating the abundance of *Bifidobacterium pseudolongum*.**

Y. L. Liu*, *Institute of Feed Science, Zhejiang University, Hangzhou, China*.

69 **How complex dietary fibers can be used to shape the human gut microbiome toward reduced inflammatory potential.**

M. L. Savo Sardaro*[1,2], S. Kuthyar[3], O. Dada[1], N. Deivassagayame[4], M. Tran[4], R. Kern[5], Y. Seidman[4], M. Atallah[1], and K. R. Amato[1], [1]*Northwestern University, Evanston, IL, USA,* [2]*University of San Raffaele, Rome, Rome, Italy,* [3]*University of California, San Diego, CA, USA,* [4]*Oakton College, Des Plaines, IL, USA,* [5]*New York University, New York, NY, USA.*

70 **Probiotic-induced restructuring of the canine gut microbiome and functional gene profiles is associated with behavioral modulation and physiological biomarkers in breeding dogs.**

P. Donnelly*, B. McAnoy, N. Dimonaco, F. Godoy Santos, S. Huws, and G. Arnott, *Queen's University Belfast, Belfast, United Kingdom.*

71 **Investigating the efficacy of a prebiotic-probiotic as an alternative to antibiotics on the growth performance of neonatal dairy calves.**

R. K. S. Lakshmi*[1,2], A. Kala[1], A. K. Verma[1], N. Agarwal[1], Md Younus Ali[3], Z. B. Dubal[1], S. E. Jadhav[1], and G. K. Gaur[1], [1]*ICAR IVRI, Bareilly, Uttarpradesh, India,* [2]*Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India,* [3]*SAARC Agriculture Center, Dhaka, Bangladesh.*

72 ***Lactobacillus*-vectored nanobodies improve broiler productivity in sub-clinical necrotic enteritis with integrated microbiome and host transcriptomic effects.**

A. Hall[1], S. Manuja[1], L. Payling[2], L. Romero[2], F. Hoerr[3], J. Shields[1], C. Hofacre[4], D. Susanti[1], D. Gangaiah[1], G. Plata[1], and A. Kumar*[1], [1]*BiomEdit, Inc, Greenfield, IN, USA,* [2]*Biofractal,*

Loulé, Portugal, [3]*Veterinary Diagnostic Pathology, LLC, Polkton, NC, USA,* [4]*Southern Poultry Research Group, Inc, Watkinsville, GA, USA.*

73 **Characterization of early-colonizing gut bacteria from neonatal calves for potential probiotic application.**
F. Viquez-Umaña, G. Alam, P. Tiwari, M. de la Paz, S. Davison, and H. Mantovani*, *University of Wisconsin–Madison, Madison, WI, USA.*

74 **Functional screening of novel propionate-producing ruminal bacteria as direct-fed microbial candidates.**
E. Fuertes*, F. Godoy-Santos, J. Pickup, and S. Huws, *Queen's University Belfast, Belfast, United Kingdom.*

75 **Optimization of protease combination under simulated chicken gastrointestinal conditions.**
H. Kim*[1], B. L. Vasanthakumari[2], and R. I. Mackie[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [2]*Kemin Industries, Waukee, IA, USA.*

76 **Effects of CLOSTAT® 500 (*Bacillus subtilis* PB6) in milk replacer on intestinal barrier function and disease incidence in pre-weaned calves.**
E. Lima Neto*[1], M. Wieghart[2], J. Traini[1], D. LaFleur[3], S. Trojan[4], and D. O' Connor[5], [1]*Kemin Industries, Inc, Des Moines, IA, USA,* [2]*All Dairy Consulting, LLC, Beldenville, WI, USA,* [3]*LaFleur Consulting, LLC, Sioux City, IA, USA,* [4]*Peak Beef Cattle Nutrition and Management Consulting, LLC, Casper, WY, USA,* [5]*DLOC Consulting LLC, Groveland, IL, USA.*

*Invited/Plenary Talks (By invitation only): Special Session: 75th Diamond Jubilee - History of the Gut Function Congress and the Discovery of Interspecies Hydrogen Transfer

1 CGIF is 75!: A history of the Congress of Gastrointestinal Function and its predecessor, the Rumen Function Conference.

R. Mackie* and S. Daniel,
Department of Animal Sciences and Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana-Champaign, IL, USA.

Since 1951, scientists have met on a biennial basis to discuss the latest advances in our understanding of the microbiology underpinning digestive function and ruminant nutrition. This tradition continues, and on April 20 to 22, 2026, we will celebrate our 75th Anniversary Diamond Jubilee meeting on the University of Illinois campus. Throughout the 1950s, bloat was a serious issue affecting animal health and productivity, and was a central theme of this founding conference. Initially the program consisted of 4 panels (Rumen Microbiology, Ruminant Nutrition, Pathophysiology, and Agronomy) that covered the factors important in the complex etiology of legume and feedlot bloat. This concept was based on the vision of the prominent thinkers and leaders in the field of physiology of digestion in the ruminant at that time. The conference eventually became known as the International Biennial Conference on Rumen Function (RFC), and broadened its program beyond bloat, to include other research in rumen microbiology, host animal physiology, toxicology, and nutrition. Over the years, RFC has served as the venue for all anaerobic microbiologists interested in gut physiology and metabolism to present their latest research, and also continued to draw a strong cadre of scientists working in ruminant physiology and nutrition. While fundamental questions regarding roles of microorganisms in ruminant digestion, and factors influencing the efficiency of ruminant digestion remain, new problems and challenges face animal agriculture. Many of these challenges and issues have a microbiological context, and are best addressed by integrative studies in microbiology, (immuno)physiology, and nutrition. To embrace these advances, the scope of the conference expanded, and in 2003 officially changed its name from Rumen Function Conference (RFC) to the Conference on Gastrointestinal Function (CGIF). The expanded scope of our conference has begun to attract many more scientists from a variety of disciplines, especially those with interests in human nutrition and biomedical sciences. After the meeting in 2011, a further name change was accepted and we are now known officially as the Congress for Gastrointestinal Function (CGIF) to reflect the international nature and history of the meeting. A concise history of the meeting will be presented to begin our 75th anniversary celebration.

2 Interspecies hydrogen transfer: Discovery, expansion, and the future.

J. Ferry*,
Department of Biochemistry and Molecular Biology, Penn State University, State College, PA, USA.

The origins of microbial ecology date back to the mid and late 1800s and early 1900s, witnessing early milestones accredited to giants in the field such as Sergei Winogradsky, who conceived the Winogradsky column, and Martinus Beijerinck, who introduced enrichment cultures, followed by the mid 1900s, which included Robert Hungate, who investigated the microbial ecology of the rumen and authored *The Rumen and Its Microbes*, stimulating research in anaerobic microbial ecology. It was in the early 1970s that Marvin Bryant, Ralph Wolfe, and Meyer (Mike) Wolin discovered interspecies hydrogen transfer (IHT) in the rumen, advancing yet another monumental milestone of principal importance in the field of microbial ecology thereby achieving the same stature of previous giants. Indeed, IHT has been foundational to a broader understanding of microbial ecology in diverse anaerobic environments that affect global warming and human health. My presentation will begin with the discovery of IHT followed by developments in diverse anaerobic

environments that have expanded the concept and amplified its importance in anaerobic microbial ecology. Finally, I will speculate on important unanswered questions about anaerobic environments that might well depend on the seminal discovery of IHT.

Key Words: hydrogen, formate, interspecies hydrogen transfer, synergistic growth

3 The Hungate1000 project: The first large scale rumen microbial genome sequencing project.

W. Kelly*, S. Leahy, and G. Attwood,
AgResearch, Palmerston North, New Zealand.

The Hungate1000 project was initiated as a community resource to generate a high-quality reference genome catalog for rumen bacteria and archaea. Although the microbial ecology of the rumen has long been the focus of research, at the beginning of the project reference genomes were available for only 14 bacteria and one methanogen, so that genomic diversity was largely unexplored. The project was made possible by funding from the New Zealand Government in support of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases and by support from the US Department of Energy Joint Genome Institute Community Science Program. A total of 410 reference genomes were generated in the Hungate1000 project thereby capturing almost all cultured species that had been taxonomically characterized from the rumen as well as several strains belonging to novel species and genera. These microbial cultures were sourced from the AgResearch Rumen Microbiology culture collection, provided by rumen microbiology researchers from North and South America, Asia, Australia and Europe, or purchased from international culture collections. The genome information is publicly available, and researchers have access to Hungate Collection strains which are being deposited in the DSMZ collection. The availability of a large-scale reference genome resource contributes to a better understanding of carbon, hydrogen, and lactate metabolism in the rumen, from the breakdown of lignocellulose, through the metabolism of substrates to short-chain fatty acids and fermentation end products, to the final step of methane formation. This information contributes to enhancing ruminant livestock productivity, limiting methane emissions, and advancing biofuel development. It also is an important component of further international collaborations which aim to bring more rumen microbes into cultivation and provide cultures for the development of genetic tools for rumen bacteria and methanogenic archaea.

Key Words: rumen bacteria, methanogens, genomes

4 The RUMEN Gateway Project: Unlocking rumen microbial diversity to transform sustainable ruminant production.

F. Santos* and The Rumen Gateway Consortia,
School of Biological Sciences/Institute for Global Food Security Queen's University Belfast, Belfast, United Kingdom.

The RUMEN Gateway Project is a global research initiative, involving over 25 institutes across 6 continents and 17 countries, launched in October 2023 and led by Queen's University Belfast, aimed at advancing the cultivation and understanding of previously uncultured rumen microorganisms. Building on the Hungate collection project (Seshadri et al. 2018 Nat. Biotechnol. **36**, 359–367, https://doi.org/10.1038/nbt.4110), it seeks to harness the rumen microbiome to drive innovation in sustainable livestock production systems. A formal collaboration agreement underscores its international importance and shared commitment to addressing a critical environmental challenge: reducing methane emissions from ruminant livestock. As a flagship initiative of the Global Research Alliance on Agricultural Greenhouse Gases, funded by the Global Methane Hub, the project focuses on isolating and characterizing previously uncultured members of the rumen microbiome. Through detailed phenotypic and genomic analyses of newly cultured isolates, the project aims to deepen understanding of ruminal fermentation processes and methane production. This knowledge will support the development of mitigation strategies, including small-molecule inhibitors, microbial feed additives, and vaccines targeting methanogenic archaea. A key strength of the project lies in its application of diverse culturomics ap-

proaches across participating laboratories. Specialists in archaea, bacteria, fungi, protozoa, and phages collaborate to apply tailored cultivation techniques, ensuring broad coverage of rumen microbial diversity. Both conventional and high-throughput anaerobic culturing methods are used to maximize recovery of previously uncultured organisms. The project aims to establish the most comprehensive open-access collection of rumen microorganisms to date, incorporating samples from a wide range of ruminant species, diets, and geographic regions. Support from the US Department of Energy's Joint Genome Institute provides essential genomic and bioinformatic resources to accelerate this effort. To date, the project has generated a biobank of over 20,000 bacterial isolates, including approximately 1,000 novel species. We are currently sequencing the novel microbial genomes to enable the consortia to assess the phenotypes of the isolates. These outputs will enhance our understanding of climate change, aid development of solutions and improve understanding of rumen nutrient cycling. Overall, the RUMEN Gateway Project represents a major step forward in decoding the complexity of the rumen microbiome. By integrating global expertise and generating open-access data resources, it is poised to deliver impactful solutions for reducing greenhouse gas emissions and improving the sustainability and resilience of livestock systems worldwide.

*Invited/Plenary Talks (By invitation only): 2026 CGIF Opening Session Invited presentations and Bryant Memorial Lecture

5 Mapping and engineering the intestinal host-microbiome interface.

H. Wang*,
Department of Systems Biology, Columbia University Irving Medical Center, New York, NY, USA.

Microbes inhabiting the gastrointestinal tract shape host metabolism, immunity, and tissue homeostasis, yet the principles connecting microbiome organization to epithelial function remain incompletely defined. This talk will describe emerging technologies to map the host-microbiome interface across space and time and methods to directly engineer resident gut bacteria in situ. I will describe micron-scale spatial metagenomics that combines microbial plot sampling with shotgun sequencing to reveal local interspecies interactions that support community assembly, stability, and function. These efforts are further paired with AI-enabled robotic culturomics to build personalized strain biobanks on-demand, isolate difficult to grow microbes, and test their interactions combinatorially. To profile host-microbiota interactions, I will describe a new fecal exfoliome sequencing approach that can track intestinal and immune dynamics along with microbiome changes over time. Finally, I will introduce metagenomic editing (MetaEdit) as a platform technology that use CRISPR for microbiome manipulations to program new functions and remove harmful traits toward next-generation microbiome therapeutics.

6 Evolution of gut microbiota in humans and mice.

A. Moeller*,
Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA.

Mammalian microbiota are deeply integrated with host phenotypes and fitness, but the evolutionary histories of these symbioses are poorly understood. In this seminar, I will show that multiple gut bacterial lineages in humans and mice trace their origins to ancestral symbioses that have co-diversified—and likely co-adapted—with host species over the past ~30 million years. Building on this evolutionary foundation, I will then investigate how gut microbiota adapt within host individuals. I will present results from recent evolve-and-resequence experiments indicating widespread adaptive evolution of gut bacterial species in wild-derived mice during transitions from low-fat to high-fat diets. Using

a combination of genome-resolved metagenomics and isolate genomics, I will show that diet-induced positive selection drives pervasive strain replacement, targets a common set of gene functions in diverse gut bacterial species, and reproducibly reshapes structural variation in gut bacterial genomes. Together, these findings shed light on how microbiota adapt within hosts over evolutionary and contemporary timescales.

7 Bryant Memorial Lecture: How cows, microbes, and evolution taught humans to drink milk.

R. Ley*,
Microbiome Science, Max Planck Institute for Biology Tübingen, Tübingen, Germany.

Humans did not evolve alone. Many members of our gut microbiome have diversified alongside human populations, forming long-term evolutionary relationships that shape how we digest food and respond to our environment. One of the clearest examples of human dietary adaptation is lactase persistence: the genetic ability to digest the milk sugar lactose into adulthood. This trait evolved independently in multiple populations following the adoption of dairying and is widely considered a classic example of gene–culture coevolution. Yet lactose metabolism also depends on the metabolic activities of the gut microbiome. In this lecture, I will discuss how host genetics and microbial metabolism jointly shape human responses to lactose. By combining human genotyping, clinical lactose tolerance testing, and gut metagenomics across populations in Europe, Africa, and Southeast Asia, we identified a subset of lactase non-persistent individuals who produce little or no fermentation gas following lactose ingestion. We refer to this phenotype as microbially acquired lactose tolerance (MALT). Microbiome analyses reveal that MALT is associated with distinct microbial consortia and metabolic functions that limit hydrogen production during lactose fermentation, including differences in hydrogenase gene content and alternative fermentation pathways. These findings suggest that diverse microbiomes can converge functionally to reduce gas production, allowing some individuals without the lactase persistence genotype to tolerate milk. Together, these results highlight how microbial metabolism can buffer host genetic constraints and illustrate how human dietary adaptation emerges from interactions between our genome and our microbiome.

Podium presentations: Session 1—Advances in Rumen Biology

8 Early-life interventions: Rewiring the rumen microbiome and shaping long-term ruminant health and performance.

D. Pitta*,
PennVet New Bolton Center, University of Pennsylvania, Kennett Square, PA, USA.

Early life represents a critical developmental window during which microbial colonization and ecological succession shape host physiology, metabolism, and productivity. Our studies of Holstein and Jersey dairy calves, pasture-raised beef calves, and lambs reveal conserved trajectories of gut microbiome maturation during the preweaning period. Early communities are highly variable but progressively reorganize into stable fermentation-oriented ecosystems as animals transition from milk to solid feed. Although this developmental progression is broadly conserved, breed, environment, and management influence microbial network architecture and are associated with differences in growth performance, metabolic efficiency, and resilience to early-life stressors. These observations prompted a central question: Can early microbial assembly be deliberately redirected to establish alternative fermentation networks before methanogenic metabolism becomes dominant? To address this, we combined ecological analyses with mechanistic mono- and coculture experiments to resolve microbial interactions governing hydrogen and carbon flow. Transcriptomic and metabolomic analyses revealed coordinated metabolic exchanges among fermenters,

acetogens, and hydrogen-utilizing microbes that control the partitioning of reducing equivalents. Guided by these insights, we engineered an early-life microbial consortium that rewires rumen redox metabolism, enabling carbon and hydrogen to be recycled within fermentation networks rather than dissipated as methane. Long-term studies demonstrate that early-life microbial programming stabilizes these rewired metabolic pathways and produces durable improvements in animal growth, metabolic efficiency, and overall health that persist well beyond the treatment period. Together, these findings show that early-life microbiome interventions can reconfigure rumen ecosystem development, offering a new framework to enhance ruminant health, productivity, and environmental sustainability.

9 Metagenomic dissection of *Asparagopsis*-mediated methane reduction reveals vitamin B12 biosynthesis disruption and functional redundancy in the rumen microbiome.

K. Lawther[1,2], N. J. Dimonaco[1], P. Donnelly[1], A. Guinguina[3], S.J. Krizsan[4], and S. A. Huws*[1],
[1]*School of Biological Sciences, Institute for Global Food Security, Queen's University Belfast, United Kingdom,* [2]*Laboratory of Microbiology, Wageningen University, Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands,* [3]*Production Systems, Natural Resources Institute Finland (LUKE), Production Systems, Natural Resources Institute Finland (LUKE), Jokioinen, Finland,* [4]*Department of Agricultural Sciences, Faculty of Applied Ecology, Agricultural Sciences and Biotechnology, Inland Norway University of Applied Sciences, Blæstad, Norway.*

Ruminant production contributes significantly to greenhouse gas emissions, with methane (CH_4) accounting for 33% of anthropogenic emissions. The feed additive, red seaweed *Asparagopsis taxiformis* (ASP) reduces CH_4 emissions by up to 80%, yet the microbial mechanisms remain poorly understood. Nordic Red cows were fed grass silage and concentrate with or without 0.5% ASP in a Latin square design, with rumen fluid sampled across 3 periods. ASP reduced CH_4 yield by 54%, with metagenomic analysis revealing mechanisms beyond methanogen inhibition, including alterations to less obvious "feeder" pathways that supply methanogenesis precursors. We observed increased abundances of genes encoding pyruvate and propionate production relative to acetate and CH4 pathways. Notably, vitamin B12 biosynthesis genes showed reduced abundance (e.g., adenosylcobinamide-GDP ribazoletransferase decreased 29.92%), representing a previously unrecognized mechanism of methane reduction. ASP also reduced dominant taxa (*Prevotella, Methanobrevibacter*) and promoted functional diversification through less dominant taxa that increased their contribution to methane-related pathways, illustrating niche displacement and functional resilience. By tracking functional shifts and their taxonomic origins, we reveal that microbiome function is robust and can be resistant to manipulation. This functional redundancy has major implications and may explain why methane mitigation strategies underperform. We reveal novel mechanisms of ASP action beyond direct methanogen inhibition, including disruption of vitamin B12 biosynthesis and precursor pathways. By tracking taxonomic origins of functional shifts, we demonstrate functional redundancy contributes to a microbiome resistant to manipulation. Effective methane mitigation strategies, therefore, require an understanding of which organisms contribute to which functions in these resilient microbial communities.

