SCIENTIFIC PROGRAM AND ABSTRACTS

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2013 CONGRESS ON GASTROINTESTINAL FUNCTION
MEETING PROGRAM

MONDAY, APRIL 15TH:

12:00-18:00 REGISTRATION **Gleacher Center, First Floor (100 Foyer)**

Please pick up your Registration materials, Name tag and Mixer drink tickets
Mount posters on boards provided

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

13:30-13:40 **Mark Morrison**, Congress Chair, CSIRO Australia and The Ohio State University
Welcome to the Opening Session of the 2013 Meeting
Introduction and Marvin P. Bryant Memorial Lecture Plaque Presentation

13:40-14:30 **Professor Rudolf Thauer**, Max Planck Institutes, Germany
Marvin P. Bryant Memorial Lecture
Interspecies hydrogen transfer and the role of flavin-based electron bifurcation: a novel mechanism of energy conservation

14:30-15:15 **Mark Morrison**, CSIRO Australia and The Ohio State University, Columbus, Ohio
Differences down-under – macropodids, methane and metagenomics

15:15-16:00 **Harry Gilbert**, Newcastle University, Newcastle-upon-Tyne, UK
Understanding the mechanisms by which the microbiota of the human large bowel utilize complex carbohydrates as a significant source of nutrients

Session break – Set up remaining posters and resume meeting on 6th floor for Welcome Mixer

16:30-18:30 **WELCOME MIXER Gleacher Center, Sixth Floor (Room 621)**
Informal poster viewing session
All attendees and guests please wear your name tag
Refreshments: Drink tickets, hors d'oeuvres and cash bar

TUESDAY, APRIL 16TH:

08:30-9:00 **Continental Breakfast** Gleacher Center, First Floor, near Tiered Classroom

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

09:00-10:30 Oral Presentations **Session 1**

09:00-09:45 **Philippe Langella,** INRA Jouy-en-Josas, France
Use of *Faecalibacterium prausnitzii* to prevent and treat gastrointestinal disorders and diseases

09:45-10:05 *Lactobacillus reuteri* 1E-1 alters expression of toll-like receptor signaling pathway genes in the IEC-6 rat intestinal epithelial cell line
M. Duersteler, K. Smith, K. Novak, E. Galbraith, and E. Davis, DuPont Nutrition and Health, Waukesha, Wisconsin, USA

10:05-10:25 Ability of yeast and yeast derivatives to reduce attachment of pathogens and mycotoxins to gut mucosa
N. Walker[1], J. Apajalahti[2], and P. Wilcock[1], [1]AB Vista, Marlborough, Wiltshire, UK; [2]Alimetrics, Espoo, Finland

10:25-11:00 **Coffee Break** Gleacher Center, First Floor, near Tiered Classroom

11:00-12:00 Oral Presentations Session 1 continued

11:00-11:20 Association of Intestinal and Systemic Inflammatory Responses with Microbial Bile Acid Deconjugation and Sulfidogenesis in High-Fat Fed Mice
F. Carbonero[1], W. Shen[2], Z. Zhou[2], M. McIntosh[2], and H.R. Gaskins[1], [1]University of Illinois, Urbana, IL, [2]University of North Carolina-Greensboro, Greensboro, NC, USA

11:20-11:40 Central Metabolic Pathways in the Syntrophic Metabolizer, *Syntrophus aciditrophicus*
Huynh Le[1], Xueyang Feng[2], Yinjie Tang[2], and Michael McInerney[1], [1]University of Oklahoma, Norman, OK, [2]Washington University, St. Louis, MO, USA

11:40-12:00 Efficient method of mRNA enrichment to study the rumen metatranscriptome
Sophie Comtet[1,2], Frédérique Chaucheyras-Durand[1,4], Pascale Mosoni[1], Jérémie Denonfoux[2], Corinne Petit-Biderre[3], Evelyne Forano[1], and Pierre Peyret[2], [1]INRA, Saint-Genès-Champanelle, [2]Université d'Auvergne, Clermont-Ferrand, [3]Université Blaise Pascal, Clermont-Ferrand, [4]Lallemand Animal Nutrition, Blagnac, France

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

Afternoon Session Gleacher Center, First Floor, Tiered Classroom

13:00-14:20 Oral Presentations Session 2

13:00-13:45 How does host genomic variation translate into gut microbiome composition?
Andy Benson, University of Nebraska, Lincoln, Nebraska, USA

13:45-14:05 **Insights into the bovine rumen plasmidome.**
A. Brown Kav[1,2], I. Sasson[1], E Jami[1,2], A. Doron-Faigenboim[1], I. Benhar[2], and I. Mizrahi[1], [1]Department of Ruminant Science, Institute of Animal Sciences,

Agricultural Research Organization, Volcani Center, Bet Dagan 50250, [2]Molecular Microbiology and Biotechnology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat-Aviv 69978, Israel

14:05-14:25 Comparative transcriptomic analysis of *Ruminococcus albus* strains 7 and 8 grown on cellulose and hemicellulose
I.H. Kwon, E.D. Henriksen, I.K.O. Cann, and R.I. Mackie, University of Illinois, Urbana, IL, USA

14:25-15:00 **Coffee Break** **Gleacher Center, First Floor, near Tiered Classroom**

15:00-16:00 Oral Presentations Session 2 continued

15:00-15:20 Proteomic analysis of the *Eubacterium rectale* membrane reveals high affinity nutrient receptors
N. Koropatkin, University of Michigan Medical School, Ann Arbor, MI, USA

15:20-15:40 Comparative Genomics of Rumen Bacteria *Selenomonas ruminantium* subsp. *ruminantium* and *Selenomonas ruminantium* subsp. *lactilytica*
E.D. Henriksen, P. Hong, I.O. Cann, and R.I. Mackie, University of Illinois, Urbana, Illinois, USA

15:40-16:00 Genome sequencing of rumen archaea: *Methanobrevibacter* sp. AbM4 genome sequence
S. C. Leahy[1,2], W. J. Kelly[2], D. Li[2], Y. Li[1,2], E. Altermann[2], S. Lambie[2], F. Cox[2] and G. T. Attwood[1,2], [1]New Zealand Agricultural Greenhouse Gas Research Centre and [2]AgResearch Limited, Palmerston North, New Zealand

16:00-16:20 Remarkable similarity among bacteria isolated from 4 hosts after 8 week enrichments of feces with cellulose and xylan/pectin.
C. J. Ziemer, USDA-ARS, National Laboratory for Agriculture and the Environment, Ames, Iowa, USA

16:30-18:30 POSTER SESSION MIXER Gleacher Center, Sixth Floor (Room 621)

WEDNESDAY, APRIL 17TH:

08:30-9:00 **Continental Breakfast** Gleacher Center, First Floor, near Tiered Classroom

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

09:00-10:25 Oral Presentations Session 3

09:00-09:45 Role of Dietary Oligosaccharides in Gastrointestinal Development and Host Defense in the Piglet Model.
Sharon Donovan, University of Illinois, Urbana, Illinois, USA

09:45-10:05 Effect of Dietary Genistein on Ovalbumin-induced Allergic Reactions in a Mouse Model of Food Allergic Diarrhea
J.-H. Wang, S.-W. Fan, and **W.-Y. Zhu**, Nanjing Agricultural University, Nanjing, Jiangsu, China R Mackie

10:05-10:25 Impact of maternal gastrointestinal microbiota on the gastrointestinal microbiota of their infants
L. Wang[1], J. Stiverson[1], T. Williams[2], C. Shaw[1], M. Morrison[1,3], and Z. Yu[1]
[1]The Ohio State University, Columbus, Ohio, USA, [2]Abbott Nutrition, Columbus, Ohio, USA, [3]CSIRO Livestock Industries, St Lucia, QLD, Australia

10:25-11:00 **Coffee Break Gleacher Center, First Floor, near Tiered Classroom**

11:00-11:40 Oral Presentations Session 3 continued

11:00-11:20 Immune Development Characterization of Gastrointestinal Tissue from High and Low Gaining Steers Under Feedlot Management Conditions K.N. Novak, **E.A. Galbraith**, M.J. Hundt, and E. Davis, DuPont Nutrition and Health, Waukesha, Wisconsin, USA

11:20-11:40 Effect of galacto-oligosaccharide supplementation on the microbial community composition of the rumen and colon of pre-weaning milk-fed Holstein calves
J. Castro, A. Gomez, B. White, and J.K Drackley, University of Illinois, Urbana, IL, USA

11:40-12:00 The Western Lowland Gorilla (*G. gorilla gorilla*) Gastrointestinal Microbiome Sheds Light on their Overall Ecology and Conservation
A.M Gomez[1], C.J. Yeoman[2], B.A. White[1], A. Todd[4], R. Stumpf[1], K.E Nelson[5], M. Gillis[5], M. Torralba[5], F. Carbonero[1], H.R Gaskins[1], B.A. Wilson[1], K. Ptrzelkova[3], and S.R Leigh[6], [1]University of Illinois, Urbana, Illinois, USA, [2]Montana State University, Bozeman. Montana, USA, [3]Czech Academy of Sciences, Brno, Czech Republic, [4]World Wildlife Fund, Bangui, Central African Republic, [5]Craig Venter Institute, Rockville, Maryland, USA, [6]University of Colorado, Boulder, Colorado, USA

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

Afternoon Session Gleacher Center, First Floor, Tiered Classroom

13:00-14:25 Oral Presentations Session 4

13:00-13:45 Control of Food Intake by Metabolism of Fuels - A Comparison Across Species
Mike Allen, Michigan State University, East Lansing, Michigan, USA

13:45-14:05 Animal-specific recovery patterns of rumen bacterial community after rumen content exchange
M. Zhou, B. Ghoshal, P. Stothard, and L.L. Guan, University of Alberta, Edmonton, AB, Canada

14:05-14:25 Increasing Levels of Dietary Wet Distillers Grains plus Solubles Effects on Rumen Bacterial Population Dynamics
G. M. Shipp[1], D. W. Pitta[2,5], S. L. Ivey[3], B. Milligan[3], J. C. MacDonald[4,1], and W. **E. Pinchak**[5], [1]Texas A&M AgriLife Resaerch, Amarillo, TX, [2]University of Pennsylvannia, Kennet Square, PA, [3]New Mexico State University, Las Cruces, NM, [4]Univestiy of Nebraska, Lincoln, NE, [5]Texas A&M AgriLife Research, Vernon, TX, USA

14:25-14:45 **Coffee Break Gleacher Center, First Floor, near Tiered Classroom**

14:45-15:20 Oral Presentations Session 4 continued

14:45-15:05 *Saccharomyces cerevisiae* fermentation product stabilized rumen microbial communities of lactating dairy cows during subacute ruminal acidosis
S. Li[1], E. Khafipour[1], I. Yoon[2,1], M. Scott[2,1], and J. C. Plaizier[1], [1]University of Manitoba, Winnipeg, MB, Canada, [2]Diamond V, Cedar Rapids, IA

15:05-15:25 Mixed Rumen Microbes Respond to Excess Carbohydrate by Synthesizing Reserve Carbohydrate and Spilling Energy
T. J. Hackmann and J. L. Firkins, The Ohio State University, Columbus, OH, USA

15:25-15:45 Immunogenic inhibition of mixed culture ruminal bacteria as a means to reduce lipolysis and fatty acid biohydrogenation *in vitro*
H. D. Edwards[1], R. C. Anderson[2], W. L. Shelver[3], N. A. Krueger[2], D. J. Nisbet[2], and S. B. Smith[1], [1]Texas A&M University, College Station, Texas, [2]USDA/ARS, College Station, Texas, [3]USDA/ARS, Fargo, North Dakota, USA

15:45-15:55 CLOSING REMARKS AND INVITATION TO CGIF 2015

16:00 BUSINESS MEETING

ABSTRACTS OF INVITED, PODIUM AND POSTER PRESENTATIONS

Bryant Memorial Lecture

Interspecies hydrogen transfer and the role of flavin-based electron bifurcation, a novel mechanism of energy conservation

Rudolf K. Thauer*, *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany.*

Marvin Bryant's paper from 1968 on interspecies hydrogen transfer in a co-culture of a bacterium fermenting ethanol to acetic acid and H_2 and of a methanogen growing on H_2 and CO_2 remains today one of the groundbreaking findings in microbiology. Whereas the methanogen, later named *Methanobacterium bryantii* in his honor, could grow alone on H_2 and CO_2, growth of the ethanol fermenter was dependent on the methanogen because ethanol fermentation to acetic acid and H_2 by proton reduction is only exergonic when the H_2 concentration is kept very low by the methanogen. The concept of obligate syntrophic proton reducers was born and revolutionized our understanding of how plant biomass is fermented in the intestinal tract of animals and other anoxic environments. From the year of our first meeting in 1974 until his death in 2000, we met many times in Urbana and at conferences both in the USA and in Europe. Through our many discussions, I became interested in H_2-dependent methanogens and in how H_2-forming microorganisms can produce H_2 from NADH, which in the living cell has a redox potential only near −280 mV, making the reaction thermodynamically highly unfavorable. But it took until 2008 for the mystery to unravel: in H_2-forming and H_2-consuming anaerobes, endergonic and exergonic redox reactions can be coupled by the mechanism of flavin-based electron bifurcation catalyzed by cytoplasmic enzyme complexes. This novel energy-coupling mechanism will be the focus of this Bryant Memorial Lecture. Marv would surely have been delighted to hear that we are starting to mechanistically understand what he discovered more than 40 years ago.

Invited Presentations and Selected Podium Presentations

Differences downunder: macropodids, methane and metagenomics

M. Morrison[1,2] and the CAFHS Microbial Biology and Metagenomics group[1]; [1]CSIRO Division of Animal, Food and Health Sciences, St Lucia, QLD, Australia; and [2]The Ohio State University, Columbus, OH, USA.

The somewhat controversial findings during the 1980s that the Australian macropodids (kangaroos and wallabies) produce a fraction of the methane emissions compared to ruminant livestock was recently confirmed by new studies performed with captive animals in Denmark. Although macropodid adaptations to herbivory, which differ from those of ruminants, are likely to provide some explanation for the "low methane" phenotype; the foregut microbiota has also evolved under these selective pressures to sustain an effective hydrolysis and fermentation of plant biomass. We have applied a broad range of metagenomic and conventional microbiological approaches that provide a range of new insights into the structure-function relationships of the macropodid prokaryote microbiota. In addition to a preponderance of PUL-like gene clusters associated with "cellulases and xylanases" recovered from the metagenomic DNA libraries; the numbers of methanogenic archaea present in the macropodid foregut are substantially less when compared to domesticated ruminants. Furthermore, the seventh order of methanogens, the *Methanoplasmatales,* and *Methanosphaera spp.* appear predominant. The relevance of these latter findings is emphasized via our mutualistic use of (meta)genomic sequencing and culture-based studies. We have found that isolates from both genera are obligately heterotrophic, and some strains of *Methanosphaera* spp. from the macropodid appear capable of using short-chain alcohols in place of H_2 to support methanogenesis and growth, unlike the available isolates from ruminants and the human gut. Our

metagenomic sequence and bioinformatics analyses have also supported our "reconstruction" of the physiological features of some key bacteria representing deep "new" branches in the phylogenetic lineages of Proteobacteria and Lachnospiraceae. Such knowledge enabled the axenic cultivation of one of these key bacteria, which was subsequently confirmed to be a member of the *Succinovibrionaceae.* Our collective studies to date not only augment the microbiological bases for the "low methane" phenotype of these animals beyond the realm of homoacetogenesis, but also reveal and raise interesting questions as to how the Bacteria and Archaea have evolved to colonize and persist in this interesting host-microbe mutualism.