Key Words: rumen, microbiome, B12, *Asparagopsis*, methane

10 Smart Underwear: A novel wearable for long-term monitoring of gut microbial gas production via flatus.

B. Hall*,
Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA.

Gut microbial gas production is among the most direct and continuous readouts of microbiome metabolic activity, yet no tools have existed for its longitudinal, non-invasive measurement. To address this gap, we developed the Smart Underwear, a compact wearable device that passively monitors hydrogen gas in flatus by attaching to the exterior of any type of underwear. In a

week-long user experience study with 19 healthy adults, the device demonstrated strong compliance and comfort, while objective measurement revealed a mean of 32 flatus events per day, substantially higher than the 10 to 20 events typically cited from self-report studies, with striking interindividual variation ranging from 4 to 59 events per day. To validate sensitivity to diet-induced changes, we conducted the GUMDROP study, a crossover trial in 38 participants comparing microbiome activity after consuming readily absorbed sugars versus inulin, with the Smart Underwear detecting elevated hydrogen production following inulin consumption in 94.7% of participants. Building on these results, we launched the Human Flatus Atlas, a nationwide study aimed at defining the true baseline of human flatus patterns, in which participants wear the Smart Underwear for multiple days and photograph meals, enabling direct correlation of diet with gut fermentation activity. With over 8,000 enrollment survey completions to date, this study represents the first large-scale objective characterization of human flatulence and its relationship to diet and the gut microbiome.

11 Metabolic ecology as a framework for understanding and controlling bioactive metabolite production in the gut microbiome.

S. Light*,
University of Chicago, Biological Science Division, Chicago, IL, USA.

Gut microbes occupy specialized metabolic niches defined by utilization of host-derived or dietary substrates, employing strategies that produce bioactive metabolites influencing host health. Yet predicting which microbes produce which metabolites and controlling their levels remains challenging. Through screening and comparative genomics, we have characterized metabolic niches occupied by diverse, understudied gut microbes. By linking these niches to microbial physiology, we have identified examples illustrating how dietary factors can interface with metabolic preferences to control bioactive metabolite production in complex gut communities. Based on these insights, we are seeking to develop strategies to rationally control metabolite levels, with potential applications for microbiome-targeted therapies.

Podium presentations: Session 2—Advances in Rumen Biology

12 Microbial adaptation to combined feeding strategies alters ecosystem structure and fermentation.

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Rumen methanogenesis represents a major loss of dietary energy in dairy cattle, and strategies that redirect hydrogen away from methane production may improve rumen efficiency. Feed additives such as algae and methane inhibitor 3-nitrooxypropanol (3-NOP) have been shown to affect methanogenic archaea, while dietary manipulation through fat source selection can alter bacterial fermentation; however, their combined effects on the rumen ecosystem remain unclear. We hypothesized that combining feed additives with different fat sources would produce synergistic shifts in rumen fermentation and microbial community structure. To test this, we evaluated combinations of fat sources (soybean, canola, or linseed) and feed additives (cultivated macroalgae, 3-NOP) using 16S rRNA gene sequencing and fermentation profiling. Across studies, most additive-fat combinations did not significantly alter fermentation outcomes. In contrast, the inclusion of 3-NOP together with any fat source produced measurable metabolic effects and reduced the relative abundance of *Methanobrevibacter,* by approximately 50%. Although α diversity was unaffected, modest shifts in β-diversity and treatment-associated changes in the relative abundance of specific microbial taxa were observed. Dietary fat source alone had minimal effect on both fermentation and microbial community structure. These results indicate that the

methane inhibition via 3-NOP drives targeted shifts in methanogenic populations without broad restructuring of the rumen microbiome, and that co-supplementation with dietary fats does not enhance these effects. This suggests limited synergy between these strategies under the conditions evaluated and highlights the specificity of microbial responses to methane-targeting interventions.

13 Differential rumen responses in neonatal ruminants to volatile fatty acids, glucose, and physical stimulation: Insights into the drivers of early rumen development.

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The transition from milk to solid feed is a critical stage for rumen development in young ruminants, yet the distinct contributions of chemical stimuli, such as volatile fatty acids (VFAs), compared with physical stimulation, remain poorly understood. This study aimed to disentangle these influential factors during the pre-ruminant phase, specifically evaluating the roles of chemical signals (VFAs), simple energy provision (glucose), and physical stimuli in initiating functional rumen maturation. Using a novel, non-invasive oral tube delivery technique and 28 newborn male lambs, we evaluated rumen development, host tissue transcriptomics, and the gastric microbiota in response to a mixed VFA solution, an isoenergetic glucose solution, and sponge (a physical stimulus). VFA gavage proved to be the most effective treatment. It significantly increased rumen and reticulum weights, promoted papillae length and width. Transcriptomic analysis revealed upregulated expression of genes involved in epithelial development. These effects coincided with an improved host energy status, as evidenced by modulated hepatic gene expression, and a shift in the rumen microbiota toward VFA-producing and probiotic genera, such as *Porphyromonas* and *Ligilactobacillus*. In contrast, the isoenergetic glucose treatment induced a dysbiotic microbiota enriched with potential pathogens (e.g., *Streptococcus*) and triggered a host inflammatory response, despite partially stimulating papillae growth. Physical stimulation alone exerted a limited effect on papillae morphology and was associated with indicators of a negative energy balance, suggesting it is insufficient and potentially detrimental for initiating rumen development. In conclusion, our findings provide clear evidence that chemical signaling by VFAs is the primary driver of functional, healthy rumen maturation and of the establishment of a beneficial host-microbe interplay in neonatal lambs. These results demonstrate that the chemical signals (VFAs), rather than physical abrasion or simple energy provision, are the key determinant guiding the transition toward a functional rumen system.

Key Words: rumen development, volatile fatty acid, rumen microbiota, epithelial maturation, lamb

14 Host genetic variation in *SPINK5* regulates rumen epithelial barrier function and shapes heritable microbial communities through SCFA-mediated mechanisms in dairy cattle.

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Ruminants, through long-term natural evolution, have developed a nutritional strategy reliant on rumen microbial fermentation to generate short-

chain fatty acids (SCFAs) for energy acquisition. However, substantial interindividual differences in microbial composition persist among dairy cows, affecting feed efficiency and methane production. Although genome-wide association studies (GWAS) link genetic polymorphisms to microbial abundances, the physiological mechanisms through which host genetics regulate rumen microecology remain unexplored, hindering precision breeding. Integrating genomic, microbiomic, and metabolomic data from 603 lactating Holstein cows, we found genetic clustering significantly influenced rumen microbial β-diversity, explaining 4% to 22% of variation. Species-level heritability analysis identified 54 heritable microbial taxa ($h^2 \geq 0.2$), collectively representing 23% of bacterial and 44% of methanogenic abundance. Fermentation parameters showed high heritability, with propionate exhibiting the highest genetic control ($h^2 = 0.670$). GWAS identified a chromosomal hotspot containing 13 significant SNPs, fine-mapped to SPINK5. Functional validation revealed SPINK5, expressed in the spinous layer of rumen epithelium, controls keratinization and barrier permeability. SPINK5 knockdown accelerated keratinization by upregulating keratin genes, strengthening epithelial barriers and reducing SCFA absorption, while overexpression increased permeability. Co-occurrence networks showed moderate SCFA concentrations promoted maximum connectivity among heritable microbes. This study reveals a physiological mechanism whereby host genetic variants regulate rumen microbiota through SPINK5-mediated epithelial barrier control. SPINK5 functions as a molecular rheostat modulating epithelial permeability and paracellular SCFA absorption, thereby controlling intraluminal fermentation environments that shape heritable microbial communities. These findings provide actionable genetic markers for genomic selection programs, offering strategies to enhance rumen fermentation efficiency and reduce methane emissions.

Key Words: rumen microbiota, heritability, SCFA concentrations, GWAS, SPINK5

Podium presentations: Session 3—The human microbiome/host-microbe interactions in health and disease

15 Toward microbiome-informed personalized medicine: Rescuing the non-responders.

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The human gut microbiota plays a key role in determining individual responses to interventions, from diet, to lifestyle, to pharmaceuticals. In the past, medicine has largely focused on population-wide interventions, where patients are given a single drug or treatment for a given indication. While this approach has yielded substantial gains in population health, many individuals appear to be underserved by these one-size-fits-all solutions. Clinical non-responders have been left to fall through the cracks of our healthcare system. Rescuing these non-responders is a critical challenge of our era. Over the past several years, my group has shown several ways in which the gut microbiota, independent of host genetics or demographics, influences how people respond to interventions: from statins to diet and aging. I will discuss some of the computational and experimental tools we have developed to dissect and engineer these host-microbial systems, and how I envision expanding these tools into microbiome-mediated personalized medicine.

16 Niche dimensionality drives microbial community structure.

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Niche dimensionality links environmental complexity to ecosystem structure. Although niche theory is often invoked in investigations of microbiomes, most models assume very high-dimensional coexistence, effectively sidestepping the

role of dimensionality. However, direct estimates of niche dimensionality of microbiome have been lacking. Here we use joint species distribution modeling (JSDM) to infer niche dimensionality from relative abundance data alone. In paired 16S rRNA–metabolomics datasets, inferred dimensionality closely tracked the complexity of the metabolic environment, validating our abundance-only approach. Across nearly 200 human gut microbiome studies, lower dimensionality coincided with greater inter-species competition (metabolic niche overlap) and reduced biodiversity, consistent with the macroecological niche dimensionality hypothesis. Consumer/resource simulations reproduced these empirical relationships when both species-intrinsic metabolic tradeoffs and species-extrinsic environmental tradeoffs were imposed, with the latter dominating. Dimensionality was reduced in stressed or diet-simplified microbiomes and correlated broadly with ecological stress markers; negatively with prevalence of generalists and positively with microbial load. Together, these results establish niche dimensionality as a measurable and previously overlooked driver of microbial community structure.

Key Words: niche dimensionality, competition, low dimensional microbiomes

17 Revealing the pervasive landscape of MGE-host interactions in situ with single-cell genomics.

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Mobile genetic elements (MGEs), including plasmids and viruses, drive microbial evolution and ecosystem dynamics, yet their distribution, host range, and functions remain poorly understood, especially among uncultivated lineages. Using ~60,000 single-cell amplified genomes (SAGs) from host-associated and environmental microbiomes, we identified MGEs internal to or attached to individual cells, directly linking them to their hosts. Between 25% and 75% of cells contained at least one MGE, with gut-derived SAGs showing the highest MGE load. Although most MGEs exhibited narrow, species-specific host ranges, a subset spanned multiple species and higher taxonomic ranks. We detected MGE clusters and complete, nearly identical genomes repeatedly associated with hosts across phyla, suggesting both genuinely broad host ranges and recurrent DNA entry events. We also identified microbial species acting as major donors or recipients in antibiotic resistance gene (ARG) exchange, and MGEs, including unclassified types that mediate extensive horizontal transfer of auxiliary functions. Together, these findings illuminate MGE–host associations and underscore that the genetic content of microbial cells is fundamentally linked to their MGEs in nature.

Key Words: mobile genetic elements, horizontal gene transfer, single cell genomics

18 *Bacteroides intestinalis*-driven arabinoxylan fermentation mitigates inflammatory and metabolic dysfunction.

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Consuming a Western diet, characterized by high-fat, high-sugar, and low-fiber foods, has been linked to compromised gut microbiota function and the rising metabolic and inflammatory disorders that affect millions of people worldwide, causing enormous human suffering and economic burden. In contrast, a higher intake of dietary fiber is associated with a more diverse gut microbiome and a reduced risk of these diseases. The health benefits of fermentable fibers depend on fiber-utilizing gut microbes that convert complex polysaccharides into bioactive metabolites with protective effects. Yet, the fiber-deficient Western diet has reduced or eliminated many fiber-fermenting taxa from the gut microbiota, diminishing the benefits of dietary fiber for many individuals. To address this gap, we aim to develop synergistic synbiotics that pair fiber-fermenting microbes with their preferred substrates as therapeutic dietary supple-

ments. We recently made an exciting finding: the human gut commensal *Bacteroides intestinalis* ferments insoluble wheat arabinoxylan (inWAX) in vivo, a complex polysaccharide abundant in dietary fiber, thereby enhancing host resistance to inflammatory bowel diseases and improving glucose tolerance in high-fat diet-induced obesity. Mechanistically, the intervention selectively increases the hepatic and microbial production of anti-diabetic and anti-steatotic bile acid species and promotes the microbial transformation of anti-inflammatory and antioxidant phenolic compounds. This metabolic shift is accompanied by coordinated transcriptomic reprogramming in the colon and spleen, activating gene networks that support triglyceride metabolism, circadian regulation, and immune protection. Our findings identify *B*. *intestinalis* as a key driver of dietary fiber-driven metabolic and immune benefits, and underscore the therapeutic potential of the *B. intestinalis*–inWAX synbiotic for mitigating inflammatory and metabolic diseases.

Key Words: *Bacteroides intestinalis*, insoluble wheat arabinoxylan, fermentation, bile acid remodeling, phenolic compound transformation

19 A functional role for a microbial cortisol-metabolizing enzyme in the human gut.

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Gut bacteria are known to metabolize endogenous and exogenous compounds into a variety of metabolically active compounds that affect host physiology and disease predisposition. *Clostridium scindens* was previously found to have a cortisol-induced operon (DesABCD), encoding 2 cortisol metabolizing enzymes, including DesC, an NADH-dependent 20α-hydroxysteroid dehydrogenase that converts cortisol to 20α-dihydrocortisol. However, the physiological effects of this metabolite on the host remain unknown. After resolving the structure and reaction mechanism of DesC, we generated a loss-of-function mutant lacking cortisol-metabolizing activity. We conducted an in vivo study in germ-free mice colonized with *E. coli* expressing either functional or mutant *DesC* gene and treated with cortisol, 20α-dihydrocortisol or vehicle control. RNA sequencing of cells collected from the small and large intestine revealed that microbial production of 20α-dihydrocortisol induced pro-inflammatory conditions in the mouse gut, with upregulation of inflammatory and cancer-related pathways across multiple cell types. To examine the direct effects of 20α-dihydrocortisol, primary mouse intestinal epithelial cells were isolated and treated with either cortisol or 20α-dihydrocortisol. RNA sequencing revealed activation of the MAPK/ERK signaling pathway, accompanied by increased pro-inflammatory cytokine expression and inhibition of cell cycle-regulating genes in the 20α-dihydrocortisol-treated cells. Western blot analysis and immunostaining confirmed MAPK/ERK signaling pathway activation. To investigate the molecular mechanism underlying these effects, we used siRNA to identify an orphan nuclear receptor: Nuclear Receptor family 4 member 3 (NR4A3), which mediates 20α-dihydrocortisol signaling. Isothermal titration calorimetry (ITC) demonstrated that 20α-dihydrocortisol, but not cortisol, binds the ligand-binding domain of NR4A3. Together, these findings demonstrate that bacterial production of 20α-dihydrocortisol promotes gut inflammation through NR4A3-mediated signaling pathways. This study highlights a novel mechanism by which gut microbiota-derived metabolites modulate host physiology.

Key Words: gut microbiome, cortisol metabolism, 20α-dihydrocortisol, NR4A3 signaling, hydroxysteroid dehydrogenase

Podium presentations: Session 4—Animal nutrition

20 Abrupt dietary shifts as a pre-harvest food safety intervention strategy lead to profound shifts in the ruminant gastrointestinal microbiome in feedlot cattle.

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Foodborne pathogens such as *Escherichia coli* O157:H7 can live in the gut of cattle. Inclusion of dried distillers grains and solubles (DDGS) increased *E. coli* O157:H7 shedding and altered the gut microbial population. Abruptly changing from high-concentrate to high-forage diets reduced *E. coli* O157:H7, but effects on the gut microbiome remain unknown. The effects of abrupt dietary shifts from a high-concentrate and DDGS-containing to forage rations on the ruminal and fecal microbial populations were evaluated. The first study used 32 Angus cattle (359 ± 62.2 kg) in a randomized complete block design. Pre-switch treatments were: (1) 0% DDGS (CTRL), (2) 20% DDGS, and (3) 40% DDGS. A positive control group (PCTRL) received the 40% DDGS diet throughout, whereas all other groups switched to endophyte-free alfalfa and tall fescue hay. Principal component analysis (PCA: 51.95% total variance explained) identified changes in bacterial genera, α diversity, and performance metric. PCTRL treatment maintained a distinct microbial consortium with greater *Prevotella* ($P \leq 0.048$). In contrast, hay-fed cattle exhibited increased ($P \leq 0.036$) populations of *Butyrivibrio_A_168226,* post-switch. Pielou's evenness and Shannon diversity index were lowest ($P \leq 0.006$) in PCTRL. Cattle on 20% and 40% DDGS diets had higher ($P \leq 0.049$) fecal shedding of *E. coli*/coliforms. Switching to forage lowered *E. coli* and coliform counts, and lead to clear microbiome changes. In study 2, cattle (n = 27) were abruptly switched from a high-concentrate feedlot ration to (1) high-concentrate (CTRL), (2) low-quality forage (LQF; Bermuda grass hay), and (3) high-quality forage (HQF; endophyte-free alfalfa and tall fescue hay). Microbial α diversity did not differ ($P \leq 0.05$) until 22 d after the dietary switch. Overall, forage-based diets enhance microbial diversity through complex substrates, with forage quality having the greatest effect over longer adaptation periods. Despite the benefits in reducing *E. coli* populations in the gut of food animals, abruptly switching from high-concentrate to forage-based ration is not a practical solution to improve food safety commercially. Furthermore, nutrient composition is crucial to unraveling the mechanisms associated with effective preharvest pathogen reduction strategies.

Key Words: food safety, pre-harvest, microbial ecology

21 Effects of supplementing the homoacetogen *Blautia pseudococcoides* as an alternative hydrogen sink on in vitro rumen fermentation and methanogenesis.