Understanding the mechanisms by which the microbiota of the human large bowel utilize complex carbohydrates as a significant source of nutrients

H. J. Gilbert, Newcastle University, Newcastle-upon-Tyne, UK.

Dietary and host complex carbohydrates play a central role in human health by defining the structure of our large bowel microbial community (human microbiota). The utilization of complex carbohydrates by the human microbiota is dependent on their efficient hydrolysis to their component sugars by extensive repertoires of enzymes expressed by this microbial community. The identification of such enzymes from genomic data is therefore critical to understanding the mechanism by which complex carbohydrates can be used to manipulate the human microbiota through prebiotic and probiotic strategies. In this seminar I will show how structural biology, in harness with detailed biochemical analyses, can be exploited to provide a predictive biology platform designed to identify glycan degrading enzymes in the human microbiota[1]. The seminar will focus on the enzyme systems of the Bacteroidetes of the human microbiota that catalyze the degradation of dietary plant and microbial polysaccharides, exemplified by pectins[1], xylans and yeast mannans, and host glycans such as high mannose N-glycans[2]. The lecture will emphasise how the biochemical properties of both the enzymes and associated non-catalytic glycan binding proteins are adapted to ensure efficient deconstruction and utilization of these glycans[3]. Specifically, the lecture will illustrate how the enzymes on the surface of Bacteroidetes recognize only extended substrates, ensuring that the resultant oligosaccharides are efficiently inserted into the periplasm, where more active glycoside hydrolases complete the deconstruction process. The seminar will also demonstrate how the analysis of large polysaccharide utilization loci, which encode the glycan degrading enzymes[4], has shown how these organisms are adapted to metabolize extensive variations of core glycan structures.

SESSION 1

Use of *Faecalibacterium prausnitzii* to prevent and to treat gastrointestinal disorders and diseases

S Miquela*, R Martinab*, F Chaina, S Hudaulta, C Bridonneaua, J Lu[b], J Jury[b], JJ Gratadoux[a], S Blugeona, E Verdu[b], P Bercik[b], H Sokolacd ,LG Bermúdez-Humarána, M Thomasa and **P Langellaa.** aCommensal and Probiotics-Host Interactions Laboratory, UMR1319 Micalis, INRA, Jouy-en-Josas, France, [b]Farncombe Family Digestive Health Research Institute. McMaster University. 1200 Main St West, H.Sc. 3N6. Hamilton, Ontario, Canada. cERL INSERM U 1057/UMR7203, Faculté de Médecine Saint-Antoine,Université Pierre et Marie Curie (UPMC), Paris 6, France, dService de Gastroenterologie, Hôpital Saint-Antoine, AssistancePublique – Hôpitaux de Paris (APHP), Paris, France. * equal contributions

Diminished prevalence and abundance of Faecalibacterium prausnitzii has been reported in intestinal disorders as inflammatory bowel disease (IBD)1 and irritable bowel syndrom (IBS)2. F. prausnitzii is the first anti-inflammatory commensal bacterium identified on the basis of human clinical data and validated in acute high-dose TNBS colitis model3. Here, chronic low-grade inflammation, as observed in IBS patients, was first induced in conventional mice by performing two cycles of low-dose DNBS challenge. No significant difference was observed in classic inflammation parameters. However, serotonin levels, gut permeability and cytokines profiles were improved with in mice treated with *F. prausnitzii* or its

supernatant. Both *F. prausnitzii* and supernatant were recently tested in murine maternal separation models and preliminary results will be presented. We then designed a chronic IBD model by two cycles of high-dose DNBS-induced colitis. Interestingly, both *F. prausnitzii* and its supernatant had significant protective effects after colitis reactivation. To decipher the mechanisms involved in these anti-inflammatory effects, F. prausnitzii monoxenic mice were obtained using both intragastric and intrarectal administrations of F. prausnitzii (but only in 19% of treated mice). Escherichia coli, also involved in IBD dysbiosis, was co-inoculated with F. prausnitzii as a companion strain. After an adaptation period, a stable F. prausnitzii population level slightly lower than E. coli was observed. E. coli/F. prausnitzii dixenic mice and E. coli monoxenic mice were challenged by TNBS to induce acute colitis. In E. coli/F. prausnitzii dixenic mice Disease Activity Index (DAI), histological scores, MPO activity and cytokinic responses were significantly decreased. The highest DAI decrease was obtained in mice with the highest F. prausnitzii implantation (>108 CFU/g of faeces). These results confirm the high potential of *F. prausnitzii* as a potential probiotic for both IBS and IBD patients.

Lactobacillus reuteri 1E-1 alters expression of toll-like receptor signaling pathway genes in the IEC-6 rat intestinal epithelial cell line

M. Duersteler*, K. Smith, K. Novak, E. Galbraith, and E. Davis, *DuPont, Waukesha, WI, USA.*

Lactobacillus reuteri 1E-1 (previously identified as *Lactobacillus brevis*) is a direct-fed microbial that has been effective in improving growth performance, altering gastrointestinal microbiota, and influencing immunity in pigs. Interaction with toll-like receptors (TLR) on intestinal epithelial cells is a possible mode of action for these effects. The objective of this study was to determine the effect of *L. reuteri* 1E-1 on expression of TLR pathway signaling genes in the IEC-6 rat intestinal epithelial cell line. IEC-6 cells (3×10^5) were treated with *L. reuteri* 1E-1 (10^7 cfu) with and without LPS (10ng) challenge for one hour. Quantitative PCR was used to measure the fold change in expression of 18 genes associated with TLR2, TLR4, and TLR9 signaling cascades. *L. reuteri* 1E-1 down-regulated ($p<0.05$) expression of TLR9, CD14, MyD88, CASP8, IRAK4, TRAF6, MAPK9, TNF-a, and MIP-2, and up-regulated ($p<0.05$) expression of NF-kB relative to the untreated control. With an LPS challenge, *L. reuteri* 1E-1 down-regulated ($p<0.05$) expression of TLR2, TLR9, CD14, CASP8, SARM1, IRAK4, TRAF6, MAPK14, IRF7, and MIP-2 compared to LPS alone. The LPS challenge caused few differences in the effect of *L. reuteri* 1E-1. The fold change in expression of TNF-a, MIP-2 and MAPK9 was higher ($p<0.05$), and expression of TLR2 was lower ($p<0.05$) in cells treated with *L. reuteri* 1E-1 and LPS compared to *L. reuteri* 1E-1 alone. The down-regulation of TLR pathway signaling molecules and inflammatory cytokines by *L. reuteri* 1E-1 regardless of an LPS challenge indicate that the interaction between *L. reuteri* 1E-1 and TLRs on intestinal epithelial cells may result in reduced inflammation, which could be the mode of action for improved growth performance and alteration of gastrointestinal microbiota.

Ability of yeast and yeast derivatives to reduce attachment of pathogens and mycotoxins to gut mucosa

N Walker*[1], J Apajalahti[2], and P Wilcock[1], [1]*AB Vista, Marlborough, Wiltshire, UK*, [2]*Alimetrics, Espoo, Finland.*

Pathogens and mycotoxins can have a negative impact on animal health and performance. Several studies have demonstrated the potential of yeast and yeast derivatives to reduce the degree of attachment of pathogens and some mycotoxins to gut mucosa. Instead they are attached to the surface of the yeast and either cleared from the gut or their rate of absorption into the bloodstream slowed. The aim of the study was to test the capacity of a range of yeast and yeast derivatives to prevent pathogens and mycotoxins attaching to gut mucosa. Test products included 2 strains of *S. cerevisiae* (V and B), a yeast autolysate (P); an enzyme-hydrolysed yeast (EHY) and a commercial blend of yeast, yeast wall and clay (U).