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Ruminants convert plant biomass into metabolically available energy via the rumen microbiome, a diverse ecosystem comprising bacteria, archaea, fungi, protozoa, and bacteriophages. Rumen fermentation produces hydrogen (H_2), which is primarily utilized by methanogenic archaea that convert it into methane (CH_4). As CH_4 is a potent greenhouse gas, substantial efforts have aimed to mitigate enteric CH_4 emissions from ruminants. However, direct inhibition of rumen methanogenesis leads to H_2 accumulation in the rumen, negatively affecting fibrolytic rumen microbes and the host animal. An alternative H_2 utilization pathway in the rumen to remove excess H_2, is reductive acetogenesis. Although thermodynamically less favorable than methanogenesis, reductive acetogenesis conserves

energy as acetate, a volatile fatty acid (VFA) that the animal can use for milk and meat production. In this study, we hypothesized that (1) increasing the concentration of hydrogenotrophic acetogens in the rumen would enhance their competitiveness against methanogens for H_2 utilization, and (2) supplementing hydrogenotrophic acetogens in the rumen when methanogenesis is inhibited would redirect excess H_2 toward acetate formation. To test this, the hydrogenotrophic acetogen *Blautia pseudococcoides* was supplemented at increasing concentrations (0%, 1%, 5%, and 10%), with and without the addition of bromoform (a known methanogenesis inhibitor) at 0.02% DM in an 18-d semi-continuous in vitro rumen fermentation system. Total gas, CH_4, and H_2 production were measured every 2 d, as well as total VFAs and acetate concentration. According to our results, the addition of *B. pseudococcoides* showed a trend toward decreased CH_4 concentration ($P < 0.10$) and numerically increased acetate concentration, whereas total gas production and H_2 concentration remained unchanged. Co-supplementation with bromoform significantly decreased CH_4 and H_2 production and increased acetate concentration ($P < 0.05$), particularly at the highest *B. pseudococcoides* inclusion level (10%). Total VFA concentration remained unchanged. These results suggest that combining *B. pseudococcoides* with bromoform may redirect the excess H_2 generated during methanogenesis inhibition toward acetate production, representing a promising strategy to mitigate enteric CH_4 emissions in ruminants.

Key Words: reductive acetogenesis, methanogens, bromoform supplementation, hydrogen sink, methane mitigation

22 Effect of dietary supplementation of bovine-derived *Bifidobacterium longum* ssp. *longum* on the gut bacterial establishment in colostrum-compromised calves.

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Insufficient colostrum feeding leads to gut dysbiosis (reduced beneficial bacteria and increased pathogen abundance) in newborn calves. Probiotics are reported to improve gut health, but findings are controversial due to the lack of host-specific products tailored to calves and limited understanding of their mode of action in vivo. This study assessed the effects of a bovine-derived *Bifidobacterium longum* ssp. *longum* cocktail on temporal gut bacterial dynamics in calves receiving insufficient colostrum at birth. Twenty-seven newborn Holstein calves were randomly assigned to either optimal colostrum feeding (OC, n = 9) receiving 4 L of colostrum replacer (CR, 200 g of IgG) or suboptimal colostrum feeding (SOC, n = 18) receiving 1 L of CR (50 g of IgG), within 2 h of birth. From d 2 to d 14 postpartum, OC calves received a placebo, while SOC calves were assigned to either a placebo (SOC, n = 9) or a cocktail of 5 *Bifidobacterium longum* ssp. *longum* strains (5×10^9 cfu/d; PRO, n = 9) administered during morning feeding. Fecal samples were collected at d 2, 7, 14, 21, 28, and 35 to quantify bacterial groups using quantitative PCR (qPCR) and to profile bacterial community using 16S rRNA gene amplicon sequencing. *Bifidobacterium* spp. abundance increased from d 2 to d 35 in all groups ($P < 0.001$), with a greater fold increase in PRO (~1,596-fold) than SOC (~119-fold). MaAsLin2 identified more differentially abundant bacterial taxa between SOC and PRO (10 taxa) than between OC and PRO (1 taxon) from d 7 to d 35, indicating that PRO shifted the gut bacterial composition of SOC calves toward that of OC calves. Specifically, higher abundance of *Sharpea* spp. and *Succiniclasticum* spp., and lower abundance of *Tyzzerella* spp. and *Eisenbergiella* spp., were observed in PRO compared with SOC. Moreover, OC (554 ± 33.4, $P = 0.006$) and PRO (593 ± 30.8, $P = 0.04$) displayed similarly lower richness (observed ASVs) than SOC (697 ± 30.6) on d 35, whereas OC and PRO did not differ ($P = 0.61$), suggesting that PRO restored the microbial diversity pattern toward that in OC calves. These findings suggest that PRO may reverse, at least partially, the gut dysbiosis caused by insufficient

colostrum feeding. Further studies are needed to understand whether PRO affected gut microbial metabolic function in relation to their influence on calf health.

Key Words: bovine-derived probiotic, colostrum-compromised calf, gut bacteria

Computational approaches and applications

23 Characterization of cooperative network structure of rumen bacterial communities centered on *Aristaeella* spp. (*Christensenellaceae* R-7 group) and its role in fermentation.

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Rumen bacteria form a core microbial community that supports host energy supply through diverse metabolic functions. Within the rumen microbial community, bacteria with different functions interact to form complex metabolic networks that cooperatively drive rumen fermentation. Ruminant production efficiency, including feed energy loss due to methane emission, is closely related to rumen fermentation, and understanding microbial interactions is essential for optimizing it. In this study, we aimed to determine the co-occurrence network structures of the bacterial community, which were linked to fermentation characteristics. To achieve this, in vitro rumen cultures were conducted under different conditions that induced distinct fermentation characteristics. Bioinformatic analyses were performed on the datasets of the fermentation parameters and microbial communities across the respective culture conditions. *Aristaeella* spp. (previously known as *Christensenellaceae* R-7 group) consistently showed high relative abundance and condition-dependent network centrality. The microbial assemblages surrounding *Aristaeella* spp. varied markedly among fermentation conditions. Network structures centered on *Aristaeella* spp. were strongly associated with elevated methane production, whereas under low-methane conditions, *Aristaeella* spp. was embedded in distinct communities without a clear association with methane production. These results suggest that *Aristaeella* spp., a major hydrogen producer in the rumen, may act as a central player influencing electron flow and metabolic pathways through cooperative interactions with co-occurring microorganisms. In the methane-associated networks, *Aristaeella* spp. co-occurred with several functionally distinct bacterial groups, suggesting potential cooperative interactions within the rumen bacterial community. Notably, associations with acetogenic bacteria were related to reduced methane production, suggesting a redirection of hydrogen from methanogenesis to acetogenesis. Together, the present study indicates that *Aristaeella* spp. can interact with various members of the rumen bacterial community and may influence methane production through hydrogen utilization.

Key Words: rumen microbiota, microbial interaction, methane production, co-occurrence network, hydrogen metabolism

24 Method choice matters—Identifying intestinal fungi and protists from shotgun metagenomic data.

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The intestines host diverse microorganisms, including fungi and protists. These eukaryotes can interact with bacteria or contribute to disease but methods to detect them remain poorly characterized. Shotgun metagenomics is a powerful approach for simultaneously quantifying microbes across domains. Although many methods can detect eukaryotes from these data, their performance on intestinal eukaryotes is unclear. Furthermore, it is uncertain if eukaryotic count data should be normalized separately from bacterial counts that follow distinct distributions. We simulated synthetic metagenome libraries from mock intestinal communities. We then assessed how effectively existing methods can identify intestinal eukaryotes using various reference databases. We next simulated count data for microbes across domains (archaea, bacteria, eukaryotes, and viruses) and injected various time trends, which we sought to detect with different types of linear mixed models (LMM) after we normalized counts separately for each domain or together. The mapping based MiCoP and k-mer based Kracken2 were significantly ($P \leq 0.05$) and dramatically more accurate (F1 score) than MetaPhlAn4, EukDetect, or human mycobiome scan. This accuracy required use of the most recent and expansive Kracken2 database for both

methods. Use of the MiCoP database, the standard Kraken2 database, or the 2024 version of the expanded Kraken2 database all significantly ($P \leq 0.05$) decreased the accuracy of both tools. Performance when classifying fungi versus protists was not significantly ($P > 0.05$) different for any method save for EukDetect, which was significantly ($P \leq 0.05$) better at detecting fungi. After detection, normalizing each domain separately made a small but significant ($P \leq 0.05$) impact on the ability to detect known fluctuations in rare microbes. The most effective LMM for doing so varied by domain but included MaAslin2 with the negative binomial distribution and the hurdle based MaAslin3. These findings highlight the importance of selecting appropriate methods to detect intestinal eukaryotes and emphasize the importance of using up-to-date databases. By normalizing rare microbes away from other domains, investigators can increase their ability to detect patterns in the abundance of rare microbes that may be contributing to intestinal health or disease.

Key Words: microbiome, fungi, methods, metagenomics

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

25 3-Nitropropionic acid induces biphasic microbial restructuring and redirects hydrogen flux during in vitro rumen fermentation.

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Nutritional strategies targeting rumen microbial modulation are promising for enteric methane mitigation; however, methanogenesis inhibition may cause hydrogen accumulation and reduced fermentation efficiency. 3-Nitropropionic acid (3NPA), a plant-derived organic nitro compound, has hydrogen electron-accepting potential in the rumen, yet its microbial effects remain poorly understood. We evaluated fermentation dynamics and microbial restructuring in vitro using control and 3NPA-treated incubations over 48 h under a 50:50 concentrate-to-forage diet. 3NPA caused complete methane suppression from 3 to 12 h and reduced gas production by 13% to 29% during early fermentation (3–6 h). Formic acid was only detected in treated samples between 3 and 12 h. Butyrate increased by 34% at 24 h, propionate was enhanced at 48 h, and the acetate to propionate ratio declined, indicating a shift toward propionate favoring pathways. Microbial analysis revealed that the *Bacteroidota*/*Bacillota* ratio declined below 1 after 24 h in treated samples, coinciding with methane inhibition and increased butyrate production. Methane suppression was associated with a significant reduction in *Methanobrevibacter*. Hydrogen metabolism associated taxa were differentially affected by 3NPA; *Selenomonas, Blautia, Anaerovibrio, Pseudobutyrivibrio, Oribacterium* increased markedly, whereas *Succiniclasticum, Ruminococcus, Treponema, Butyrivibrio* decreased ($P < 0.01$). Fiber degraders showed a similar pattern; *Fibrobacter* and *Rikenellaceae RC9 gut group* increased, whereas *Ruminococcus* and *Treponema* declined, indicating functional redundancy as fiber digestibility was maintained ($P > 0.05$). *Denitrobacterium*, associated with 3NPA degradation, was consistently enriched across incubation. Early fermentation (0.5–6 h) showed transient pH reduction and enrichment of *Streptococcus.* From 12 to 48 h, enrichment of hydrogen consuming and butyrate or propionate associated taxa, including *Sutterella, Desulfovibrio, Prevotellaceae YAB2003*, coincided with stabilized fermentation. Collectively, 3NPA induced a biphasic microbial response characterized by

early metabolic reprogramming followed by stabilization, activating multiple hydrogen sinks and redirecting electron flow away from methanogenesis without impairing fiber degradation.

Key Words: 3-nitropropionic acid, methane inhibition, hydrogen redirection

26 The transcriptional plasticity of rumen microbes underlies growth performance and methane output in hay-fed beef cattle.

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Improving ruminant productivity while minimizing greenhouse gas (GHG) release is a major challenge for sustainable agriculture. However, the physiological mechanisms underlying within-herd variation in growth performance and methane emissions are not well understood, hindering the development of effective solutions. In this work, we analyzed the composition and transcriptional activity of the rumen microbiome and their relationship with growth and methane output in 53 hay-fed beef heifers. Although microbiome features measured at the DNA level showed only modest associations with performance or methane emissions, animals that gained more weight than expected from their feed intake or emitted less methane than expected from their weight gain showed increased transcription of genes involved in microbial growth and energy metabolism. Notably, these expression differences were observed across most rumen species, suggesting a common selective pressure and evidencing widespread transcriptional adaptability of rumen microbes. We hypothesized that higher ruminal passage rates driven by rumination differences between animals select for faster microbial growth rates, leading to increased productivity of microbial biomass and higher residual weight gain with reduced methane emissions. Interestingly, we also found that weight gain was negatively associated with digestibility independently of feed intake. This suggests that nutrient availability to rumen microbes, possibly mediated by increased surface area and particle colonization, may rise more rapidly than passage rate as a function of rumination, enhancing microbial biomass production but reducing overall feed digestibility. Altogether, our results suggest that within-herd differences in animal performance and GHG emissions are partly mediated by concerted transcriptional reprogramming by rumen microbes, potentially driven by ruminal passage rates.

Key Words: rumen microbiome, growth performance, beef cattle, methane emission, metatranscriptomics

27 Genetic toolkits for rumen bacteria to advance methane control.

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Bacteria are the most abundant members of the rumen microbiota. They play a crucial role in breaking down plant biomass to soluble sugars, which are further fermented to VFAs, CO_2, and H_2. Methanogens in the rumen reduce CO_2 to methane leading to enteric methane emission and loss of energy to the host animal. The lack of genetic tractability within this community limits both understanding and methane mitigation efforts. This study focused on the development of genetic toolkits for 11 predominant bacterial strains including major plant cell wall degraders and lactate metabolizers. To ensure identity and purity, clonal cultures were generated through multiple rounds of colony purification and confirmed by 16S rRNA gene sequencing. Whole genomes of the bacteria were then sequenced and methylome analysis conducted to identify their methylation patterns. This enabled us to design and synthesize DNA lacking these restriction sites, evading degradation by host re-

striction systems and allowing introduction into the bacterial host. In addition, genes encoding the native methyl transferases were identified, cloned and expressed in *Escherichia coli.* These *E. coli* strains will be used to protect plasmid DNA before transformation into the target organism. Through antibiotic sensitivity testing, potential antibiotic selectable markers were identified for the genetic toolkits. The plating efficiency of each of the studied bacteria was also assessed to ensure a high transformation efficiency. The development of these genetic tools will enable the manipulation of rumen microbes, which will bridge the gap to understanding and controlling the processes driving methane production.

Key Words: rumen bacteria, genetic toolkits, methylome analysis, methane mitigation

28 Inhibition of methanogenesis with biochar in combination with nitrate in continuous culture of ruminal microbes.

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Biochar (BC) has redox-active chemical groups. We hypothesized that the potential for added NO_3^- to compete for aqueous H_2 [H_2(aq)] to suppress methanogenesis would be enhanced by NO_3^- embedding in the BC matrix. Rumen fluid was collected from 2 cannulated Holstein cows to inoculate 5 dual-flow continuous culture fermenters in 5 periods in a 5 × 5 Latin square design. All fermenters were fed 50 g (dry matter [DM]) of a pelleted 50:50 forage:concentrate diet every 12 h. The 5 treatments were control, BC (2% DM), NO_3^- (1.5% DM), BC + NO_3^- (2% DM both), and BC + NO_3^- (1% DM both). Liquid and solid outflow rates were set to 10% and 5%/h. Periods were 8 d of adaptation followed by 4 d of sampling. All treatments decreased ($P \leq 0.03$) CH_4 production, with the 1% BC + NO_3^- combination resulting in the largest reduction (87%, $P < 0.01$). Both BC and 1% BC + NO_3^- reduced daily H_2 emission while 1.5% NO_3^- increased ($P = 0.07$) the emission. We concluded BC interacted with the ruminal H_2 pool to enhance NO_3^- reduction with H_2(aq) while minimizing H_2 emission. None of the treatments affected ($P > 0.10$) neutral detergent fiber degradability (NDFD). Lack of differences in NDFD suggests that CH_4 per unit of NDFD was decreased considerably by BC and NO_3^-. The 1% BC + NO_3^- and 1.5% NO_3^- increased ($P = 0.01$) propionate production by 15% and 21%, respectively, highlighting NO_3^- as an electron acceptor both alone and when combined with low doses of BC. All treatments containing NO_3^- increased ($P \leq 0.10$) total branch-chained volatile fatty acids (BCVFA), and the 1% BC + NO_3^- combination decreased ($P \leq 0.08$) NH_3-N concentration. The NO_3^-/NO_2 concentrations declined to near-zero by 2 h after feeding, indicating a rapid reduction across all NO_3^- doses. Further information is forthcoming to help elucidate microbial mechanisms underpinning the BC and NO_3^- interaction.

Key Words: ruminants, biochar, nitrate, methane, hydrogen

29 Isolation of rumen acetogens and methanogens from bovine and ovine rumen contents.

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Methanogenic archaea are the main users of hydrogen in the rumen, but there are few pure cultures of ruminal methanogens available. Ruminal organisms able to carry out reductive acetogenesis are of particular interest, as they may provide an alternative sink for hydrogen but cultures of these organisms are also lacking. Culturing a wider diversity of ruminal acetogens and methanogens will help in understanding their physiologies, ecological roles, and metabolic capabilities and will complement the large amount of metagenomic data that is accumulating from rumen microbiome studies. The aim of this study was to isolate previously uncultured ruminal acetogens and methanogens from both cattle and sheep. Rumen contents were collected and

maintained under anaerobic conditions and inoculated into a dilution medium. Ten-step, 10-fold dilution to extinction series were performed before inoculating diluted sub-samples into 6 different types of media designed to target both methylotrophic and hydrogenotrophic methanogens and acetogenic bacteria. After a period of incubation of 2 to 4 wk, depending on growth in the different media, visual growth scoring, and measurements of methane and acetate production were used to estimate the abundance of target organisms, employing a most probable number method. In calf rumen samples, methanogens were estimated at 10^7 cells/mL, whereas mixotropic growth on substrates forming acetate with reduced hydrogen production yielded an estimate of 10^8 cells/mL. The richness of the sheep samples appeared to be lower at 10^6 cells/mL for mixotrophic acetate production and 10^5 cells/mL for methanogens. Samples from the higher dilution tubes exhibiting growth were inoculated into agar roll tubes and after incubation, isolated colonies were picked to purify strains. The enrichments were also preserved frozen with 10% glycerol at ⁻80°C to allow subsequent isolations. A greater diversity of methanogen cultures will enable a broader understanding of these important rumen microorganisms and allow better targeted approaches to decreasing their methane-forming activities. Obtaining more acetogenic cultures will allow detailed studies of their reductive acetogenic activities and their potential to act as alternate hydrogen users as part of methane-reducing technologies.

Key Words: rumen, methane, acetogen, methanogen, cultivation

Immunology (Including host-microbe interactions)

30 Fermented food associated aromatic amino acid microbial metabolites regulate macrophage inflammatory tone and function.

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Fermented foods provide increasingly appreciated health benefits, but mechanisms driving these effects are largely unknown. Aromatic amino acids are metabolized by select lactic acid bacteria (LAB) into downstream metabolites, termed aryl lactates, phenyllactic acid, 4-hydroxyphenyllactic acid, indole-3-lactic acid. Aryl lactates are produced by microbes in fermented foods and reach bioactive concentrations in commercial products. Previous work in our laboratory showed that feeding aryl-lactates to mice via drinking water at food relevant concentrations (0.25 mg/mL per aryl lactate), resulted in lower liver steatosis and sings of inflammation in the liver of diet-induced obese (DIO) mice. This led us to hypothesize that aryl-lactates signal via the innate immune system to regulate liver inflammation and limit co-morbidities associated with DIO. Here we determine how aryl-lactates affect macrophage physiology using human THP-1 macrophage cell line. THP-1 express the hydroxycarboxylic acid receptor HCA3, and the nuclear aryl hydrocarbon receptor AhR, 2 candidate aryl-lactate receptors. Using Presto-Tango GPCR and nuclear reporter assays, we identified stereospecific receptor engagement: D-PLA and ILA acted as HCA3 agonists, whereas L-PLA and 4-HPLA were inactive. In contrast, only ILA activated AhR, indicating receptor- and stereochemistry-specific signaling. We next examined how aryl-lactates affect macrophage efferocytosis, a key function regulating debris clearance. PMA-differentiated THP-1 macrophages were treated with PLA or ILA (10 μ*M*) for 48 h, then co-incubated with CFSE-labeled apoptotic Jurkat T cells (2:1) overnight to assess efferocytosis. Both ILA and PLA significantly increased apoptotic cell clearance (% efferocytosis, $P < 0.05$). We next examined whether aryl-lactates modulate macrophage polarization during polarization toward M0, M1, or M2 states. In cells treated with ILA (10 μ*M*) we observed significant shifts in polarization-associated cytokine expression, consistent with immunomodulatory activity. Ongoing siRNA and pharmacological antagonist studies are defining receptor-specific mecha-

nisms governing macrophage efferocytosis and polarization programs.

Key Words: aryl lactates, macrophages, HCA3, AhR, fermented foods

31 Identification of arabinoxylan-responsive bacteria in the gut microbiota via D-amino acid metabolism probe technology.

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Arabinoxylan, a common dietary fiber, is recognized for its ability to influence gut health and metabolic regulation via microbiota-mediated mechanisms. Despite its established benefits, the specific bacterial taxa that respond to arabinoxylan intervention have yet to be systematically characterized. In this study, we employed an integrative approach combining 16S rRNA sequencing with fluorescent D-amino acid (FDAA)-based metabolic labeling to identify bacteria that preferentially metabolize arabinoxylan. Our results showed that arabinoxylan supplementation led to increased fecal short-chain fatty acid (SCFA) concentrations and enhanced expression of antimicrobial peptides in the colon. These changes were accompanied by a marked restructuring of the gut microbial community, particularly an enrichment of taxa involved in carbohydrate metabolism. FDAA-based sorting coupled with fluorescence-activated cell sorting revealed a pronounced expansion of *Bacteroidetes* in mice receiving 10% arabinoxylan. Among these, *Bacteroides acidifaciens* emerged as a putative responder, showing strong correlations with acetate and propionate levels. Collectively, these findings provide new insight into the selective microbial targets of arabinoxylan and offer a framework for developing microbiota-directed functional foods.

Key Words: arabinoxylan, gut microbiota, fluorescent D-amino acid, SCFA

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

32 Vitamin B12-mediated regulation of BtuB receptor-dependent bacteriophage L6jm infection of *Salmonella enterica* serovar Choleraesuis.

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Salmonella enterica serovar Choleraesuis is a major zoonotic pathogen causing systemic infections in pigs and posing risks to human health. Rising antibiotic resistance has prompted interest in bacteriophages (phages) as alternative antimicrobials. In this study, a virulent bacteriophage, L6jm, was isolated from pig intestinal mucosa and digesta samples using *Salmonella enterica* serovar Choleraesuis 13312 as the host. Biological characterization revealed L6jm was a polyvalent phage capable of infecting 9 *Escherichia coli* strains (including one enterotoxigenic *E. coli* strain), exhibiting optimal multiplicity of infection (MOI) of 0.01, with good heat resistance and pH tolerance. Genomic analysis classified L6jm as a member of the *Tequintavirus* genus and confirmed the absence of virulence or drug-resistance-related genes. Given that phages adhering to the mucus layer may provide a non-host-derived defense by limiting bacterial colonization, we evaluated L6jm adhesion to intestinal epithelial cell lines (IPEC-1, Caco-2, and MDCK) and its potential to protect host cells from bacterial invasion. L6jm preferentially adhered to mucus-producing intestinal epithelial cells in a dose-dependent manner. However, pre-adhesion did not significantly protect host cells from bacterial invasion, and its antibacterial activity was strongly completely lost in DMEM/F12 medium, indicating nutrient-dependent regulation of phage infection. Screening of medium compo-

nents identified vitamin B12 (VB12) as a key inhibitory factor, markedly reducing phage adsorption rate and lytic activity. Deletion of the VB12 transporter gene *btuB* substantially decreased the adsorption rate of L6jm and abolished plaque formation. Moreover, VB12 suppressed L6jm-induced *btuB* mRNA levels, suggesting competitive inhibition of phage adsorption via receptor occupancy. These findings reveal that phage-host interactions in the gut are regulated by nutrient availability and provide mechanistic insight into the potential use of phages for controlling enteric pathogen infections.

Key Words: *Salmonella enterica* serovar Choleraesuis, bacteriophage, biological characterization, vitamin B12, phage receptor

33 Cross-platform and cross-database comparative analysis of 16S rRNA sequencing analysis for high-resolution profiling of rumen bacterial communities.

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Technological advances in full-length 16S rRNA gene sequencing have improved taxonomic resolution in rumen microbiome studies, however, a considerable fraction of detected taxa remains unclassified. In addition, discrepancies among sequencing platforms and reference databases often result in inconsistent microbial profiles. The current study evaluated to identify the optimal combination of sequencing platform, analytical workflow, and reference database to improve rumen bacteriome classification using short-read and full-length 16S rRNA gene sequences generated from rumen samples collected from 2 beef cattle populations. Short-read sequencing was performed using Illumina NextSeq2000 and processed with QIIME2. Full-length 16S rRNA sequencing was performed using PacBio Revio (PacBio-16S) and Nanopore MinION (ONT-16S); PacBio-16S data were analyzed using QIIME2 and Emu, while Nanopore data were analyzed using EPI2ME and Emu. Taxonomic profiling was conducted across 5 databases: SILVA 138.2, SILVA 138.2 with Hungate1000 collection, NCBI, Greengenes2 (2024.9), and GTDB (10-RS226). The comparisons showed that PacBio-16S processed with Emu exhibited the highest number of classified reads among all analytical workflows and GTDB consistently produced the highest number of uniquely classified taxa. At the genus level, *Prevotella* was predominant in Illumina and PacBio-16S datasets under GTDB but was underrepresented in ONT-16S workflows. At the species level, PacBio-16S combined with Emu showed reliable resolution of *Prevotella* species under GTDB, whereas Greengenes2 frequently resulted in misclassification. Overall, our results demonstrate that platform type, workflow choice, and database selection strongly influence rumen bacteriome profiles, and PacBio-16S analyzed with Emu and GTDB was as the most reliable workflow for achieving high-resolution taxonomic classification of rumen bacteriome.

Key Words: rumen, bacteriome, full-length 16S rRNA gene sequencing, taxonomic resolution, reference database limitation

34 Analysis of rumen microbial composition of Scottish Blackface sheep consuming pasture- or concentrate-based diets using culture-based and culture-independent metagenomic techniques.

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Understanding rumen microbial ecology is vital for enhancing feed efficiency and mitigating methane emissions. This study compared the rumen microbial communities in 10 Scottish Blackface sheep (n = 5/diet) either grazed on perennial ryegrass or fed ad libitum concentrate diets. Post-slaughter, rumen fluid, solid digesta, and papillae were analyzed using 16S rRNA/ITS sequencing and anaerobic (80% N_2, 10% H_2, and 10% CO_2) culturing methods (direct plating and enrichment) on 6 rumen-simulating media (M2, PYG, M10, PC, MRS, Orpin). Bray–Curtis

PCoA indicated that diet and rumen fraction significantly influenced microbial composition (R^2 = 0.336, P = 0.001), with diet as the primary driver. In 2 diets formed distinct clusters, papillae-associated communities were clearly separated from digesta, especially under concentrate feeding. Alpha diversity (richness and Shannon index) was significantly higher in the papillae and solid digesta of grazing sheep ($P < 0.05$), whereas fluid communities remained more stable across diets. Grazing promoted higher relative abundances of fibrolytic and butyrate-producing genera, including *Xylanibacter*, *Ruminococcus*, and *Butyrivibrio*. Conversely, concentrate feeding increased *Fibrobacter* and *Treponema* in the solid fraction. VFA analysis showed acetate as the dominant product, followed by propionate and butyrate. All VFAs reached significantly higher concentrations in grazing sheep. Fungi play a key role in lignocellulose degradation and nutrient availability, yet their ecology is still not fully understood. In grazing animals, *Feramyces* predominated, whereas *Neocallimastix* became dominant under concentrate diets. Notably, *Cecomyces* showed consistently higher relative abundance in concentrate-fed sheep across all fractions, indicating a possible adaptation to such diets. Anaerobic culturing produced 140 pure isolates. Sanger sequencing confirmed the presence of *Enterococcus*, *Lactobacillus*, and *Streptococcus* spp., including *Lactiplantibacillus plantarum*, a potential direct-fed microbial candidate. In conclusion, diet and rumen niche strongly dictate microbial structure and fermentation. Integrating sequencing with targeted culturing provides a robust framework for characterizing microbial populations leading to the development of sustainable strategies to improve ruminant productivity and methane mitigation.

Key Words: rumen microbiome, anaerobic culturing, sheep

35 Community context reshapes microbial proteomes and reduces functional overlap.

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Microbial coexistence in complex communities requires mechanisms that minimize competition and optimize resource use. Here, we show that bacteria modulate protein abundance in response to specific community members, reducing functional redundancy and promoting metabolic complementarity. Using synthetic gut-derived consortia exposed to distinct carbon sources, we systematically profiled proteomic responses of individual species across isolate, pairwise, and 4-member communities. We found that biotic interactions, rather than abiotic conditions, were the dominant drivers of proteomic variation. These interactions led to reproducible, partner-specific expression shifts that significantly reduced functional overlap and were frequently associated with increased community productivity. Our findings reveal that microbes dynamically reshape their realized niche through protein abundance plasticity, enabling them to partition metabolic space and stabilize community structure. This study provides a mechanistic link between microbial interaction networks, regulatory flexibility, and coexistence, offering a generalizable framework for understanding and engineering functional microbial ecosystems.

Key Words: metaproteomics, biotic interaction, functional redundancy, microbial productivity, microbial coexistence

37 UHPLC-QTOF-IMS metabolomics-based phytochemical characterization of high-altitude Himalayan green leafy crops and determination of gastro-intestinal digestibility of antioxidants.

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The indigenous communities in the high-altitude regions of Indian Himalayan Region, a biodiversity hotspot, rely on wild edible plants (WEPs) for their nutritional needs. We evaluated the nutritional and bioactive properties of frequently consumed indigenous wild green leafy vegetables (GLVs), viz., *Amaranthusspinosus* (Spiny amaranth), *Urtica dioica* (stinging nettle), and *Zanthoxylum armatum* (winged prickly ash).

UHPLC-QTOF-IMS-based targeted and non-targeted metabolomics identified antioxidants and anti-inflammatory compounds in these GLVs, viz., flavonoids (quercetin, kaempferol, epicatechin, rutin), phenolic acids (protocatechuic acid, ferulic acid, caffeic acid, gallic acid) and oxygenated lipids (oxylipins), particularly hydroxy fatty acids such as 9-hydroxy-10,12-octadecadienoic acid, and 9-hydroxyoctadecatrienoic acid in stinging nettles. The GLVs possessed moderate fiber content ranging from 3.3 to 8.60 g 100 g^{-1} promoting digestion and supporting healthy gut flora. Further the GLVs, particularly amaranth and nettles, were abundant in essential micronutrients such as iron (40–70 mg 100 g^{-1}) and zinc (3–5 mg 100 g^{-1}). These essential nutrients and phytochemicals have been reported to protect the gastrointestinal mucosa, reduce oxidative stress, and support gut barrier integrity. Supporting this hypothesis, the hydro-alcoholic extracts of GLVs exhibited strong free radical scavenging and reducing power activities (IC_{50} values <200 µg mL^{-1}). The overall phytochemical abundance and antioxidant activity demonstrated by the GLVs were much higher compared with conventionally consumed vegetables such as broccoli, carrot, and spinach. The in vitro gastrointestinal digestibility of polyphenols in these GLVs ranged between 18% and 30% with phenolic acids showing 1.5-fold higher intestinal bioaccessibility compared with flavonoids. Among the GLVs, spiny amaranth exhibited higher polyphenol accessibility. Incorporation of these GLVs in the diet would enrich the nutrient quality vis-à-vis therapeutic benefits, primarily anti-inflammatory properties supporting gut health. The GLVs could be explored for their role as prebiotics in modulating gut microflora and improve the gut health.

Key Words: green leafy vegetables, gastrointestinal bioaccessibility, polyphenols, antioxidants, Western Himalayas

38 Diet-influenced hydrogen accumulation, VFA shifts, and methane responses to 3-nitrooxypropanol in a dual flow continuous culture rumen fermentation system.

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Methane (CH_4) has a global warming potential approximately 80 times higher than carbon dioxide (CO_2) over 20 years. Enteric fermentation contributes 27% to 31% of anthropogenic methane emissions, and methane emission represents an energy loss in ruminants. The availability of hydrogen (H_2), as an electron donor in hydrogenotrophic methanogenesis, determines the direction of hydrogen flow within rumen fermentation pathways and is modulated by diet. This study investigated responses to the specific methanogenic inhibitor, 3-nitrooxypropanol (3-NOP; 99.7% purity), using a dual-flow continuous culture fermenter. A 2 × 2 factorial design with 8 fermenters evaluated the effects of diet type (forage vs. high-concentrate) and 3-NOP supplementation (10, 15, 20 mg 3-NOP/kg DM). 3-NOP was applied in stepwise dose increments with intervening recovery periods. Biogas, dissolved hydrogen (dH2), metabolites, and volatile fatty acids (VFAs) were monitored. 3-NOP reduced methane production in a dose-dependent manner, with a greater response on the high-concentrate diet (34%–52% reduction) compared with the forage diet (24%–61% reduction). Despite lower methane concentrations on the high-concentrate diet, increased total biogas production resulted in comparable or higher methane output to the forage diet. This inhibition led to increases in hydrogen accumulation, with gaseous H_2 increasing up to 5- to 39-fold (forage) and 14- to 69-fold (concentrate), as dH_2 reached up to 0.55 m*M*. During intervening recovery periods without 3-NOP (8.4 turnovers), methane production recovered to more than 80% of that observed in control fermenters. Total VFA concentrations were higher under the high-concentrate diet, with no significant differences between 3-NOP treatments. 3-NOP supplementation decreased acetate concentration while increasing propionate, butyrate, and isovalerate concentrations. Specifically, the highest propionate and isovalerate concentration was observed on the high-concentrate diet supplemented with 20 mg 3-NOP/kg DM. Methanogenesis inhibition increases hydrogen accumulation, altering hydro-

gen distribution among fermentation products. Understanding hydrogen flux is essential for optimizing methane mitigation, and multi-omics analyses currently underway will further clarify the underlying microbial mechanisms involved.

Key Words: methanogenesis inhibition, hydrogen metabolism, 3-nitrooxypropanol, in vitro fermentation

39 Life without an enolase does not disadvantage *Butyrivibrio* and *Pseudobutyrivibrio* species growth on glucose.

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Analysis of 405 rumen bacterial genomes from the Hungate 1000 project demonstrated that some strains of *Butyrivibrio* and *Pseudobutyrivibrio* lack a complete Embden-Meyerhoff-Parnas (glycolytic) pathway due to the absence of the enolase gene. These organisms are key contributors in the degradation of xylan and pectin in ruminant feed, fermenting the resulting hexose and pentose sugars principally to butyrate and they form part of the core rumen microbiome globally, largely independent of diet. How these successful organisms generate ATP despite an incomplete glycolytic pathway is unknown. In this study closely related pairs of *eno*$^{-/+}$ strains were selected from each of the genera *Butyrivibrio* and *Pseudobutyrivibrio.* RNaseq analysis of strains grown on glucose did not identify transcripts that could directly compensate for the absence of an enolase but revealed a viable alternative pathway enabling carbon flux from glucose to pyruvate that bypasses the requirement for an enolase in all strains, whether *eno*$^{+}$ or *eno*$^{-}$. Growth rates and fermentation end products in batch cultures of the 4 strains grown on glucose were almost identical between the matched *eno*$^{-/+}$ pairs. The theoretical ATP yield for each of the *Butyrivibrio* and *Pseudobutyrivibrio eno*$^{-}$ strains was 1.65 and 1.52 per glucose, respectively, in contrast to 3.45 and 3.76 ATP per glucose for the *eno*$^{+}$ strains. However, more cell mass was produced per glucose by both *eno*$^{-}$ strains compared with the *eno*$^{+}$ ones, suggesting that lack of an enolase is no disadvantage to growth, and that there are additional ATP forming steps that could not be determined from the genomes. Together these results indicate that absence of enolase does not impose a growth constraint in *Butyrivibrio* and *Pseudobutyrivibrio* and implies that these bacteria use a non-canonical pathway for glucose metabolism and ATP production that has not yet been fully elucidated.

Key Words: rumen, loss of enolase, *Butyrivibrio*, *Pseudobutyrivibrio,* carbon flux

40 Establishing a working culture collection of anaerobic rumen bacteria and methanogenic archaea for the development of genetic toolkits.

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Methane production from enteric fermentation in ruminant livestock is helping to drive disastrous climate change. Although the rumen microbiome has been studied for many years, we still lack genetically tractable members of this community that would aid in the development of understanding and advancement of methane mitigation strategies. This project aims to create genetic tools for rumen bacteria and methanogenic archaea involved in the production and utilization of hydrogen produced during rumen fermentation. This research project is facilitated by the development and maintenance of a comprehensive collection of 30 methanogen strains and 79 bacterial strains, belonging to such phyla as *Bacteriodota*, *Bacillota*, and *Pseudomonadota*. Multiple media compositions were tested to determine which were able to support growth of the most strains, resulting in the selection of M2GSC and a modified BY medium as the most useful.