Fresh pig gut mucosa was isolated, washed and irreversibly bound to micro-titre wells. To test pathogen attachment, 3 doses of each product were added in quadruplicate, with mannose included as a test. Radiolabelled cells of *S. typhimurium* and *E. coli* were added and incubated for 1h. The reaction mix was discarded, wells washed, scintillation liquid added and wells counted. Attached pathogens were expressed as a percentage of the control incubation. Statistical significance was analysed using Student's T-test. To test mycotoxin attachment, 2 doses of each product were tested in quadruplicate. Radiolabelled aflatoxin, zearalenone, ochratoxin and vomitoxin were added to the mix and incubated for 2h. Solids were removed by centrifugation and the amount of unattached mycotoxin was analysed in the supernatant by measuring radioactivity with liquid scintillation counting. Results showed that all of the products significantly ($P<0.001$) reduced the attachment of pathogens and mycotoxins in a dose dependent manner. The majority of the products were better than mannose against *E. coli*, especially EHY which was the most effective, indicating mannose is not necessarily the only factor within these products to affect *E coli*. Yeast strains V and B had different effects due to their glucan and mannan content. For prevention of aflatoxin attachment, the inclusion of clay in the commercial blend was the most effective choice. However, the clay seemed to increase Salmonella attachment. In conclusion, different yeast and yeast derivatives can have varying effects on preventing the attachment of pathogens and mycotoxins to gut mucosa.

Association of Intestinal and Systemic Inflammatory Responses with Microbial Bile Acid Deconjugation and Sulfidogenesis in High-Fat Fed Mice

F Carbonero*[1], W Shen[2], Z Zhou[2], M McIntosh[2], and HR Gaskins[1], [1]*University of Illinois, Urbana, IL, USA*, [2]*University of North Carolina-Greensboro, Greensboro, NC, USA.*

Recent studies in mice have demonstrated that consumption of a diet enriched in fat representative of a typical Western diet induces colonic inflammation potentially mediated by the microbiome. We tested the hypothesis that a high fat diet rich in saturated fatty acids stimulates sulfate and sulfite reducing bacteria (SRB) and 7 alpha-dehydroxylating bacteria (7ADB) producing respectively hydrogen sulfide and secondary bile acids, and that these increases correlate with inflammatory responses and disruption of gut barrier function. Forty, 3 wk-old C57BL/6J male mice were fed a low fat (LF: 10% of kcals; n=20) or high fat diet (HF: 60% of kcals; n=20) for 6 or 20 weeks. Mucosa and digesta samples were collected from the ileum, cecum and colon and used for microbial DNA extraction. Matching intestinal samples and visceral and subcutaneous white adipose tissue (WAT) depots were used to measure mRNA abundance for candidate inflammatory genes using qRT-PCR. Functional gene based and 16S rRNA gene qPCR assays were performed to quantify SRB (dissimilatory sulfite reductase (*dsrAB*) and 16S rRNA genes of four SRB genera), the taurine-degrading, sulfite-reducing *Bilophila wadsworthia* and 7ADB. Overall, the functional genes for SRB, *Bilophila wadsworthia* and 7ADB were significantly more abundant in samples from HF-fed mice after 20 weeks and this increase was detected for SRB and 7ADB after 6 weeks. More specifically, the increase was generally more marked in digesta samples. The most significant increases, including the genes cited previously and the SRB genera, were observed in cecal and colonic digesta as well as distal colonic mucosa but were not observed for proximal colon. The expression of markers of macrophage infiltration and inflammation were significantly more abundant in the ileum at 20 weeks. High fat feeding reduced the abundance of the tight junction protein, ZO-1, at the apical area of the ileal epithelium at 6 and 20 weeks. Genes encoding multiple markers of macrophage inflammation were also upregulated in visceral WAT after 6 and 20 weeks of high fat feeding. These data indicate that long-term consumption of a HF diet has a distinct effect on the composition and function of the intestinal microbiome and associated intestinal and systemic inflammation.

Central Metabolic Pathways in the Syntrophic Metabolizer, *Syntrophus aciditrophicus*

Huynh Le*[1], Xueyang Feng[2], Yinjie Tang[2], and Michael McInerney[1], [1]*University of Oklahoma, Norman, OK, USA*, [2]*Washington University, St. Louis, MO, USA.*

In many anaerobic ecosystems, the interaction amongst several microorganisms is imperative due to the thermodynamic limitations of key reactions during carbon metabolism, also known as syntrophy. The anaerobic metabolism of fatty and aromatic acids by *Syntrophus aciditrophicus* (SB) grown in coculture with *Methanospirillum hungatei* (JF1), a hydrogen- and formate-using methanogen, provides a model to study syntrophic cooperation. [^{13}C]-assisted pathway analyses were conducted to elucidate the enzyme systems involved in the central metabolism and key biosynthesis steps in SB. The isotopomer labeling patterns in proteinogenic amino acids were determined by gas chromatography-mass spectroscopy. Replicate cultures were grown with [1-^{13}C]-acetate and [1-^{13}C]-sodium bicarbonate under each of the following conditions: SB alone on crotonate, SB and JF1 on crotonate, and SB and JF1 on benzoate. Labeled carbons were detected in all amino acids, indicating a global utilization of acetate and bicarbonate as carbon sources. [^{13}C]-assisted pathway analysis of SB indicated that pyruvate was synthesized from acetyl-CoA via pyruvate carboxylase and that oxaloacetate was synthesized by carboxylation of pyruvate. Similar labeling patterns in leucine and isoleucine in cells grown with [1-^{13}C]-acetate and [1-^{13}C]-sodium bicarbonate with unlabeled crotonate suggested the use of the citramalate pathway for leucine and isoleucine. Isotopomer labeling patterns also suggest the use of the 4-hydroxybutyrate pathway for glutamate synthesis. Constitutively expressed proteins homologous to a (*Re*)-citrate synthase in *Clostridium kluyveri* and a citramalate synthase in *Geobacter sulfurreducens* were detected when SB was grown with crotonate, benzoate, and cyclohexane carboxylate as electron donors. [^{13}C]-assisted pathway analysis showed the importance of acetate and bicarbonate as carbon sources, the use of an alternate pathway for leucine and isoleucine biosynthesis, and a novel route for glutamate biosynthesis.

Efficient method of mRNA enrichment to study the rumen metatranscriptome

Sophie Comtet*[1,2], Frédérique Chaucheyras-Durand[1,4], Pascale Mosoni[1], Jérémie Denonfoux[2], Corinne Petit-Biderre[3], Evelyne Forano[1], and Pierre Peyret[2], [1]*INRA, Saint-Genès-Champanelle, France*, [2]*Université d'Auvergne, Clermont-Ferrand, France*, [3]*Université Blaise Pascal, Clermont-Ferrand, France*, [4]*Lallemand Animal Nutrition, Blagnac, France.*

The rumen is inhabited by a complex community of prokaryotic and eukaryotic microorganisms which ensures essential functions for the ruminant. Global transcriptomic studies such as functional microarrays or RNA-Seq represent suitable approaches to investigate microbial functions in the rumen. However, the high proportion of ribosomal RNAs (rRNAs) in total RNAs (95 to 99%) can hinder the detection of low abundant transcripts. Therefore, enrichment of messenger RNAs (mRNAs) is necessary to increase the sensitivity of DNA microarrays and to avoid producing unused sequences by next generation sequencing. The aim of this work was to develop an efficient method dedicated to rumen samples for the subtraction of rRNAs from total RNAs samples. The method is based on the use of magnetic beads and probes of forty-three bases allowing the capture of rRNA molecules. Probes were designed to target all rumen microorganisms already identified, including bacteria, archaea, protozoa and fungi. In this way, a database dedicated to rumen ecosystem was constructed with rRNA sequences (16S, 18S, 23S and 28S) of good quality from the SILVA database. Ribosomal RNA sequences from the same genus were aligned and iterative alignment of genus alignments was performed for each kingdom. Probes were determined in conserved regions highlighted by the Clustal W alignments; this permitted to reduce capture probes complexity and to improve rRNA removal efficiency. Overall coverage of probes was evaluated on the SILVA database and specificity was checked with HiSpOD in order to avoid mRNA withdrawal. The *in silico* strategy enabled the design of seventeen probes targeting all rumen microbes. The pool of capture probes is currently being implemented to remove also plant rRNAs which may be present in the rumen of

grazing animals. This method of mRNA enrichment is now applied to analyze the effect of abiotic or biotic factors on targeted functions in the rumen and could easily be adapted to other ecosystems.