Routine testing was also conducted to determine best practices for long-term culture storage and viability after cryofreezing for each strain. Managing this working reference collection required the development of a highly specific inventory system that is both easy to update and provides an accurate location for stored cultures and their further distribution to our research partners. This was completed by developing indexing functions that automate compilation of storage data and output easily comprehensible tables. The upkeep and continual improvement of this reference collection library allows for quick dissemination of strains to collaborators, and is imperative to the facilitation of future research both within and beyond the Genetic Toolkits project.

Key Words: culture collection, rumen bacteria, methanogenic archaea, ruminant microbiome

41 Transcriptional analysis of arabinan utilization and acetate production via heterolactic fermentation in ruminal *Streptococcus*.

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The *Streptococcus bovis*/*equinus* complex (SBEC) is a commensal bacterial group found in the GI tract of herbivores. This group is a major contributor to rumen acidosis, a gastrointestinal disorder in ruminal livestock, via lactate production through starch fermentation. Our previous work found that *Streptococcus equinus* (formerly *S. bovis*) ATCC33317 utilizes pectic arabinan as a substrate. However, the arabinan utilization mechanisms of SBEC remain unknown. We found that *S. equinus* ATCC33317 only grew on arabinan among the polysaccharides contained in pectin. In addition, ion chromatography analysis indicated that *S. equinus* ATCC33317 utilized arabino-oligosaccharides with more than 3 degrees of polymerization, whereas it did not utilize arabinose or arabinobiose. Acetate was the primary end product in arabinan fermentation, but lactate was the major end product in glucose, galactose, and soluble starch fermentation. These results suggest that *S. equinus* ATCC33317 may specialize in the degradation and metabolism of polymerized arabinan. Transcriptomic analysis revealed 21 differentially expressed genes in the same gene cluster that were upregulated in the arabinan group. This gene cluster encodes 2 transcriptional regulators, 4 enzymes related to heterolactic fermentation, 3 sugar transporters, and 7 glycoside hydrolases. Notably, 6 of these hydrolases were annotated as GH43, GH51, and GH121 family enzymes, which are specifically associated with arabinan degradation. Collectively, these results suggest that this gene cluster contributes to the arabinan utilization system in *S. equinus* ATCC33317. Genome comparison of 108 strains belonging to SBEC found that this gene cluster was conserved among strains isolated from the GI tract of foregut-fermenting herbivores. This result may reflect an evolutionary adaptation of certain SBEC to this ecological niche. Altogether, our results highlight a previously unrecognized pectic arabinan utilization mechanism via heterolactic fermentation in SBEC, providing novel insights into the metabolic diversity and ecological niche of this bacterial group in the GI tract of foregut-fermenting herbivores.

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Key Words: *Streptococcus bovis/equinus* complex, arabinan, heterolactic fermentation, foregut-fermenting herbivore

42 Microbial correlates of fecal lactate concentration and evaluation of the genomic potential for lactate utilization in a *Schwartzia*-related MAG from Japanese draft horses.

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Feeding large amounts of concentrate to racehorses increases hindgut lactate accumulation and the risk of acidosis. To prevent this disorder, dietary starch intake thresholds have been proposed for Thoroughbreds, and lactate-utilizing bacteria have been explored and applied as probiotics. However, nutritional and microbial studies on Japanese draft horses—one of the largest horse breeds used for Ban'ei racing, held in Obihiro, Hokkaido—remain limited. Here, we identified bacterial taxa associated with fecal lactate concentration and investigated the lactate-utilizing potential of a metagenome-assembled genome (MAG) enriched in horses with low lactate concentrations. Fecal samples were collected from 76 Japanese draft horses. Based on fecal lactate concentration (<1 m*M*, LL; ≥1 m*M*, HL), 9 matched pairs with comparable starch intake (kg/100 kg body weight per meal), age, and sex were selected for 16S rRNA gene analysis. ANCOM-BC revealed that *Streptococcus*, uncultured *Weeksellaceae*, *Escherichia-Shigella*, *Corynebacterium*, and *Lactobacillus* were enriched in HL. Overall, the HL group was dominated by lactate-producing and facultative anaerobic bacteria. In contrast, the LL group had a higher abundance of *Schwartzia*. To date, only *S. succinivorans*, a propionate-producing bacterium, has been reported within this genus. An ASV assigned to *Schwartzia* in this study showed 93.7% sequence similarity to *S. succinivorans*. Therefore, shotgun metagenomic sequencing was performed on 3 LL individuals, yielding a high-quality *Schwartzia*-related MAG (95.8% completeness, 2.82% redundancy), which was subsequently analyzed for its potential in lactate utilization. However, this MAG possessed genes associated with the propionate-producing pathway via succinate, whereas no genes related to lactate transport or utilization were detected. Accordingly, future studies should conduct more comprehensive analyses to clarify the ecological role of this taxon, including its potential contribution to lactate mitigation, by examining genes involved in oxygen consumption and substrate competition with lactate-producing bacteria enriched in the HL.

Key Words: fecal lactate accumulation, *Schwartzia*, metagenomic analysis

43 Comparative fecal microbiome analysis of Japanese Dosanko and Japanese draft horses reveals functional and resistome differences.

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The equine gut is an underexplored model for understanding microbiome interactions, especially among lesser-known breeds. We profiled fecal metagenomes from 2 Japanese horse breeds: the endangered, native Dosanko horse (n = 5) fed high-fiber diets with minimal antibiotic exposure, and Japanese draft horse (n = 3) with more conventional rearing methods. Taxonomic profiling via MetaPhlAn4 revealed that Dosanko horses exhibited increased abundance of methanogenic archaea, including *Methanocorpusculum* and *Methanobrevibacter* and the bacterial genus *Limimorpha*, whereas Japanese draft horses were enriched in saccharolytic genera such as *Akkermansia*, *Prevotella* and *Firm-16*. Microbial communities were not significantly different by α-diversity metrics but were significant by β-diversity (Bray-Curtis, PERMANOVA, P = 0.038) with distinct clustering with PCoA analysis. Functional analysis using EggNOG mapper showed enrichment of core methanogenesis (*mcr*, *fwd/fmd, mtr*) and hemicellulolytic genes in Dosanko horses, whereas draft horses had relatively more cellulose-degrading genes, reflecting

host diet differences. Resistome profiling using CARD RGI revealed significant differences in antimicrobial resistance gene (ARG) composition between breeds (Bray–Curtis, PERMANOVA, $P = 0.042$). Despite lower reported antibiotic exposure, Dosanko horses carried several ARGs, including *ant(6)-Ia*, *cfxA2*, and *vanH*/*vanT* cluster variants, with prevalence differences up to 100% between breeds. These findings indicate that breed, diet, and environment shape equine gut microbial composition, functional capacity, and resistome structure. Enhanced fiber degradation and methanogenesis in Dosanko horses likely reflect adaptation to high-fiber diets, whereas unexpected ARG patterns suggest ecological and historical influences beyond current antibiotic use. This study highlights the need for targeted equine microbiome research to inform nutrition management, antimicrobial stewardship, and environmental impact assessment.

Key Words: equine microbiome, methanogenesis, antimicrobial resistance, fiber degradation, shotgun metagenomics

44 Isolation and partial characterization of a cellulolytic consortium derived from the rumen of a grazing cow.

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The hydrolysis of complex plant polysaccharides by the ruminal microbiota is essential the nutrition of the host ruminant. Although there are well-known fibrolytic bacteria isolated from the rumen, growing evidence suggests that fiber degradation is more efficiently performed by interacting with microbial groups. The aim of this study was to isolate and identify rumen anaerobic bacteria able to degrade cellulose. Fresh rumen fluid from a grazing Aberdeen Angus cow was serially diluted under anaerobic conditions and inoculated into liquid medium with Whatman filter paper as a cellulose source. After 5 d of cultivation at 39°C, the highest dilution showing visible filter paper degradation was serially diluted and subcultured in roll-tubes containing either cellobiose or ball-milled paper as source of carbon. Forty isolated colonies from roll-tubes were tested for filter paper degradation, but none exhibited detectable cellulolytic activity. Therefore, the highest dilution of the rumen fluid displaying cellulolytic activity was selected for DNA extraction to characterize the bacterial composition. Near full-length 16S rRNA gene sequencing using Oxford Nanopore Technology showed a bacterial consortium taxonomically affiliated with the phyla *Bacillota* (*Ruminococcus* sp., *Aminopila* sp.), *Bacteroidota* (*Bacteroides ovatus*/*xylanisolvens*), *Actinomycetota* (*Eggerthella* sp.), and *Pseudomonadota*, with a fraction of sequences classified as unknown. The culture supernatant exhibited cellulolytic activity against carboxymethyl cellulose, evidenced by degradation halos on Congo red–stained agar plates and by reducing sugar release in liquid assays quantified by DNS method. The consortium remained functionally active over 10 successive passages. These results suggest that the observed cellulolytic activity arises from the metabolic interactions within the microbial consortium, potentially involving *Ruminococcus* sp. in association with other community members. This relatively simple system could represent a valuable model to study metabolic interactions underlying ruminal fiber degradation.

Key Words: rumen microbiota, cellulolytic consortium, anaerobic bacteria

45 Metagenomic profiling using long reads PacBio sequencing revealed key metabolic functions of rumen microbiome in beef cattle across seasonal grazing.

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The majority of rumen microbiome studies have been conducted under controlled dry lot feeding conditions, limiting our understanding of microbial dynamics under natural grazing systems. Here, we investigated how seasonal grazing environments shaped rumen microbiome composition and metabolic potential in beef cattle with divergent feed efficiency. Rumen samples were collected from 48 beef cattle across a year-round cow–calf production cycle spanning 4 management phases: dry lot in March, planted pasture in June, summer native rangeland in August, and fall native rangeland in November. Rumen-derived DNA was sequenced using PacBio HiFi long-read metagenomics to reconstruct metagenome-assembled genomes (MAGs) and characterize functional shifts in the rumen microbiome. In total, 46 MAGs (completeness >80%, contamination <10%) were reconstructed and combined with 12,758 publicly available rumen MAGs, followed by species-level dereplication at 95% average nucleotide identity, yielding 6,713 species-level representative MAGs. A nonredundant gene catalog was constructed to characterize hydrogen (H_2) metabolism pathways and their seasonal dynamics. DESeq likelihood ratio analysis identified 26 H_2-related genes and associated MAGs that were significantly affected by grazing season (FDR <0.05), including genes encoding HydB, FeFe hydrogenases, and *FrdA*, encoded by taxa such as UBA1367, *Onthomonas*, and *Cryptobacteroides*. These functional changes were observed during transitions from dry lot to pasture-based systems, and between summer and fall on native rangelands, suggesting an association between grazing environment and rumen H_2 metabolism. This work expands our understanding of rumen microbiome composition beyond conventional feeding systems and provides MAGs and gene catalogs for developing microbiome-based strategies to improve feed efficiency in grazing cattle.

Key Words: feed efficiency, grazing system, MAG, hydrogen metabolism, rumen microbiome

46 Diet quality and microbial tryptophan metabolism in colorectal cancer risk.

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Black Americans have a higher colorectal cancer (CRC) burden and well-documented diet disparities. Our preliminary analysis also showed that individuals with lower diet quality identified as Black. Microbial metabolism of dietary tryptophan creates immunomodulating indole and indole metabolites. However, the effect of diet quality on dietary tryptophan and the microbial enzyme tryptophanase (*tnaA*) is not known. We evaluated this relationship and predicted differences in *tnaA* structures across gut microbes. We recruited 163 participants from the Chicago area (mean age 59.6 ± 6.2 years; 52% Black; 57% females), gathered two 24-h dietary recalls, and assessed diet quality using Healthy Eating Index (HEI)-2015, where <51 was defined as lower diet quality. Fasting serum and stool samples were analyzed by UPLC-MS and stool microbiome with shotgun metagenome sequencing. Alphafold2 was used to predict *tnaA* structures. Multivariate linear regression models were used to evaluate associations between dietary sulfur amino acids, tryptophan intake, race, and tryptophan metabolites, including interaction terms, $\alpha = 0.05$. Dietary tryptophan intake was similar across race and diet quality groups. Participants with lower diet quality who identified as Black had higher circulating serotonin ($P < 0.001$), lower circulating tryptophan ($P < 0.01$), and higher fecal Indole-3-acetic acid (IAA; $P < 0.01$). Additionally, race was a significant modifier in the interaction between dietary cysteine and dietary tryptophan in predicting fecal indole-3-propionic acid (IPA; $P < 0.05$). Fecal indole and indole-3-lactic acid (ILA) did not vary by race or diet quality. Enzyme *tnaA* harboring genera *Porphyromonas*, *Akkermansia*, and *Lachnospiraceae*

were associated with HEI ≥51, and *Escherichia*, *Fusobacterium*, *Odoribacter*, and *Lachnospiracae* were associated with HEI <51. Homology analysis of *tnaA* sequences using representative species within associated genera ranged from 25% to 87%. However, predicted structures of *tnaA* revealed structural similarities across these microbes, shown by pTM ~0.94; mean pLDDT 95–97. Conclusion: Despite comparable levels of dietary tryptophan consumption, lower diet quality among Black individuals resulted in higher fecal IAA and circulating serotonin and lower circulating tryptophan. Our results demonstrate that these differences in tryptophan metabolism were not driven by *tnaA* isoenzymes, requiring exploration of other mechanisms.

Key Words: colorectal cancer, tryptophanase, cancer disparities, diet quality, indole

47 Improving rapid identification of rumen bacteria: Development and evaluation of a rumen specific MALDI-TOF MS database.

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In recent years, efforts to expand pure ruminal culture collections have been made with the intention of improving the understanding of ruminal fermenting microbes. This understanding is essential for advancing the development of direct-fed microbials (DFMs), which hold promise for improving livestock productivity and mitigating environmental impact. Identification methods are usually laborious and costly. In contrast, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been widely used in clinical applications for the rapid and accurate identification of pathogenic species providing an alternative to traditional methods. However, its accuracy is limited to recognizing microorganisms that are already included in its database. This study presents the development and evaluation of a customized MALDI-TOF MS database, aiming to improve the identification of novel bacterial isolates and optimizing the expansion of the ruminal biobank of pure cultures. Pure cultures of rumen bacterial were cultivated on Hobson's M2 agar under anaerobic conditions at 39°C until growth was observed by the formation of colonies on agar and a colony from the plate was analyzed using the Autobio AutoMS1600 MALDI-TOF MS system. The manufacturer library construction method was followed. Once spectra from an isolate were incorporated into the database, colonies from the same plate were picked and processed following the manufacturer's extended method for initial validation. The spectra generated were processed using both the original AutoBio database and the rumen customized database. The efficacy of the database was then evaluated using 115 freshly isolated ruminal bacteria. Currently, the rumen-specific MALDI-TOF MS database includes 231 bacterial isolates in pure culture with identification based on partial 16S rRNA gene sequencing. Accurate identification of rumen bacterial species increased from 50% to 84% with the creation of the rumen database. Testing with the freshly isolated samples showed a 73% increase of the identification efficiency compared with the inbuilt database. In conclusion, the customized rumen-specific MALDI-TOF MS database is valuable for expanding culture collections by reducing the number of novel isolates that require identification through sequencing.

Key Words: bacteria, microbiome, identification, culturing

48 The effects of tail docking status on fecal microbiome adaptation to pasture in Polyplay ewe lambs.

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Tail docking is commonly performed in lambs within the first days to weeks of life to reduce flystrike, though evidence for its efficacy is limited and conflicting. Acute pain and tissue inflam-

mation during this critical developmental window may alter immune programming and microbial colonization, with potential consequences for disease susceptibility, including increased vulnerability to gastrointestinal nematode infection. Therefore, we evaluated the effects of neonatal tail docking on the fecal microbiome and parasite load. Polypay twin ewe lambs (n = 56) were assigned within pairs to tail docking or no docking at 1 to 2 d of age. Lambs transitioned to pasture at 3.6 ± 0.3 (mean ± SD) months of age. Fecal samples were collected prepasture (6/16/22), immediately postpasture (8/3/22), and 2 weeks postpasture (8/17/22) for 16S rRNA sequencing (Illumina MiSeq) and fecal egg count (FEC; Modified McMaster). The effects of tail docking status, FEC, and collection date on the fecal microbial diversity were analyzed with generalized linear models. Differential abundance analysis was performed with ANCOM-BC2, and microbial co-occurrence networks were constructed with Spiec-Easi to assess differences in microbial community structure. Docked lambs had a greater increase in FEC on pasture and postpasture compared with the undocked lambs ($P < 0.05$). Microbial diversity ($P < 0.05$) increased, and community structure shifted ($P < 0.05$) following pasture for both docked and undocked ewes. Although tail docking had no effect on either microbial diversity or overall community structure, FEC influenced community composition ($P < 0.05$). *Alistipes* and *Desulfovibrio* were enriched after pasture ($P < 0.01$), and with every one-unit increase of FEC, *Clostridium* increased and *Akkermansia* was depleted ($P < 0.01$). Co-occurrence network structure developed relative to time on pasture and was affected by tail docking status ($P < 0.05$). Docked lambs had higher median node degree, closeness, and betweenness centrality and network density, whereas undocked ewes had more modular networks with increased average local and global cluster coefficients ($P < 0.05$). These results suggest that docked lambs may have impaired microbial adaptability to dietary changes, potentially increasing parasitic infection risk, which also negatively affects the fecal microbiome.

Key Words: lamb, microbiome, pasture, tail-docking, parasite

49 Enterosignature dynamics of healthy and diarrheic dairy calves during the preweaning period.

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The gut microbiota is essential to the health and physiological development of dairy calves during the neonatal period. However, the specific microbial signatures associated with diarrheic transitions remain unclear. This study aimed to identify fecal enterosignatures (ES) in calves during the preweaning period and evaluate their longitudinal dynamics in healthy versus diarrheic animals. Fecal samples were collected longitudinally from 56 calves during the first 8 weeks of life. Calves were classified as Healthy (n = 20) or Diarrhea (≥1 episode, n = 36). Genera present in ≥20% of samples were retained, and abundance data were normalized to relative abundances, centered log-ratio transformed, and analyzed using linear mixed-effects models (FDR 5%, Benjamini–Hochberg). Five distinct enterosignatures (ES1–ES5) were identified, cumulatively explaining 86.6% of the variance in genus abundances. ES1 was dominated by *Prevotella* (63.23%) and ES2 by *Phocaeicola* (53.5%). *Bacteroides* (29.5%) was abundant in ES3, with contributions from *Lactobacillus* (14.0%), whereas ES4 was dominated by *Clostridium* (27.2%) and *Enterococcus* (18.9%), and ES5 was co-dominated by *Fusobacterium* (42.5%) and *Prevotellamassilia* (42.4%). At week 1, the relative abundance of ES4 was significantly higher in calves that subsequently experienced diarrhea (44.8% vs. 18.2%; $P = 0.01$), whereas ES5 was more prevalent in healthy calves (7.0% vs. 1.8%; $P = 0.01$). No significant differences between health status groups were detected for ES1–ES3 ($P > 0.05$). Longitudinally, ES1 (*Prevotella*-dominated) increased in healthy calves after week 1 ($P \leq 0.02$), indicating progressive microbial maturation. ES5 increased in both groups ($P \leq 0.02$), whereas ES3 declined over the study period ($P \leq 0.027$). Notably, ES4 peaked at week 1 and decreased sharply thereafter in both groups ($P < 0.001$). These results demonstrate that early

life diarrhea in calves is associated with distinct baseline enterosignatures. Although health status significantly influenced microbial composition during the first week, the microbiome underwent progressive restructuring, leading to increased community similarity by the eighth week. These findings suggest that early-life microbial development is driven by a combination of disease-associated shifts and inherent developmental maturation.

Key Words: calf microbiota, gut maturation, calf health, microbiome

50 Carbon-responsive phase variation drives adaptive regulation in *Bacteroides thetaiotaomicron*.

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Bacteroides thetaiotaomicron is a prominent member of the human gut microbiota and encodes a diverse array of Sus (starch utilization system) operons that facilitate the utilization of a wide range of dietary and host-derived glycans. Precise regulation of these carbon-utilization-related genes is crucial for niche establishment and persistence within this competitive ecosystem. One regulatory strategy employed by bacteria involves invertase-mediated DNA inversion at inverted repeat sequences positioned within intergenic or intragenic regions, thereby modulating gene expression. Here, we demonstrate that tandem duplication of N-acetylglucosamine-6-phosphate deacetylase (*nagA*), together with regulation mediated by an intragenic inversion within one duplicated copy, represents a strategy for adaptation and survival in the gut. Using computational prediction algorithms, we further predict that the *nagA* variant containing the invertible region preferentially interacts with dTDP-4-dehydrorhamnose reductase (RfbD), an enzyme involved in dTDP-4-rhamnose biosynthesis, a precursor of capsular polysaccharide (CPS) production. Although *nagA* sequences among *Bacteroides* species are highly conserved (>96% sequence identity), the presence, genomic location, and length of invertible repeat regions vary substantially. Moreover, in species possessing *rfbD* homologs (>70% sequence identity), residues predicted to mediate polar interactions with NagA are replaced by oppositely charged or neutral residues, suggesting that the NagA–RfbD interaction may be unique to *B. thetaiotaomicron*. Collectively, our findings suggest that phase variation in *B. thetaiotaomicron* is dynamically modulated by environmental carbon availability in the gut, enabling adaptive regulation that supports colonization, persistence, and survival.

Key Words: tandem duplication, phase variation, bacterial evolution, gut adaptation, carbon utilization

51 A functionally selected *Acinetobacter* sp. phosphoethanolamine transferase gene from the goose fecal microbiome confers colistin resistance in *Escherichia coli*.

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Polymyxins are last-resort antibiotics for infections caused by multidrug resistant gram-negative bacteria including *Acinetobacter baumannii*. This makes the rise of bacteria exhibiting colistin resistance through modification of lipid A concerning and suggests that it is important to document potential sources of the corresponding resistance genes. This study searched for potential emerging colistin-resistance genes from the environment by using a functional metagenomic selection for colistin resistance of a goose fecal microbiome. We found that the selection captured *Acinetobacter* sp. DNA fragments which all contained *eptA* genes. We confirmed their ability to confer significant colistin resistance in *E. coli via* modification of lipid A in the outer membrane.

Furthermore, we found evidence for mobilization of closely related *eptA* genes in *Acinetobacter* strains, marking them as potential *mcr* genes or their precursors. This study highlights the goose fecal microbiome as a potential source for colistin resistance in the environment.

Key Words: antibiotic resistance, colistin, microbiome, MCR

52 Capsule-mediated sensitivity to antipsychotic drugs reveals a new vulnerability in *Escherichia coli*.

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Bacterial capsular polysaccharides (CPS) are dense surface coatings that protect against host immune defenses and environmental stressors. These structures are important determinants of bacterial fitness in environments such as the gut, where bacteria face constant chemical, viral and immunological pressure. Nonantibiotic drugs, especially psychiatric medications like phenothiazines (PTH), can accumulate in the gut at concentrations sufficient to affect microbial growth. How bacterial surface structures modulate sensitivity to such compounds remains poorly understood. Here, we show that the K5 CPS of *Escherichia coli* Nissle 1917 (EcN), rather than conferring protection, sensitizes the bacterium to PTHs. In screening *E. coli* strains for sensitivity to a representative PTH thioridazine (TDZ), we found EcN to be the most sensitive and that this sensitivity worsened in a minimal medium. We experimentally evolved EcN under physiological gut concentrations of TDZ, revealing convergent mutations targeting the K5 CPS locus across independent populations. Phenotypic assessments of CPS display validated correlations between CPS expression and TDZ sensitivity, as well as between CPS expression and growth in minimal medium. Subsequent transcriptomic profiling and knockout-complementation experiments confirmed that CPS is required for full PTH sensitivity. Finally, an expanded investigation of several other PTH drugs demonstrated CPS-mediated sensitivity for most PTHs. Taken together, these results reveal that a canonically protective bacterial surface structure can invert its functional role under nonantibiotic drug pressure, rendering bacteria more vulnerable rather than more resistant. This demonstrates that CPS are not universally beneficial fitness factors and raises the possibility that drug-mediated selection shapes CPS expression and genetic encoding in the gut microbiome. More broadly, our findings suggest that the chemical environment of the gut, including widely prescribed nonantibiotic medications, may be an underappreciated driver of microbial surface remodeling and community composition.

Key Words: capsular polysaccharide, *Escherichia coli*, antipsychotic, nonantibiotics

53 Longitudinal gut microbiome restructuring associates with pain burden following kidney transplantation.

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Kidney transplant recipients (KTRs) experience improved renal function; however, many report persistent pain burden. Evidence suggests that the gut microbiome influences inflammatory signaling, but longitudinal species-level changes associated with post-transplant pain remain poorly defined. In this study, we performed shotgun metagenomic sequencing on fecal specimens from a longitudinal cohort of 31 adult KTRs sampled pretransplant and 3 mo post-transplant to characterize microbiome restructuring and its relationship to pain interference. We observed significant shifts in microbial community composition between visits (q-value < 0.2). Across time points, individuals with higher pain interference exhibited depletion of butyrate-producing and anti-inflammatory taxa, including *Faecalibacterium*, *Anaerostipes*, and *Dorea*, even after accounting for each participant's baseline microbiome composition and repeated measures within subjects. These findings suggest that persistent pain after kidney transplantation is associated with depletion of short-chain fatty acid–producing bacteria, supporting a potential link between altered microbial inflammatory regulation and symptom trajectories. This expanded longitudinal metagenomic analysis builds upon prior work and further supports gut microbial composition as a potentially modifiable target for alleviating symptom burden in KTRs.

Key Words: kidney transplantation, gut microbiome, inflammatory signaling, shotgun sequencing, short-chain fatty acid

54 Enrichment of a novel methanogen from the bovine rumen.

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Methanogens are obligate methane-producing archaea that colonize the rumen and other gut compartments of ruminant livestock and convert the byproducts of rumen fermentation (CO_2 and H_2) into methane. The methane production is energetically unfavorable for the host animal, and the gas, when exhaled, makes a large contribution to greenhouse gas emissions from animal agriculture. Despite their importance, the range of cultured methanogens isolated from the rumen is limited and there is a current emphasis on the isolation and cultivation of a wider diversity of this functionally important group of rumen microbes. To contribute to this effort, we attempted isolation of novel methanogens from the rumen of a beef animal (2-year-old Angus/Simmental steer fed a diet containing 90% corn silage plus 10% supplement) housed at the beef farm at the University of Illinois. Using rumen contents as inoculum and *Methanobrevibacter smithii* medium (MSM) containing 5% rumen fluid (with a headspace of 80% H_2 + 20% CO_2) as growth medium, we performed 5 rounds of enrichments in the liquid medium. After the fifth round, we streaked the enriched culture on MSM-agar plates, picked single colonies, and further purified them twice by streaking. Finally, the clonally purified isolate, which produced methane and exhibited autofluorescence upon UV exposure, was characterized by microscopy and sequence analysis. Scanning electron microscopy showed majority of cells to be small oval- and disc-shaped cocci. Whole genome sequencing revealed this methanogen to be a species of *Methanocorpusculum*, with Sourmash analysis assigning it as metagenome-genome assembled *Methanocorpusculum* sp023396065. Although analysis of nucleotide identity (ANI) was 99.93% between the new methanogen and *Methanocorpusculum* sp023396065, there were several significant differences including additions and deletions. We therefore provisionally named this novel hydrogenotrophic methanogen *Methanocorpusculum ruminantium* GMH1 which, to the best of our knowledge, is the first *Methanocorpusculum* sp. isolated from the rumen.

Key Words: methanogen, rumen fermentation, enrichment of rumen microbes, *Methanocorpusculum*, greenhouse gas emissions

55 Sensing and regulation of the utilization of dietary polysaccharides in Bacteroides spp.

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Dietary fibers are main components of human diet and have a major impact on gut microbiota composition and diversity. Common dietary fibers such as arabinoxylan, arabinan, and pectin are energy-rich polysaccharides. However, they are generally nonmetabolizable by the human host due to the absence of the genes encoding the requisite degradative enzymes in the host genome. However, the human colon harbors microbiota equipped with a wide range of carbohydrate-active enzymes (CAZymes) capable of degrading dietary fiber. These enzymes include glycoside hydrolases, polysaccharide lyases and carbohydrate esterases. In the major colonic bacterial phylum *Bacteroidota*, enzymes responsible for polysaccharide degradation are encoded in gene clusters called polysaccharide utilization loci (PULs). *Bacteroides* spp. genomes contain numerous PULs for the degradation and uptake of diverse carbohydrates, typically regulated by a hybrid 2-component system (HTCS) polypeptide. Using RNA-seq analysis, we identified the PULs responsible for the degradation of arabinoxylan, pectin and arabinan in *Bacteroides intestinalis* grown on the respective polysaccharides, as well as their associated HTCS regulators. Each HTCS protein contains N-terminal sensor and Y–Y–Y domains (Sensor_Y_Y_Y) and C-terminal histidine kinase, response regulator, and helix–turn–helix domains (HK_RR_HTH). Using immunostaining with polyclonal antibodies raised against the N- and C-terminal halves of the HTCS, we showed that the Sensor_Y_Y_Y domain is membrane-associated, whereas the HK_RR_HTH domain is localized in the cytoplasm. We expressed and purified the recombinant Sensor_Y_Y_Y proteins from several colonic *Bacteroides* spp. and demonstrated by isothermal titration calorimetry that they specifically bind to the degradation products of the polysaccharides corresponding to their respective PULs. In addition, untargeted metabolomics was used to identify the metabolites associated with PUL upregulation in members of the colonic *Bacteroidota*. This enables us to link polysaccharide sensing and degradation to potential health and nutritional benefits for the host. These findings provide mechanistic insights into modulating human health and nutrition through the administration of probiotic, prebiotic, and synbiotic.

Key Words: polysaccharide utilization, dietary fiber degradation, *Bacteroidota*, HTCS

77 Persistent auxiliary microbiome of early colonizers shapes the developing rumen ecosystem.

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The early-life assembly of the rumen microbiome is a critical developmental process with lasting consequences for host physiology and greenhouse gas emissions. Using high-resolution longitudinal metagenomics in calves tracked from birth to 3 yr (~800 d), we reconstructed 2,873 high-quality metagenome-assembled genomes, including 517 novel genomes predominantly detected in early life. These genomes, spanning 274 genera, define a diverse and functionally distinct auxiliary microbiome. Unlike transient early colonizers described in other ecosystems, this auxiliary community persists into adulthood, retaining ecological and functional relevance despite reduced abundance while contributing to

the metabolic capacity of the mature rumen ecosystem. Temporal clustering revealed strong associations with dietary transitions and functional enrichments in environmental sensing, nutrient biosynthesis, and volatile fatty acid metabolism. Metabolic network analyses further demonstrated that auxiliary genomes complement non-auxiliary community members in key biosynthetic and fermentative pathways, indicating coordinated ecosystem-level interactions. These findings suggest that early colonizers act as ecosystem engineers, shaping the developmental trajectory of the rumen microbiome.

Nutrition and metabolism of livestock, humans, and companion animals

56 *In vitro* evaluation of feed additive combinations for methane emission mitigation.

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Reducing enteric methane (CH_4) from ruminant livestock is essential for sustainable agriculture, as it is both a major greenhouse gas and a loss of dietary energy. Individual feed additives (FAD) with antimethanogenic potential often show variable, dose-dependent responses, thus research has focused on stacking additives with complementary modes of action to achieve more consistent CH_4 mitigation. This study evaluated, in vitro, individual and combined effects of 3 FAD: a blend of essential oils, bioflavonoids, and tannins (A); nitrate (B); and calcium peroxide (C), on CH_4 production and rumen fermentation dynamics. Treatments were tested at low, medium, and high doses, plus stacked AB, AC, BC, and ABC with controls (CON), tested in triplicate. Batch fermentations were conducted in 3 independent runs using perennial ryegrass silage (1.6 g) incubated in 100-mL serum bottles with 60 mL of a 1:1 (vol/vol) mixture of Van Soest buffer and pooled rumen fluid (5 cattle donors, post-slaughter). Bottles were incubated at 39°C for 48 h with continuous shaking (110 rpm). Gas production and headspace pressure were recorded at 4, 24, and 48 h, and CH_4 concentration was quantified by GC-MS. Rumen fluid was analyzed for pH, ammonia, and VFA. Data were analyzed using PROC GLM in SAS (SAS Institute Inc.) with treatment, dose, time and run as fixed effects; model assumptions were verified, and Tukey's post hoc tests were applied when $P < 0.05$ was found. C was highly effective at all doses. At 24 h, CON produced 4.2 mL of CH_4, but C at high dose produced only 0.7 mL. At 48 h, CON CH_4 averaged 4.6 mL against 0.7 mL for C-high, both ≈ −85%. B showed a dose-dependent effect, reducing CH_4 at 24 h from 4.2 to 2.9 mL (≈ −32%) and at 48 h from 4.64 to 2.9 mL (≈ −38%). A had minimal impact at 24 h and modest reductions at 48 h (≈ 7%–15%). In stacking, high reduction occurred only when C was included: at 24 h, AC, BC, and ABC reduced CH_4 from 4.2 to ≈0.6 mL (≈ −85%), and at 48 h, from 5.1 to ≈0.6 mL (≈ −89%). By contrast, AB provided minimal mitigation (≈ −8% at 48 h). Treatments with C consistently lowered pressure and ammonia accumulation, indicating altered fermentation. The acetate:propionate ratio increased transiently but returned toward CON by 48 h, suggesting microbial adaptation. Overall, C was the main driver of CH_4 suppression and stacking with A or B maintained rather than enhanced this effect, supporting further in vivo evaluation of calcium-peroxide-based mitigation strategies.

Key Words: fermentation, ruminant nutrition, stacking

57 Dietary transition from high-forage to finishing ration reshapes the ecology of *Neocallimastigomycota* in the rumen.

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The anaerobic gut fungi (AGF) of the rumen belong to the phylum *Neocallimastigomycota* and account for a significant proportion of the rumen microbial biomass. They play a central role in the digestion of lignocellulose during consumption of high forage diets. AGF physically penetrate and secrete potent hydrolytic enzymes during the microbial colonization of feed particles when they enter the rumen. It is generally believed that AGF play little to no role in the digestion of high-starch, grain-based diets. The growth of AGF is inhibited at the acidic pH levels that occur in the rumen during the consumption of the high-starch diets typically used in feedlots. However, there are limited data to test this hypothesis and directly characterize how the ecology of AGF changes during the transition from a high-forage to a high-concentrate diet. To address this knowledge gap, we have used a metataxonomic approach to understand the temporal shift in the AGF community of the rumen over a 268-d feeding period where the diet changes from high forage to high grain. Rumen samples were collected at 4 time points via esophageal tube and metataxonomic sequencing of the rRNA LSU was preformed using metagenomic DNA extracted from rumen fluid. The shift from a high-forage to a high-grain diet significantly reduced the number of AGF ASVs from a mean of 30 to 10 ($P < 0.001$). There was a significant difference in the β-diversity of samples between the backgrounding and finishing periods using both weighted and unweighted metrics ($P < 0.001$). This indicates that the identity and relative abundance of the anaerobic fungi in the rumen change during this transition. A taxonomic reorganization was observed, with high-forage diets selecting a diverse community of several well-characterized fungal genera, including *Neocallimastix*, *Pecoramyces*, and *Cecomyces*, alongside uncultured groups. In contrast, the AGF community in samples from the high-grain diet were dominated by a small number of ASVs assigned to uncultured genera. These findings suggest that uncultured AGF may play a role in the digestion of high-grain diets. The apparent dominance of several uncultured taxa under these conditions indicates that efforts to isolate AGF from these samples may provide opportunities to characterize novel taxa and expand our understanding of the diverse roles these organisms play in ruminant feed digestion.

Key Words: rumen, *Neocallimastigomycota*, anaerobic gut fungi, microbial ecology, cattle

58 Genomic insights into tryptophan metabolism in lactic acid bacteria of bovine origin.

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In the gut, bacteria can metabolize tryptophan, an essential amino acid, producing both detrimental and beneficial metabolites. Among these metabolites, indolelactic acid, indolealdehyde, and indole acetic acid are known to positively influence host immunity and gut function. Aromatic amino acid aminotransferase (ArAT) is a key enzyme involved in tryptophan metabolism that leads to indolelactic acid and indolealdehyde production in lactic acid bacteria (LAB). However, research on this metabolic pathway has been largely focused on human-derived isolates, and the metabolic potential of this pathway in LAB of bovine origin remains poorly characterized. This study aimed to determine whether lactobacilli isolates of bovine gut origin possess the genomic potential for tryptophan metabolism to produce beneficial indole derivatives and to evaluate their relevance as probiotic candidates. Fourteen Lactobacilli isolates were isolated from the hindgut of newborn calves, and their genomes were sequenced using Illumina MiSeq. The annotation of ArATs was performed utilizing the NCBI database to retrieve both protein sequences and the protein-coding nucleotide sequences from the domain of Bacteria. The protein and DNA sequences were then aligned with each other using MAFFT with the FFT-NS-PartTree-1 multiple sequence alignment strategy. Phylogenetic trees were constructed using the FastTree program and visualized using the iTOL tool to assess the conservation of ArAT at both amino acid and DNA levels. In total, 88,532 protein and 89,137 nucleotide sequences were identified for ArAT from the NCBI search. Furthermore, phylogenetic trees revealed that this enzyme was diverse

and poorly conserved at both amino acid and DNA levels within the domain of Bacteria. Ongoing analysis involves downloading all lactobacilli genomes and bovine isolate data sets to identify the presence and conservation of ArAT, aiming to determine their potential key tryptophan metabolic pathways for future probiotic implications.