SESSION 2

How does host genomic variation translate into gut microbiome composition?
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The assemblage of microbes in the vertebrate gut behaves like a complex trait, subject to a wide array of environmental influences as well as genetic variation among the host population. Host genomic loci that influence microbiome composition have been identified in Quantitative Trait Locus (QTL) mapping studies in mouse models, showing that at least a portion of the variation in microbiome composition can be attributed to variation at specific loci in host genome. Studies to date have used individual taxa or taxonomically related groups of taxa as "traits" though these approaches consistently identify pleiotropic loci that influence multiple taxa, suggesting that host genetic variation exerts a broad effect on microbiome composition through effects on groups of taxa that have or contribute unique functionality. A combination of high-throughput shotgun metagenome sequencing and sophisticated resource populations of recombinant-inbred and outcross mouse lines are now being used to determine if host genetic architecture enriches simply for taxa or functionally important sets of genes/pathways represented in groups of taxa. Separate studies that incorporate dietary variables in an intercross model were also recently completed and identified eight QTLs that show gene X diet interactions. The fact that diet can modify the effects of some genetic loci on microbiome composition underscores the need to study microbiome composition and assembly in light of the complex combinations of genetic architecture and environmental factors that together shape these climax communities.

Insights into the Bovine Rumen Plasmidome
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Plasmids are self-replicating genetic elements capable of mobilization between different hosts. Plasmids often serve as mediators of lateral gene transfer, a process considered to be a strong and sculpting evolutionary force in microbial environments. Our aim was to characterize the overall plasmid population in the environment of the bovine rumen, which houses a complex and dense microbiota that holds enormous significance for humans. We developed a procedure for the isolation of total rumen plasmid DNA, termed rumen plasmidome, and subjected it to deep sequencing using the Illumina paired-end protocol and analysis using public and custom-made bioinformatics tools. A large number of plasmidome contigs aligned with plasmids of rumen bacteria isolated from different locations and at various time points, suggesting that not only the bacterial taxa, but also their plasmids, are defined by the ecological niche. The bacterial phylum distribution of the plasmidome was different from that of the rumen bacterial taxa. Nevertheless, both shared a dominance of the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Evidently, the rumen plasmidome is of a highly mosaic nature that can cross phyla. Interestingly, when we compared the functional profile of the rumen plasmidome to two plasmid databases and two recently published rumen metagenomes, it became apparent that the rumen plasmidome codes for functions, which are enriched in the rumen ecological niche and could confer advantages to their hosts, suggesting that the functional profiles of mobile genetic elements are associated with their environment, as has been previously implied for viruses.

Comparative transcriptomic analysis of *Ruminococcus albus* strains 7 and 8 grown on cellulose and hemicellulose

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Ruminococcus albus is known as a specialist bacterium for the degradation of both cellulose and hemicellulose in the rumen. However, depending on strain, their phenotypic and fibrolytic abilities are variable and no information is available on a comparison of genomic and transcriptional differences between strains of *R. albus* that underly this phenotypic variation. The regulation of genes involved in polysaccharide degradation in both strains grown on either cellobiose, phosphoric acid-swollen cellulose (PASC), or wheat arabinoxylan (WAX) were compared using RNA sequencing approaches. Both strains of *R. albus* demonstrated similar growth rates, substrate degradation, and accumulation of fermentation end-products when grown on cellobiose, PASC, and WAX. However, examination of the transcriptional profiles from RNA-seq analysis showed distinct patterns between both strains. During the growth with PASC relative to cellobiose, *R. albus* 7 had more up-regulated genes than *R. albus* 8. Based on annotation using NCBI, CAZy, and Pfam databases, putative glycoside hydrolase (GH) genes of total up-regulated genes in *R. albus* 7 were higher than ones in *R. albus* 8. We observed that *R. albus* 7 had a higher cell yield and fermentation end-products than *R. albus* 8 when grown on PASC. In contrast, when grown on WAX relative to cellobiose, both strains had different transcriptional profiles from ones grown on PASC. *R. albus* 8 had more up-regulated genes and putative GH genes than *R. albus* 7. It was also shown that *R. albus* 8 had more highly induced genes than *R. albus* 7 when comparing transcription profiles on WAX with PASC. This transcriptional profile suggests that *R. albus* 8 has a higher potential to degrade hemicellulose in the plant cell wall. This study provides critical insight into the degradation of cellulose and hemicellulose based on analysis of genetic and transcriptional differences between the two *R. albus* strains.

Proteomic analysis of the *Eubacterium rectale* membrane reveals high affinity nutrient receptors

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The butyrate-producing Firmicute *Eubacterium rectale* is a prominent member of the healthy human gut microbial community, and tends to be found in lower abundance in individuals suffering from inflammatory bowel diseases. The abundance of this organism in the human gut microbiota is increased when the host consumes a diet rich in resistant starch; however, recent *in vitro* studies have demonstrated that *E. rectale* cannot grow well on resistant starch but grows quickly when a primary starch degrader is included. This suggests that *E rectale* is adept at scavenging partially degraded or solubilized starch. In order to determine what proteins contribute to this ability, we performed a proteomic analysis of the cell wall/membrane of *E. rectale* grown on two different starches as well as glucose. One of the most abundant proteins during growth on starch was Eur_01830. Eur_01830 is expressed from a putative operon encoding an ABC transporter as well as a predicted alpha-amylase Eur_01860. We performed a biochemical and structural analysis of Eur_01830 revealing it is a high-affinity maltoligosaccharide-binding protein as part of an ABC transporter. This protein has little detectable binding to maltose, but binds maltooligosaccharides, including the glucoamylase inhibitor acarbose, with high affinity ($K_a \sim 10^6$). Transcriptional analysis of the genes encoding Eur_01830, Eur_01860 and Eur_21100, an abundant 150kDa cell anchored GH13 enzyme, reveal specific induction of these proteins on both maltose and starch, and repression during growth on wheat arabinoxylan. These proteins are likely responsible for *E. rectale*'s ability to competitively scavenge starch in the human intestinal tract, and future work is directed at better understanding the molecular details of this response. This study is the first molecular level analysis of this important human gut symbiont.

Comparative Genomics of Rumen Bacteria *Selenomonas ruminantium* subsp. *ruminantium* and *Selenomonas ruminantium* subsp. *lactilytica*

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Selenomonas ruminantium is one of the most numerically dominant and metabolically versatile bacteria in the rumen. As a significant producer of propionate, *S. ruminantium* strains are extremely important for the nutrition of beef and dairy cattle. Propionate is principally produced by decarboxylation of succinate, but can also be produced by fermentation of carbohydrates, including glucose, cellobiose, xylose, and arabinose released by fibrolytic bacteria. Certain strains of *S. ruminantium* can also ferment mannitol, glycerol, or lactate, with the latter activity used to designate subspecies. To better understand the role of selenomonads in propionate production and lactate turnover, draft genomes of *S. ruminantium* subsp. *ruminantium* GA192 and *S. ruminantium* subsp. *lactilytica* HD4 were sequenced and assembled *de novo*. The GA192 draft genome is 3.5 mb total with 65 gaps on three scaffolds, while the HD4 draft genome is 3.18 mb total with 57 gaps on three scaffolds. These genome sizes are slightly smaller than that of *S. ruminantium* subsp. *lactilytica* TAM6421, the only other sequenced member of this species, but the differences in size can be attributed to plasmid diversity between the strains. Some synteny is observed between all three strains, more so between the *lactilytica* subspecies, HD4 and TAM6421, and the overall functional genome composition between the three strains is similar. However, the HD4 and TAM6421 genomes contain putative cytochrome-c dependent L-lactate dehydrogenases (EC 1.1.2.3) that are likely the genetic basis for lactate utilization, and these sequences were not found in either the assembled sequence or raw reads from GA192. As the cycling of lactate greatly affects ruminant health, understanding microbial production and fermentation of lactate could have significant impacts. The genome sequences further reveal the metabolic potential of *S. ruminantium,* with genes enabling the use of numerous monosaccharides, organic acids, and sugar alcohols, as well as confirmation that production of propionate occurs through the methylmalonyl pathway. The results from this work will help to provide a more robust classification for the genus, and, more importantly, determine the metabolic roles of *S. ruminantium* in the rumen.