Key Words: tryptophan metabolism, aromatic amino acid aminotransferase, *Lactobacillus*, cattle

59 Ultra-processed foods link to gut microbial sulfite metabolism genes.

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Ultra-processed foods (UPF) contain sulfur additives, such as sulfites and caramel coloring, which may influence gut microbial sulfur cycling. Key sulfite metabolism genes include anaerobic sulfite reductase (*asr*) and dissimilatory sulfite reductase (*dsr*), but links to ultra-processed diets are underexplored. We tested whether UPF intake, particularly from ultra-processed beverages (e.g., sugar-sweetened and alcoholic drinks), correlates with microbial gut sulfite-metabolism gene abundance (*asr*, *dsr*, *cysJ*) and sulfite-reducing taxa. We studied 138 adults (age 45–75 years) recruited from 2 urban academic medical institutions in Chicago, IL. Participants completed two 24-h dietary recalls, and stool samples were collected within 5 d of the first interview and stored at −80°C. Dietary data were classified using the NOVA system by 2 independent coders (with a third resolving discrepancies) and recategorized into 18 UPF subcategories. UPFs were screened for sulfur additives, including sulfate, sulfite, and caramel coloring, using name-brand and off-brand ingredient lists. Fecal DNA was shotgun sequenced, whereas sulfite metabolism genes (*dsr, asr, cysJ*) were identified using hidden Markov model libraries, and abundance was correlated with the percentage of total energy from UPFs (NOVA group 4 kcal/total kcal × 100) using unadjusted Spearman correlations. Among participants, 13% of consumed foods contained caramel coloring. *Asr* and *dsr* genes showed significant positive correlations with percent total kcal from UPFs (n = 138; $P < 0.02$ and $P < 0.005$, respectively). Sugar-sweetened beverages and alcohol showed the strongest associations with sulfite metabolism gene abundance. This analysis relied on unadjusted correlations, so findings represent associations, not causation. Ongoing work includes chemical assays to quantify sulfur species in beverages and in vitro experiments to test metabolism of beverage-derived sulfites by representative microbial isolates.

Key Words: ultra-processed foods, sulfite metabolism, gut microbiome, NOVA classification, sulfur additives

60 Discriminating peptides from ammonia and branched-chain volatile fatty acids on nutrient digestibility, bacterial protein synthesis, and bacterial communities in continuous culture.

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We questioned if peptides (PEP) could be distinguished from ammonia and branched-chain volatile fatty acids (BCVFA) to optimize fiber degradation and microbial protein synthesis. Dual-flow continuous cultures were used in a 6 × 6 Latin square with 6 treatments. We formulated 2 diets differing in rumen-degraded protein (RDP) based on NASEM (2021, *Nutrient Requirements of Dairy Cattle*, 8th rev. ed., https://doi.org/10.17226/25806): negative (NCON; 8.7% RDP) and positive (PCON; 10.2% RDP) controls. In 4 more diets, low RDP diets were all supplemented with PEP (0.24 g N/d) to have similar RDP as PCON; these treatments were arranged factorially with low (LU) or high (HU) urea (0.15

or 0.40 g/L) buffers and without or with 2.15 mmol/d of each of the 3 BCVFA. Periods had 8 d of adaptation and 4 d of sampling. Liquid and solids dilution rates were 10% and 5%/h, respectively. Treatment was a fixed effect, and period and fermenter were random effects. Contrasts were NCON versus 4 PEP treatments, PCON versus 4 PEP treatments, and main effects and interaction of urea and BCVFA within the 4 PEP treatments. HU increased ($P < 0.05$) bacterial N, EMPS, and PEP outflow compared with LU (all within PEP treatments). Supplementation of PEP increased ($P < 0.05$) cellulose digestibility by 6.5 percentage units compared with PCON. Responses to BCVFA were minor, suggesting supplemental BCVFA did not compensate for inadequate NH_3-N but also were not negative with high NH_3-N provided by HU combined with PEP. Total bacterial AA flow increased ($P < 0.01$) with PEP supplementation versus NCON and with HU versus LU. Increasing urea increased ($P = 0.09$) total nonbacterial AA flow, particularly Pro, suggesting inhibition of peptidase as supported by increased recovery of peptides (passing through 10-kDa filters). DNA was extracted, amplicon sequencing variants were clustered, and taxa were classified using SILVA version 132.8. PEP increased ($P = 0.05$) observed ASV, Fisher index, and Faith's phylogenetic diversity compared with NCON, but evenness was not different among treatments. Increasing urea in the 4 PEP treatments increased ($P < 0.01$) *Bacillota* and decreased ($P = 0.05$) *Bacteroidota*, with many associated changes among genera. Supplementation with PEP promoted a more balanced bacterial community, whereas increasing urea inhibited peptidolysis with minor effects when BCVFA were adequately provided by PEP.

Key Words: peptide, ammonia concentration, branched-chain VFA, continuous culture, rumen microbial protein

61 Dietary modulation of rumen microbial structure and functional potential influences stress physiology and performance in beef bulls.

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Dietary modulation of gastrointestinal function influences health and performance in beef cattle, however effects of summer feeding strategies on rumen microbial structure and functional potential remain unclear. This study evaluated 4 summer systems in Holstein bulls: grazing only (G), pasture plus 2 kg/d concentrate (G2), grazing with ad libitum concentrate (GAL), and housing with silage and ad libitum concentrate (H). Growth performance, carcass traits, behavioral reactivity and temperament, hair cortisol concentration (HCC), and rumen microbiome profiles were assessed (n = 54). Following summer treatments, animals transitioned to a common winter diet. Shotgun metagenomic sequencing of rumen contents enabled comprehensive taxonomic profiling alongside KEGG ortholog and pathway-level functional analyses. Summer dietary treatment significantly altered rumen microbial community composition at the genus level (Bray–Curtis PERMANOVA, $R^2 = 0.556$, $P = 0.001$). Functional gene-level composition based on KEGG ortholog profiles was strongly differentiated by dietary treatment during summer (Bray–Curtis PERMANOVA, $R^2 = 0.63$, $P = 0.001$). Differential abundance analysis identified 1,466 KEGG orthologs significantly differing between treatments during summer ($q < 0.25$), particularly in pathways related to carbohydrate metabolism, glycan degradation, immune signaling and stress response. Bulls in the grazing-only system (G) exhibited higher HCC during summer (8.38 ± 0.72 pg/mg) compared with G2 (5.74 ± 0.71) and GAL (4.98 ± 0.61; $F_{3,49} = 4.68$, $P = 0.01$), and lower slaughter live weight at finishing (500.77 ± 7.70 kg) relative to G2 (533.50 ± 12.55), GAL (556.50 ± 16.06) and H (548.62 ± 11.24; $F_{3,50} = 6.19$, $P = 0.001$). Microbial and endocrine differences largely converged following winter dietary standardization, although carcass disparities persisted. Associations between specific microbial taxa, HCC, and behavioral reactivity were observed. These findings demonstrate

that summer dietary strategy modulates rumen microbial structure and functional capacity, with systemic stress, behavioral, and production consequences. Nutritional transitions during grazing represent a critical window for shaping gastrointestinal function and host resilience in beef bulls.

Key Words: rumen microbiome, shotgun metagenomics, dietary transition, stress physiology, beef cattle

62 Gut *Akkermansia muciniphila* attenuates obesity via modulating bile acid metabolism.

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Global health is increasingly challenged by the growing prevalence of obesity and its associated complications. *Akkermansia muciniphila*, the paradigm for next-generation beneficial microorganisms, has garnered attention as an alternative to pharmacological approaches for inhibiting obesity, despite the poorly understood underlying mechanisms. In this study, we demonstrate that *A. muciniphila* could significantly reverse obesity-related phenotypes and propose a novel mechanism of "*A. muciniphila*-bile acid metabolism" mediated by epigenetic modification N^6-methyladenosine (m^6A). Specifically, enrichment of *A. muciniphila* leads to generate more indole-3-lactic acid (ILA). Increased ILA is being transported to liver via the bloodstream and declines the levels of m^6A in 12α-hydroxylase (*Cyp8b1*) mRNA by upregulating expression of fat mass and obesity-associated protein (FTO). This process positively extends the half-life of *Cyp8b1* mRNA and subsequently inhibits its decay mediated by YTHDF2. Enhanced expression of CYP8B1 facilitates cholesterol convert to cholic acid (CA), which in turn drastically suppresses adipogenesis via activating the farnesoid X receptor (FXR) in adipose tissue. This work introduces a novel therapeutic target for regulating appropriate fat deposition and expands upon the current limited understanding of the mediator function of m^6A modifications in microorganism-influenced bile acid metabolism.

Key Words: *Akkermansia muciniphila*, obesity, mRNA m^6A, cholic acid

63 Lower-gut delivery of brassica-derived isothiocyanates links vegetable intake to GLP-1, appetite, and metabolic health.

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Brassica vegetables (e.g., broccoli, kale) are rich in glucosinolates (GSLs), which can be hydrolyzed by plant myrosinase into bioactive isothiocyanates (ITCs) that contribute to the health benefits of brassica intake. Cooking largely inactivates myrosinase, shifting GSL hydrolysis toward microbiota-dependent ITC formation in the intestine and increasing distal-gut ITC exposure, where GLP-1-secreting enteroendocrine cells are enriched. Here, we tested whether targeted lower-gut exposure to ITCs stimulates GLP-1 signaling and influences appetite regulation. We first screened several ITCs in vitro, including allyl ITC (AITC) and sulforaphane (SF), as well as the parent GSL of AITC (sinigrin; SN), for their ability to stimulate GLP-1 secretion in the mouse enteroendocrine cell line STC-1. To evaluate physiological relevance in vivo, we employed 2 complementary models: (1) a cecal injection model coupled with portal vein sampling to capture acute GLP-1 responses to lower-gut ITC exposure, and (2) a terminal-ileum cannulation model enabling real-time distal delivery in awake animals followed by 24-h food intake measurement. In STC-1 cells, ITCs, but not GSL, increased GLP-1 secretion in a dose-dependent manner, with AITC and SF exhibiting the greatest potency. In vivo, cecal exposure to SF, but not AITC, significantly increased portal GLP-1. Consistently, SF reduced 24-h food intake across multiple time points in cannulated rats, whereas AITC had no effect. Together, these data indicate that SF exposure in the distal gut can modulate gut hormone secretion and suppress appetite. Distal gut delivery of SF is sufficient to elevate GLP-1 and reduce 24-h food intake, supporting a GLP-1-mediated satiety mechanism that may be leveraged to help prevent or treat obesity and related metabolic disorders when ITC formation occurs in the distal intestine. Moreover, the divergence between in vitro and in vivo responses suggests that brassica vegetables are not nutritionally equivalent for appetite/metabolic signal-

ing, and that personalized benefit may depend on optimization of the gut microbiota toward maximal ITC (i.e., SF) production at the right gut location.

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Key Words: GLP-1, isothiocyanate, appetite, obesity

64 Association of *Undaria pinnatifida* rhizoid chemical composition with ruminal methane mitigation.

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In recent years, reducing methane emissions from the livestock industry has become a global challenge, and feed additives such as seaweed that have methane-reducing effects have been studied. Previously, we found that aerobically fermented seaweed liquid from the *Undaria pinnatifida* rhizoid reduced ruminal methane production via in vitro tests. In addition, this liquid contained 3-nitropropionic acid and picolinic acid, which have a ruminal methane-mitigating effect. We hypothesized that the synergistic effect of these compounds contributed to the results described above. However, the degradation mechanism of the *U. pinnatifida* rhizoid during fermentation remains unclear. An additional challenge was the low reproducibility of the methane-reducing effect of this liquid. In this study, we focused on the metabolite of *U. pinnatifida* rhizoid, and investigated the factors contributing to the decreased reproducibility. In experiment 1, we conducted metabolome analysis of 10 samples of dried *U. pinnatifida* rhizoid by CE-TOFMS. In experiment 2, we made the fermented *U. pinnatifida* liquid using rhizoid samples from experiment 1, and conducted in vitro testing. The *U. pinnatifida* rhizoids were fermented for up to 12 weeks, and 7 time point samples (0, 1, 2, 3, 4, 5, and 8 weeks) for each fermented *U. pinnatifida* liquid were used for the in vitro test. One replicate tube from each sample was incubated at 39°C for 24 h. The concentration of short chain fatty acids (SCFA) was monitored by HPLC. The composition of gas was analyzed by gas chromatography. In experiment 1, the chemical composition differed among the *U. pinnatifida* rhizoid samples, and were classified into 4 groups by hierarchical clustering. In experiment 2, although the methane production ratio decreased in 6 samples, no clear relationship was observed with respect to the fermentation period of the *U. pinnatifida* rhizoids or the clustering groups. Furthermore, the ratio of each SCFA did not differ among the treatment groups. These results showed that there was a weak association between the composition of *U. pinnatifida* rhizoids and the methane-mitigating effect of the fermented liquid. These weak correlations suggest that the methane-reducing effect is more strongly influenced by other factors than by the metabolite composition of *U. pinnatifida*. Future studies will characterize the bacterial and fungal communities in each of the 10 fermentation fluids.

Key Words: 3-nitorpropionic acid, seaweed, methane mitigation

65 Investigating the source of liver abscess pathogens in the gastrointestinal tract of ruminants.

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Liver abscesses are polymicrobial and the origin of their causative pathogens may be from gut locations other than the rumen. The objective of the study was to investigate the prevalence of liver abscess pathogens within the gastrointestinal tract of ruminants without liver abscesses. Experiment 1 (EXP1) consisted of 11 ewes (BW = 49 kg), fitted with ruminal and cecal cannulas. Rumen, cecal, and fecal samples (n = 126) were collected and microbial DNA was extracted. Full-

length 16S rRNA sequencing was performed on PacBio Revio platform. The sequencing results were used to determine relative abundance of 5 liver abscess pathogens: *Fusobacterium necrophorum*, *Trueperella pyogenes*, *Bacteroides pyogenes*, *Bacteroides heparinolyticus*, and an unidentified strain of *Bacteroides (Bacteroides NA*). In experiment 2 (EXP2), 6 feedlot bulls and 8 heifers finished on a common, corn-based feedlot diet, were slaughtered. Samples (n = 75) were collected from the rumen, cecum, and rectum after evisceration. Epimural (n = 40) and digesta (n = 35) samples were collected from each site and microbial DNA was extracted. A real-time qPCR assay was completed for both experiments to target 2 subspecies of *F. necrophorum* and *F. varium*. In EXP1, 16S sequencing results indicated *F. necrophorum* had the greatest relative abundance in the cecum. Furthermore, real-time qPCR from EXP1 showed *F. necrophorum* ssp. *funduliforme* to have greater prevalence in the cecum than rumen or feces. In EXP2, targeted liver abscess pathogens were not observed in cecal and fecal samples from feedlot cattle. However, *Fusobacterium* ssp. *necrophorum* was observed in 9% of the rumen epimural samples and 23% of rumen digesta samples. In conclusion, both experiments indicated a low abundance or absence of liver abscess pathogens within the gastrointestinal tract of ruminants without liver abscesses, suggesting these organisms may not be consistently detected in gut microbial communities.

Key Words: liver abscess, pathogen, ruminant

Prebiotics, probiotics, and DFM development

66 A *Bacillus*-based direct-fed microbial (DFM) mixture remodels the gut microbiome to augment respiratory health of *Salmonella* infected pigs.

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Salmonella enterica serotype Choleraesuis and porcine reproductive and respiratory syndrome virus (PRRSV) are significant pathogens that contribute substantially to morbidity, mortality, and economic losses in commercial pig production. Although *Bacillus*-based direct-fed microbials (DFMs) have been shown to reduce disease severity in these challenge models, how DFM administration reshapes the gut microbiome to support respiratory health remains unclear. Here, we investigated whether a *Bacillus*-based DFM modulates the gut microbiome and associated microbial functions along the gut-lung axis during *Salmonella* infection alone or in combination

with PRRSV. Weaned pigs were assigned control, *Salmonella* (Sal), Sal+DFM, Sal+PRRSV, or Sal+PRRSV+DFM groups. Cecal digesta and mucosa, as well as lung tissue, were analyzed using 16S rRNA gene sequencing, with shotgun metagenomics performed on cecal samples, and lung pathology assessed histologically. Both single and dual infections altered cecal microbial α and β diversity relative to controls, with enrichment of taxa previously linked to dysbiosis including *Campylobacter*, *Anaerobiospirillum*, and *Odoribacter*. DFM supplementation shifted microbial communities toward control-associated communities, as evidenced by clustering patterns in principal coordinates analysis, with the strongest effect observed in Sal-infected pigs. Pigs infected with *Salmonella* in the absence of DFM developed pronounced lung lesions, including areas of tissue hepatization, which were significantly reduced with DFM supplementation. Metagenome-assembled genome analysis revealed increased representation of genes associated with short-chain fatty acid biosynthesis, including acetate and butyrate, and a higher abundance of biosynthetic gene clusters encoding putative antimicrobial peptides, particularly sactipeptides and bacteriocins. These genomic signatures were most evident in the Sal+DFM group and coincided with improved lung histology. Collectively, these findings indicate that a *Bacillus*-based DFM alters the gut microbiome in ways that support metabolic and antimicrobial capacity and are associated with improved respiratory outcomes along the gut–lung axis. The antimicrobial peptides identified in this work may provide a useful starting point for future studies aimed at understanding the direct and indirect mechanisms by which DFMs affect the gut–lung axis and systemic health outcomes.