Genome sequencing of rumen archaea: *Methanobrevibacter* sp. AbM4 genome sequence

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Our group is sequencing the genomes of cultured representatives of rumen methanogens to define their conserved features, and to understand their role in the rumen environment for the purpose of developing methane mitigation technologies for ruminant livestock. Here, we present the genome sequence of *Methanobrevibacter* sp. AbM4, isolated and purified from the abomasum of a sheep. A phylogenetic analysis of the AbM4 small subunit ribosomal RNA gene sequence places it within the *Methanobrevibacter wolinii* species clade. Although, AbM4 is not believed to constitute large methanogen populations, it is widely distributed in ruminant species under different rumen and gut conditions. The AbM4 genome sequence is smaller than the published rumen methanogen *Methanobrevibacter ruminantium* M1 (1.99 Mb versus 2.93 Mb) and consequently encodes fewer open reading frames (1,671 versus 2,217). Overall, the composition of the AbM4 genome is very similar to that of M1 suggesting that the methanogenesis pathway and central metabolism of these strains are highly similar, and both organisms are likely to be amenable to inhibition by small molecule inhibitors and vaccine-based methane mitigation technologies targeting these conserved features. Unlike M1, AbM4 has a much reduced complement of predicted adhesin-like proteins (29 versus 105) indicating that AbM4 invests less of its genetic resources on external interactions with its environment. AbM4's better repertoire of cofactor and coenzyme biosynthetic genes also indicates it is likely to be less dependent on

other rumen microbes for the supply of cofactors for growth and survival in the rumen. These features suggest that AbM4 occupies a ruminal niche slightly different from that of M1.

Remarkable similarity among bacteria isolated from 4 hosts after 8 week enrichments of feces with cellulose and xylan/pectin

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The intestinal microbiota allows mammals to recover energy stored in plant biomass through fermentation of plant cell walls, primarily cellulose and hemicellulose. Bacteria were isolated from 8 week continuous culture enrichments with cellulose and xylan/pectin from cow (n=4), goat (n=4), human (n=4), and pig (n=6) feces. 16S rRNA genes were sequenced (ISU DNA Sequencing and Synthesis Facility) and analyzed using Bionumerics software. Bacteria isolated from the same fermenter with ≥ 99% 16S rRNA gene sequence similarity were removed in order to reduce redundancy and simplify data set; 462 cow, 363 goat, 255 human, and 575 pig isolates were included in the analyses. Of the cow and goat isolates, ~50% were < 95% and ~30% were > 95% but < 97% similar to cultured bacteria in the RDP-II, indicating limited available information on large intestinal microbiota in these ruminants. Although culture methods introduce some bias, bacteria isolated across hosts were remarkably similar in both numbers and species. *Firmicutes* and *Bacteroidetes* were the dominant phyla represented and made up 52.2% and 30.7% in cow, 43.5% and 34.2% in goat, 42.8% and 34.9% in human, and 41.9% and 32.0% in pig isolates. Other phyla represented included *Proteobacteria* (11.3% cow, 9.6% goat, 21.2% human, and 11.3% pig), *Actinobacteria* (3.5% cow, 4.7% goat, 1.2% human, and 4.0% pig), *Fusobacteria* (1.1% cow, 8.26% goat, and 9.6% pig), and *Synergistetes* (1.5% cow and 0.9% pig). While the number of isolates recovered from each carbohydrate source was similar, the distribution of isolates within phyla differed by carbohydrate type. Phylogenetic clustering by host, at the level of bacterial species, was not common. The greatest bacterial species differentiation by host was found in the *Firmicutes*. Future research will compare enriched microbiota metagenomes to isolations. These results indicate that members of distal large intestinal microbial communities have been conserved, by bacterial species, across mammals.

SESSION 3

Role of Dietary Oligosaccharides in Gastrointestinal Development and Host Defense in the Piglet Model

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Gastrointestinal diseases are a major source of morbidity and mortality in human infants and neonatal swine. Human milk and sow milk confer protection against diarrheal diseases, including rotavirus (RV). Both human and porcine milk contain oligosaccharides, which inhibit RV binding to enterocytes and promote the growth of beneficial gut bacteria *in vitro*. Herein, the role of human contain oligosaccharides (HMO) in the prevention of RV infection was evaluated in piglets. Colostrum-deprived piglets were fed with formula alone (FF), or formula containing 4 g/L HMO (40% 2'-fucosyllactose, 35% lacto-*N*-neotetraose, 10% 6'-sialylactose, 5% 3'-sialylactose and 10% free sialic acid) or prebiotics (PRE) (9:1 of short-chain galactooligosaccharides and long-chain fructooligosaccharides) for 15 days. At d10, half of the piglets were infected with $5x10^6$ FFU of group A porcine RV strain OSU. Stool consistency was monitored 3-times daily. Serum, small intestinal tissue and colonic contents were collected at 5 d post-infection (PI). Serum RV-specific IgG and IgM were measured by ELISA. The mRNA expression of RV NSP4, a marker of RV replication, was analyzed by RT-qPCR. Microbial composition of ascending colonic contents was analyzed by 454 pyrosequencing of the v1-v3 region of the 16S rRNA gene. The onset of diarrhea occurred at 36.3±1.83h PI in all infected piglets independent of diet, however, the duration of diarrhea was shorter (p=0.038) in HMO (48.8±9.8h) and PRE (53.1±11.1h) compared to FF

(80.6±4.5h). Serum RV-specific IgG and IgM were increased in infected piglets. PRE (p=0.001) and HMO (p=0.07) groups had higher concentrations of RV- IgM than FF. Mucosal RV NSP4 mRNA was significantly increased in the infected piglets, with no effect of diet. Ascending colonic microbiota was significantly different among infection and diet groups. The amount of *Bacteroidaceae* was significantly increased in the infected groups. HMO increased the amount of *Lachnospiraceae*, which contains numerous butyrate-producing bacteria. In summary, HMO and prebiotics did not prevent the RV infection, but did reduce the duration of diarrhea in piglets, possibly in part by promoting immunoglobulin response to RV infection and modulating the gut microbiota.

Effect of Dietary Genistein on Ovalbumin-induced Allergic Reactions in a Mouse Model of Food Allergic Diarrhea

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Food allergy has become increasingly prevalent over the past decades, but no effective treatment is yet available to cure food allergy. In this study, we evaluated the effect of genistein, an anti-inflammatory isoflavone, in a mouse model of ovalbumin (OVA)-induced allergy. Thirty two female BALB/c mice were randomly allocated to four groups: 1) sham-sensitized normal group, 2) OVA-sensitized control group fed with basal genistein-free diet, and the other two OVA-sensitized groups fed with basal diet supplemented with 3) 10 mg/kg and 4) 100 mg/kg of genistein respectively. Mice were systemically sensitized twice with OVA, followed by oral intubation of OVA to develop allergic diarrhea. Diarrheal incidence was lower in mice supplemented with 10 mg/kg genistein, but was higher in the 100 mg/kg genistein group as compared to the OVA-sensitized control group. Supplementation of 10 mg/kg genistein significantly increased the mRNA expression of IFN-γ and reduced that of IL13 in jejunum, whereas supplementation of 100 mg/kg genistein had no effect on the mRNA expression. Furthermore, the number of mast cells in the jejunum and colon was greatly reduced in mice fed with 10 mg/kg genistein, but significantly improved in those fed 100 mg/kg genistein as compared to the OVA-sensitized control group. However, IgE and OVA-specific IgG1 responses were not affected by genistein supplementation. In conclusion, genistein has both anti-allergy and pro-allergy effects, depending on its dose, which may act through interfering with the infiltration of intestinal mast cells of mice. The use of genistein at appropriate dosage may have positive effects for the treatment of OVA-induced allergic diarrhea.