67 Rumen-native microbe supplementation to consistently improve dairy cow productivity.

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Next-generation probiotics aim to enhance the impact of microbiome interventions by employing host-derived microbial strains that are compatible with the existing microbiome. To identify candidate strains associated with improved production, we conducted a survey of the rumen microbiome in healthy dairy cows and evaluated microbial associations with milk yield and components. Four rumen-native microbes were subsequently isolated from healthy dairy cows and commercialized under the name Galaxis Frontier (GF). This consortium of live microbes, consisting of *Clostridium beijerinckii*, *Butyrivibrio fibrisolvens*, *Ruminococcus bovis*, and *Pichia kudriavzevii*, was evaluated in controlled in vivo studies across 8 independent academic trials in pregnant or lactating dairy cows. A meta-analysis of production outcomes across cohorts identified consistent effects from supplementing GF, including an increase in gross feed efficiency (+0.05 ECM/DMI; $P < 0.01$) driven by fat yield (+0.05 kg; $P = 0.03$) and, in most cohorts, energy-corrected milk (+2.56 kg; $P = 0.06$). Rumen metabolite profiling revealed that GF-supplemented cows tended to exhibit higher total short-chain fatty acid (SCFA) concentrations ($P = 0.05$), with significant increases in propionic acid ($P < 0.01$) and trends toward increased acetic ($P = 0.08$) and valeric acids ($P = 0.08$). Concentrations of butyric, isobutyric, and methylbutyric acids, as well as the acetate:propionate ratio, were inconsistent or unchanged. Shotgun metagenomic sequencing of a subset of rumen samples revealed that the ruminal microbiome in cows provided GF was more similar to each other than unsupplemented control cows (β-dispersion $P = 0.04$) and identified a subset of taxa that are more consistently abundant in GF cows. These taxa were correlated with ruminal SCFA concentrations, and pathway enrichment analysis suggested changes in amino acid-derived fermentation. These findings demonstrate that supplementation with rumen-native live mi-

crobes can reproducibly enhance dairy cow productivity and provide a platform for mechanistic exploration of how microbiome composition and host-microbiome interactions can converge on consistent production responses.

Key Words: next-generation probiotic, dairy cow, rumen microbiome, feed efficiency, DFM

68 Resistant starch enhances intestinal barrier function by modulating the abundance of *Bifidobacterium pseudolongum*.

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The gut microbiota, shaped significantly by dietary fiber, plays a key role in regulating host physiology and disease. A fiber-deficient Western diet promotes microbial imbalances, depleting beneficial taxa that support the intestinal barrier and exacerbating inflammation. Although fiber components such as microcrystalline cellulose, inulin, and resistant starch (RS) serve as prebiotics that support epithelial barrier function and reduce inflammation, their impact varies due to differences in solubility and fermentability. These physicochemical properties influence microbial composition and function, yet direct comparisons linking these properties to specific microbial responses and barrier protection are limited. In this study, we integrated physicochemical analysis with microbiological evaluation to assess the prebiotic effects of these fibers. We found RS to be the effective prebiotic in maintaining gut homeostasis, particularly by promoting the growth of *Bifidobacterium pseudolongum*. This microbial shift led to increased short-chain fatty acid production, which in turn enhanced colonic tight junction protein expression and strengthened the intestinal barrier, alleviating symptoms of DSS-induced colitis. Our findings underscore the significance of fiber structure in guiding microbial behavior and offer a foundation for precision dietary interventions aimed at improving intestinal health.

Key Words: prebiotic, resistant starch, probiotics, colitis, *Bifidobacterium pseudolongum*

69 How complex dietary fibers can be used to shape the human gut microbiome toward reduced inflammatory potential.

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Chronic low-grade inflammation is a central feature of human disease and is strongly modulated by diet–microbiome interactions. We evaluated whether combining structurally distinct fibers can modulate the human gut communities toward profiles associated with reduced inflammatory potential. Anaerobic batch fermentations inoculated with fecal samples from 2 healthy adults compared a β-glucan/mannan control matrix with the same matrix supplemented by inulin (fructan), pectin (heteropolysaccharide), or dextran (α-1,6-glucan). Microbiome composition (16S rRNA V4–V5) and diversity were profiled over 0–48 h; community change was assessed via α-diversity, β-diversity, and differential relative abundance. All treatments showed an early, sucrose-driven Proteobacteria increase with a transient drop in α-diversity at ~8 h, followed by fiber-specific recovery. Dextran enriched *Bacteroides* and suppressed *Proteobacteria*; inulin promoted *Bifidobacterium*, *Collinsella*, and butyrate-associated *Firmicutes*; pectin supported a broad cross-feeding consortia (e.g., *Faecalibacterium*, *Ruminococcus*, *Eubacterium*). Trajectories converged across donors by 24–48 h, indicating that fiber complexity can reduce inter-individual variation in community structure. The results suggest that multi-fiber strategies can reproducibly shift microbiomes away from an inflammatory state. These findings suggest that policy and health recommendations aimed at reducing the chronic disease risk through diet should consider specific multi-fiber food supplements rather than broadly promoting single soluble fiber products.

Key Words: fiber, 16S rRNA V4–V5, gut microbiota, prebiotics

70 Probiotic-induced restructuring of the canine gut microbiome and functional gene profiles is associated with behavioral modulation and physiological biomarkers in breeding dogs.

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Gastrointestinal microbial function plays a central role in regulating host behavior, mucosal immunity and stress responses via the microbiota-gut-brain axis (MGBA). This study investigated probiotic effects on behavioral welfare, stress biomarkers, and gut microbiome composition and function in domestic dogs housed in a licensed breeding establishment. Dogs were assigned to control or probiotic groups, and behavioral responses were assessed using validated tests (novel object test, attention bias test), alongside quantification of hair cortisol concentration (HCC), fecal glucocorticoid metabolites (FGM), and fecal secretory immunoglobulin A (sIgA). Shotgun metagenomic sequencing was used to characterize taxonomic and functional microbiome profiles. No significant treatment × time interactions were detected for HCC, FGM, or fecal sIgA. However, significant main effects of time were observed for HCC ($F_{1,52}$ = 8.18, P = 0.006) and fecal sIgA ($F_{1,52}$ = 12.59, P < 0.001), with sIgA concentrations increasing from baseline to study end independent of treatment, indicating temporal modulation of mucosal immune activity. Behaviorally, probiotic-treated dogs exhibited significantly greater exploratory behavior in the novel object test ($F_{1,26}$ = 7.98, P = 0.01) and reduced escape behavior in the attention bias test ($F_{1,26}$ = 9.12, P = 0.01) compared with controls. Shotgun metagenomic analysis revealed significant effects of treatment (Bray-Curtis PERMANOVA, R^2 = 0.038, P = 0.047) and time (R^2 = 0.040, P = 0.033) on genus-level microbial composition, with only probiotic-treated dogs exhibiting a significant temporal shift in β diversity (R^2 = 0.114, P = 0.003). At the functional gene level, a significant treatment × time interaction was detected (R^2 = 0.057, P = 0.018), driven by marked restructuring of KEGG ortholog profiles within the probiotic group (R^2 = 0.194, P = 0.002), including modulation of pathways such as tryptophan metabolism. Correlations between specific microbial genera and stress-related biomarkers further supported microbiome-host interactions. These findings demonstrate that probiotic supplementation modulates gastrointestinal microbial structure and functional gene profiles, with behavioral and mucosal immune associations.

Key Words: probiotics, gut microbiome, behavioral modulation, shotgun metagenomics, stress biomarker.

71 Investigating the efficacy of a prebiotic-probiotic as an alternative to antibiotics on the growth performance of neonatal dairy calves.

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Research shows that exposure to subtherapeutic dose of antimicrobials can lead to the emergence of antimicrobial resistance in livestock. With the growing concern of emergence of new antimicrobial resistance determinants, the need for alternative to antimicrobials is highly sought after. Although the use of probiotics to replace antimicrobials has gained traction, complementing probiotic with prebiotic is an innovative strategy to maximize host health benefits. The use of such prebiotic-probiotic combinations, hereafter referred to synbiotic formulations, has not been tested in neonatal dairy calves. Our group has developed a novel synbiotic formulation composed of a native early life colonizer, *Limosilactobacillus reuteri* RM 125, with *Helianthus tuberosus* (Jerusalem artichoke) tuber as a prebiotic. The efficacy of this formulation was initially tested under in vitro conditions to optimize dose and evaluation criteria for improved gut health parameters. Strong preliminary data led us to test the efficacy of this formulation (SYN) as an alternative to the conventional used tetracycline (TET) against a

placebo group of neonatal calves (n = 6/group). Calves were enrolled to each of the experimental diets at birth and supplemented with the respective treatments daily for 12 weeks. The synbiotic supplementation resulted in significant ($P < 0.05$) increase in average daily gain and feed intake of calves by 53.8% and 17.6% over the CON group, respectively, and feed efficiency improved from 2.77 in CON to 2.17 in SYN. Further, SYN supplementation reduced incidence (13.64% in CON vs. 5.75% in SYN) and duration of calf scours (11.5 d in CON vs. 4.5 d in SYN) and improved fecal firmness, consistency, and calf activity on a 1–4 scale, indicating that the gut environment was modulated. Fecal microbial analysis by standard pour plate technique revealed significant ($P < 0.05$) increase in fecal *Lactobacillus* and *Bifidobacterial* counts alongside improved fecal biochemical attributes (fecal pH, ammonia, lactic acid and volatile fatty acids) in SYN calves over CON and TET. Although serum antioxidant status improved with synbiotic supplementation, nutrient digestibility and rumen attributes of calves differed nonsignificantly. Although the results establish functional efficacy of novel synbiotic to replace tetracyclines feed additive further research is required to decipher the mechanistic basis.

Key Words: neonatal calf, synbiotic, diarrhea, growth performance, gut health index

72 *Lactobacillus*-vectored nanobodies improve broiler productivity in sub-clinical necrotic enteritis with integrated microbiome and host transcriptomic effects.

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Subclinical necrotic enteritis (SCNE), caused by toxin-producing *Clostridium perfringen*s, remains a significant challenge in poultry production. Traditionally managed with in-feed antibiotics, growing concerns over antimicrobial resistance necessitate effective antibiotic-free alternatives. We developed a proprietary probiotic-vectored biologic platform using engineered *Limosilactobacillus reuteri* strains designed to function through a dual mechanism in the gastrointestinal tract. Expressed nanobodies (VHHs) selectively neutralize the major virulence factors α toxin and NetB, whereas the probiotic chassis simultaneously promotes immune homeostasis and preserves intestinal barrier integrity through its intrinsic host-modulatory properties. In a controlled broiler study under SCNE challenge conditions, birds receiving engineered strains showed significantly improved feed conversion ratio and weight gain compared with prophylactic antibiotic treatment or wild-type probiotic controls. Histomorphometric analysis, jejunal microbial metatranscriptomics, and host gene expression profiling confirmed in situ nanobody expression and demonstrated reduced toxin-mediated tissue damage, attenuated intestinal inflammation, lower oxidative stress, and restoration of a metabolically efficient immune state. These findings establish probiotic-vectored nanobody delivery as a scalable, precision biologic strategy that integrates targeted pathogen neutralization with microbiome-driven host resilience for antibiotic-free control of enteric disease.

Key Words: engineered probiotics, necrotic enteritis, poultry productivity, *Lactobacillus*-vectored nanobodies

73 Characterization of early-colonizing gut bacteria from neonatal calves for potential probiotic application.

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In preruminant calves, the gastrointestinal tract microbiome is less complex than in adult cows, and early microbial colonizers play a critical role in shaping the mature ecosystem, significantly influencing host health and development. The objective of this study was to isolate native bacteria involved in the early gut colonization of neonatal calves and characterize their functional and probiotic properties. Fecal samples were collected from 13 neonatal Holstein calves (0–2 d old) and incubated in anaerobic media with rumen fluid. A total of 129 oxygen-sensitive, cata-

lase-negative isolates were isolated. Full-length 16S rRNA gene sequencing classified the isolates to the genera *Enterococcus*, *Streptococcus*, *Ligilactobacillus*, *Limosilactobacillus*, *Paraclostridium*, and *Clostridium*. Sixty-four isolates exhibiting faster growth and high biomass production in anaerobic glucose media were further phenotyped for lactose utilization and hemolytic activity against bovine erythrocytes. Twenty representative strains were subsequently screened for antimicrobial activity against common gastrointestinal pathogens (*Escherichia coli*, *Salmonella* Typhimurium, and *Clostridium perfringens*). All isolates could metabolize lactose (area under the curve = 9.43 ± 4.11), maltose (7.89 ± 3.49), and inulin (8.66 ± 3.24), with lactic acid serving as the primary fermentation end product (mean = 40.3 ± 20.8 m*M*). Eighteen isolates demonstrated varying biofilm-forming capacities (mean OD_{595} = 0.62 ± 0.33). Although most isolates inhibited the tested pathogens, the antimicrobial effect was medium-dependent; minimal activity was observed in brain heart infusion (BHI) broth, whereas significantly greater ($P < 0.05$) inhibitory activity occurred in de Man, Rogosa, and Sharpe (MRS) broth. These findings demonstrate that the neonatal calf gut harbors a diverse population of early-colonizing, lactic acid–producing bacteria with the capacity to utilize varied carbohydrate sources and form biofilms. The pronounced antimicrobial activity observed in MRS broth, but not in BHI, suggests that the probiotic potential of these isolates is closely linked to their metabolic environment. These native strains represent promising candidates for the development of calf-specific probiotics aimed at stabilizing the early life microbiome and mitigating the risk of enteric infections during the pre-ruminant period.

Key Words: calf, microbiome, probiotics, antimicrobial

74 Functional screening of novel propionate-producing ruminal bacteria as direct-fed microbial candidates.

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Direct-fed microbials (DFM) are increasingly explored as sustainable strategies to improve rumen function and reduce methane (CH_4) emissions. By introducing beneficial bacteria that redirect fermentation pathways, DFMs promote alternative H_2-utilizing routes that compete with methanogenesis. Propionate-producing pathways are particularly attractive as effective H_2 sinks. Recent culturomics initiatives have recovered previously uncultured ruminal bacteria, highlighting the broad and still underexplored microbial diversity of the rumen, yet the metabolic potential of these isolates remains poorly characterized. In this work, over 100 novel ruminal bacterial isolates were screened for volatile fatty acid (VFA) production, focusing on propionate formation. Strains were cultured anaerobically in Hobson M2 medium at 39°C and sampled at exponential phase for VFA quantification by gas chromatography. Four isolates within the *Propionibacterium* and *Selenomonas* genera showed markedly higher relative propionate production (propionate:acetate ratios of 2.2–2.6) and were selected for further characterization. The 4 selected strains were cultivated in M10 medium with glucose as sole carbon source under anaerobic conditions. Growth curves were established by optical density (OD_{600}) measurements across multiple time points, defining lag, exponential, and stationary phases. Colony-forming unit (cfu)-to-OD_{600} relationships were determined using M10 agar plate counts to standardize inoculum density and assess growth performance in liquid culture. Minimum inhibitory concentrations (MIC) for ampicillin, tetracycline, erythromycin and clindamycin were determined by E-test following EFSA FEEDAP recommendations. All isolates showed MIC values below the microbiological cut-off levels established for bacterial feed additives, indicating low risk of transferable antimicrobial resistance and supporting their safety as potential DFM candidates. These findings show that targeted functional screening combined with growth and antimicrobial resistance (AMR) profiling enables preliminary characterization of high propionate-producing ruminal bacteria as DFM candidates. This work lays the groundwork for further metabolic, genomic and in vitro rumen fermentation studies to explore their potential as

alternative H_2 sinks and applicability as DFMs for CH_4 mitigation.

Key Words: propionate, rumen, DFM, methane

75 Optimization of protease combination under simulated chicken gastrointestinal conditions.

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This study presents a novel application of statistical modeling to optimize enzyme combinations for enhancing protein digestibility of soybean meal under simulated chicken gastrointestinal conditions. Three types of proteases—aspartic protease, metalloprotease, and serine protease—were used for the study. Employing response surface methodology (RSM) with both central composite design and Box–Behnken design, we developed robust predictive models that accurately identified optimal conditions for enzyme activity and protein digestibility. The models demonstrated strong statistical reliability, with high coefficients of determination (R^2 values up to 0.94), and were validated against multiple test sets to confirm their predictive capability (R^2 = 0.95). Simulation experiments, reflecting physiological pH variations across digestive stages of crop, stomach, and intestine, underscored the practical relevance of this approach. The optimized enzyme combinations resulted in a significant improvement in overall protein digestibility compared with the control (34% enhancement; $P < 0.05$). These findings establish a rigorous and practical framework for advancing commercial poultry feed enzyme applications, providing scientific researchers and feed technologists with a reliable tool for targeted enzyme optimization.

Key Words: protease, response surface methodology, modeling, chicken, gastrointestinal tract

76 Effects of CLOSTAT® 500 (*Bacillus subtilis* PB6) in milk replacer on intestinal barrier function and disease incidence in pre-weaned calves.

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A robust body of literature shows that *Bacillus subtilis* PB6, the active strain in CLOSTAT® 500 (Kemin Industries, Inc.), improves digestive capability and strengthen intestinal barrier function. Research has demonstrated that PB6 increases the abundance of tight junction proteins such as ZO-1 and claudin-1 in the intestinal epithelium, which are essential components of gut barrier integrity. By enhancing these proteins, PB6 helps to maintain epithelial tight junctions, reducing intestinal permeability and supporting resistance against pathogenic bacterial challenges. PB6 has also been shown to inhibit pathogenic bacteria associated with the development of leaky gut. This study aimed to evaluate the effects of CLOSTAT 500 in milk replacer on the health of pre-weaned calves raised on a large Western US calf ranch receiving calves from multiple herds. Calves (n = 801) arriving from November 1 to 10, 2024, were assigned to receive *Bacillus subtilis* PB6 in their milk replacer at a rate of 500 mg/head per day for 60 d. Calves arriving from January 1 to 10, 2025, served as the control group (CON; n = 876) and received only milk replacer. Health and mortality events were tracked using DairyComp® 305 (Valley Ag Software) herd management software records. Data were analyzed using the FREQ procedure (PROC FREQ/CHISQ) of SAS statistical software (SAS Institute Inc.), with statistical significance declared at $\alpha = 0.05$. When calves received CLOSTAT 500 in their milk replacer, morbidity and mortal-

ity decreased significantly compared with control calves. Mortality between 1 and 55 d was reduced from 49 animals in the control group to 21 in the PB6 group ($P < 0.05$). Incidence of ear infections, pneumonia before 25 d of age, and idiopathic disease were all significantly reduced ($P < 0.01$). In addition, the number of healthy calves increased 36% ($P < 0.01$) in the PB6 group compared with the control group. Supplementation with CLOSTAT 500 improved calf health outcomes during the preweaning period, resulting in lower disease incidence and higher survival rates. The economic analysis indicated a return on investment of approximately 13.5:1. Results suggest that including CLOSTAT 500 in milk replacer can be an effective management strategy during early life to optimize intestinal barrier function, reduce disease pressure, and improve overall calf survivability during this high-risk period.

Key Words: *Bacillus subtilis*, calf, morbidity, mortality, mode of action

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