Impact of maternal gastrointestinal microbiota on the gastrointestinal microbiota of their infants

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The initial colonization of the sterile gut of newborns and subsequent temporal succession of the gastrointestinal (GI) microbiota are affected by many factors. In this study, we examined the impact of maternal gut microbiota and feeding on the intestinal microbiome of infants by examining co-existence of bacteria between the maternal and her infant's GI microbiota. This study involved two groups of mother-infant dyads, which were recruited before babies were born: one group consisting of 9 mothers and their exclusively breast-fed infants, while the other group having 10 mothers and their exclusively formula-fed infants. Fresh fecal samples were obtained from the mothers within 5 days after the infant's birth, and from the infants at 15 and 28 days of age. Metagenomic DNA was extracted from the fecal samples and amplicons of 16S rRNA (the V1-V3 region) were pyrosequenced using a 454 GS FLX Titanium system. The sequences were analyzed using Qiime. In general, babies at the age of 28 days have more shared OTUs with their mothers than at the age of 15 days. Some breast-fed babies and their mothers appeared to share more OTUs than formula-fed babies and their mothers. Some species of *Escherichia/Shigella*, *Streptococcus* and *Bacteroides* appeared to have more frequent co-occurrence between mothers and their

babies than other taxa. This preliminary study revealed possible sharing of bacteria between mother and infants, providing a better understanding of the factors that can affect the development of intestinal microbiome in the infants.

Immune Development Characterization of Gastrointestinal Tissue from High and Low Gaining Steers Under Feedlot Management Conditions

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Economical production of beef in commercial feedlot settings employs practices that can place a large burden of stress on cattle when the animal's immune system is still rapidly developing. Physical differences between low gaining and high gaining steers may have to do in part with the development of the innate and acquired branches of the immune system and the system's response to stress. The objective of this study was to evaluate immunological differences between low gaining and high gaining beef steers fed a common diet. Three pens, each containing 64 cattle exhibiting similar genetics such as body frame, size and breed were fed a typical commercial feedlot diet. Average daily gain was determined for feeding periods 1 (day 0-47) and 2 (day 47-111). Six steers with the highest and lowest average daily gains were chosen at the conclusion of each period for sampling (n=24), and jejunal intraepithelial lymphocytes were isolated. Populations of double positive T cells ($CD4^+CD8^+$),T helper memory lymphocytes ($CD4^+CD45RO^+$), and the T helper L-selectin (CD4+CD62L+) population were greater ($P \leq 0.05$) in high gaining steers. These populations are associated with memory and may indicate maturity in immune development of the high gaining group. The proportions of cytotoxic T cell populations ($CD4^-CD8^+$, $CD8^+CD45R^-$, $CD8^+CD45RO^-$) of low gaining steers were higher ($P \leq 0.05$) at the earlier sampling time and decreased by the later sampling time (finishing period), whereas the proportion of the same cytotoxic T cell populations remained stable over both sampling times in high gaining steers. This suggests the gastrointestinal immune system of high gaining cattle maintained homeostasis when faced with the challenges of a stressful feedlot environment, whereas these changes elicit an immune response in low gaining cattle. The same pattern of response was observed for the macrophage population ($CD172a^+MHCII^-$) and indicates the innate immune system also responds to the disruption of intestinal immune homeostasis observed in low gaining cattle during feedlot management. Although it is unknown how these differences in immune development manifested, it is likely dictated by the immune system response to microbial colonization in the gastrointestinal environment of the developing calf.

Effect of galacto-oligosaccharide supplementation on the microbial community composition of the rumen and colon of pre-weaning milk-fed Holstein calves

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Average mortality of pre-weaning dairy calves is ~8%, and ~50% of it is due to intestinal illness, mostly infectious diarrhea. Strategies to improve intestinal health during this period are required. Non-digestible oligosaccharides may function as prebiotics that modulate growth and activity of beneficial microbial populations leading to enhanced gut health. Galacto-oligosaccharides (GOS) have demonstrated such prebiotic potential. The objective of this experiment was to assess the effect of GOS supplementation on the composition of microbial communities in the rumen and colon of pre-weaning milk-fed Holstein calves. To this end, bar coded pyrosequencing of 16S rDNA was used. Thirty two newborn Holstein calves were randomly assigned to either a control or a GOS supplemented diet. Supplemented calves received ~ 0.7 g of GOS/day/kg of body weight. After 2 and 4 weeks of age, 8 calves per treatment were euthanized and digesta samples were collected from rumen and colon. Analysis of similarities (ANOSIM) revealed no differences in microbial community composition of the rumen (R=-0.004, P=0.46) and only minor differences in the colon (R=0.13, P=0.007) between GOS supplemented and non-supplemented calves. Accordingly, permutational analysis of variance (PERMANOVA) showed a statistical but

seemingly minor microbial compositional difference that took place in the colon but not the rumen of the calves (P=0.04). The interaction between prebiotic treatment and age was not significant. Bacterial species richness, as measured by the Chao1 Index, also tended to be higher for GOS supplemented calves compared to control (P=0.06) across rumen and colon. The weak dissimilarities in the colonic microbiota of GOS supplemented and non-supplemented calves were caused largely by greater abundance of *Lactobacillus Johnsonii* (12% of the dissimilarity) in control calves , whereas *Bifidobacterium* spp., *Lactobacillus reuteri* and *L. salivarus* were more abundant in GOS animals, contributing 2.8, 1.52 and 3.8 % of the dissimilarities each. Overall, GOS supplementation of pre-weaning Holstein calves exerted a limited prebiotic effect by inducing only small differences in the microbiota composition and richness of the large bowel of these animals, whereas the rumen microbial communities remained unaffected.

The Western Lowland Gorilla (*G. gorilla gorilla*) Gastrointestinal Microbiome Sheds Light on their Overall Ecology and Conservation

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An explosion of recent research has shown how the gastrointestinal microbiome (GIM) of non-human primates is shaped by several intrinsic and extrinsic factors, including host phylogeny and diet. Nonetheless, the implementation of a macro-ecological framework within which patterns of wild non-human primate GIMs can be justified is often overlooked. Here, we use a polyphasic approach, combining high-throughput sequencing of bacterial 16S rDNA, short chain fatty acid profiling and characterization of hydrogen disposal mechanisms in fecal samples (n=38) to describe the distal GIM of different groups of Western Lowland Gorillas (WLGs) at The Dzanga Sangha protected area (DSPA) in the Central African Republic. Results suggest uniqueness of the GIM composition of WLGs at DSPA compared to those reported before for other gorillas or non-human primates, reflecting dominance of lactic acid bacteria and members of the phylum Chloroflexi, while implying that their GIM and its encoded functions may be mainly modulated by a set of intertwined ecological forces that include the degree of relatedness among individuals, their foraging behavior and possibly the levels of anthropogenic disturbance in the form of habituation to human presence. Our findings support the hypothesis that the GIM is a key force modulating energy harvest mechanisms, overall physiology and health of WLGs, and places the GIM as an indicator of the ecological success and conservation of wild primates in a changing, potentially threatened niche.

SESSION 4

Control of Food Intake By Metabolism Of Fuels - A Comparison Across Species

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Animal models have been invaluable for studying aspects of food intake regulation that for various reasons cannot be observed in humans. Research with laboratory species suggests that meals can be terminated by peripheral signals carried to brain feeding centers via hepatic vagal afferents, and that these signals are affected by oxidation of fuels. Pre-gastric fermentation in ruminants greatly alters fuels, allowing mechanisms conserved across species to be studied with different types and temporal absorption of fuels. These fuels include short-chain fatty acids (FA), glucose, lactate, amino acids, and long-chain FA isomers, all of which are absorbed and metabolized by different tissues at different rates. Propionate, produced by ruminal fermentation of starch, is absorbed within the timeframe of meals and is quickly

cleared and metabolized by the liver. Propionate is utilized for gluconeogenesis or oxidized in the liver, and hypophagic effects of propionate are related to hepatic oxidation. Propionate also stimulates oxidation of acetyl CoA by anaplerosis. Fatty acids are a primary fuel oxidized in the liver across species and inhibition of fatty acid oxidation increases food intake, whereas promotion of FA oxidation suppresses food intake. Acetate is the primary short-chain FA produced in the rumen and has little effect on food intake, likely because uptake of acetate from the blood by ruminant liver is negligible. Glucose is hypophagic in nonruminants but not ruminants and unlike non-ruminant species, uptake of glucose by ruminant liver is negligible, consistent with differences in hypophagic effects between them. In addition to species differences, hypophagic effects of fuel oxidation vary with changes in metabolic state. For example, propionate is less hypophagic for cows with high glucose demand, likely because of a delay in oxidation during the timeframe of meals. Depression of food intake of cows in the peripartum period is likely caused by hepatic oxidation of non-esterified fatty acids mobilized from body reserves. Hypophagic effects of propionate are enhanced for these cows because propionate stimulates oxidation of acetyl CoA produced by beta-oxidation of non-esterified fatty acids in the liver. This presentation will address control of food intake by hepatic oxidation of fuels across species.

Animal-specific recovery patterns of rumen bacterial community after rumen content exchange

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The purpose of this study was to examine the adaptation of the rumen bacterial community after being transferred to a new host. Steers with similar body weight were paired (Pair 1: 67 and 89; Pair 2: 481 and 483) and the whole rumen contents were exchanged between steers in the same pair. The rumen content was collected before the swap (d0) and at three different time points (d1, d7, and d28) after the swap. The ruminal bacterial community composition was evaluated using pyrosequencing of the partial bacterial 16S rRNA gene, and the bacteria abundance was indicated by the copy number of the 16S rRNA gene. In Pair 1, both steers displayed similar fluctuation patterns of the bacterial community: the species richness and abundance reduced significantly at d1, and gradually recovered to their original level at d28 for both Steer67 (data of the four time points respectively: estimated operational taxonomic units (OTUs) = 1978, 1257, 1382; 2266; abundance (copies/g rumen contents) = 4.4×10^{11}, 4.6×10^{9}, 1.2×10^{11}, 1.5×10^{12}) and Steer 89 (estimated OTUs = 2647, 2321, 3002, 2551; abundance (copies/g rumen contents) = 3.5×10^{11}, 1.6×10^{11}, 9.5×10^{10}, 1.1×10^{12}). In Pair 2, the bacterial community of the two steers showed varied adaptation patterns after the swap. For Steer481, the bacterial species richness reduced significantly after rumen content exchange at d1 and did not recover at d7 and d28 (estimated OTUs = 2471, 1982, 2019, 2009); the bacterial abundance continuously increased after swap (abundance (copies/g rumen contents) =7.2×10^{10}, 1.8×10^{11}, 1.6×10^{11}, 3.4×10^{11}). For Steer483, both species richness and abundance transiently reduced at d1 and returned to its primary level at d28 (estimated OTUs = 2523, 1961, 3039, 2568; abundance (copies/g rumen contents) =1.8×10^{12}, 2.9×10^{11}, 5.4×10^{10}, 1.1×10^{12}). Our results suggest that the microbial community gradually reestablished, but the extent and pace of recovery differed significantly among individuals.

Increasing Levels of Dietary Wet Distillers' Grains plus Solubles Effects on Rumen Bacterial Population Dynamics

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In a 6x6 latin square with repeated measures, 6 ruminally cannulated feedlot steers were fed 6 finishing diets with dry rolled (DRC), steam flaked corn (SFC), or SFC replaced by 15, 30, 45 and 60% wet distillers' grains plus solubles (WDGS). Rumen samples collected at 0 and 4 h post feeding on d 21 of each period separated into fluid and solid fractions (FRAC) and archived. Genomic DNA was extracted for amplification of V4 region of 16S rDNA gene and sequenced utilizing a Roche 454 FLX platform. Phylogenetic distribution of taxa, multivariate non-metric multidimensional scaling (NMDS) and principle components analyses were employed to compare community populations and Proc MIXED repeated measures models were used to determine the influence of diet, time, FRAC and their interactions on individual rumen bacterial taxa. Different rumen bacterial communities exhibited clearly differentiated responses to diet ($P < 0.05$) and FRAC within diet ($P < 0.01$), time ($P > 0.05$) and the associated interactions. *Firmicutes* dominated and *Actinobacteria* were prevalent in solid FRAC whereas *Firmicutes* and *Proteobacteria* co-dominated liquid FRAC. *Bacteriodetes* constituted <5% of all communities and were more (P<0.05) abundant in the liquid than solid FRAC. Quantitative differences ($P < 0.05$) in *Firmicutes*, *Proteobacteria*, *Bacteriodetes*, *Synergistetes,* and *Cyanobacteria* existed among diets. The family *Lachnospiracea* that dominated the solid FRAC (~40%) were less (P<0.01) abundant in liquid FRAC (~18%). *Rumincocacceae*, increased with all levels of WDGS. Conversely, *Succinivibronaceae* decreased with increasing dietary WDGS. *Veillonellaceae*, exhibited a quadratic ($P < 0.05$) WDGS level response by decreasing above 30% WDGS. Among predominant genera, *Shuttleworthia*, *Dialister*, and *Marvinbryantia* decreased (P<0.05) with increasing levels of dietary WDGS. In contrast, *Ruminococcus*, *Succiniclasticum*, *Sporobacter*, *Anaerovorax*, and *Moryella* abundance increased (P<0.05) with increasing levels of WDGS. *Desulfobulbus* was unique to WDGS diets. In summary, decreased animal performance observed with increasing WDGS inclusion in SFC diets is correlated with changes in associated bacterial populations.

***Saccharomyces cerevisiae* fermentation product stabilized rumen microbial communities of lactating dairy cows during subacute ruminal acidosis**

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It has been suggested that *Saccharomyces cerevisiae* fermentation product (SCFP) prevents subacute ruminal acidosis (SARA) in dairy cows and is hypothesized to stabilize microbial communities in the rumen and hindgut. Eight rumen- and cecum-fistulated lactating dairy cows were used in a crossover experiment. Experimental periods consisted of 4 wk of normal feeding followed by a 1 wk grain-based SARA challenge. During normal feeding, the diet contained 42% concentrate, 28% corn silage and 30% alfalfa baleage (DM basis). During the SARA challenge, 22% corn silage in the diet was replaced with wheat/barley pellets. SCFP cows were supplemented with 14 g/d of Original XPC (Diamond V) mixed with 140 g/d ground corn, and control cows with 140 g/d ground corn only. During wk 4 and 5 of each period, rumen fluid, cecal digesta and feces were sampled. Genomic DNA was pyrosequenced using the bacterial tag-encoded GS FLX-Titanium amplicon that targeted the v1 to v3 regions of 16S rRNA. In the rumen, SARA reduced ($P < 0.05$) bacterial phylogenetic diversity and increased ($P < 0.05$) the relative abundance of Firmicutes (37.0% vs. 49.2%) and reduced ($P < 0.01$) that of Bacteroidetes (37.9% vs. 27.3%). Additionally, SARA increased ($P < 0.01$) the relative abundance of Proteobacteria (1.4% vs. 2.2%) and decreased ($P < 0.01$) that of Spirochaetes (2.1% vs. 1.2%) and Tenericutes (2.4% vs. 1.7%). Supplemental SCFP prevented the reduction ($P < 0.05$) in bacterial phylogenetic diversity and prevented the increase ($P < 0.05$) in the ratio between Firmicutes and Bacteroidetes caused by SARA. During SARA, SCFP increased ($P < 0.05$) the populations of *Treponema*, *Prevotella*, *Bacteroides*, and *YRC22* in the rumen. *Prevotella* and *Bacteroides* include many of the bacteria that are essential for carbohydrate fermentation and beneficial to rumen function. During SARA challenge, SCFP increased ($P < 0.05$) the populations of *Desulfovibrio, Moryella, Butyrivibrio, Lactobacillus* and *Coprococcus* in feces, and decreased ($P < 0.05$) those of *Mogibacterium*, and *Ruminococcus* in the cecum and feces, and decreased that of *Anaerostipes, Paraprevotella*, and *Mogibacterium* in feces. Results suggest that during SARA,

SCFP stabilizes rumen microbial communities and influences microbial populations in the cecum and feces.

Mixed Rumen Microbes Respond to Excess Carbohydrate by Synthesizing Reserve Carbohydrate and Spilling Energy

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We determined if a mixed microbial community from the bovine rumen would respond to excess carbohydrate by accumulating reserve carbohydrate, spilling energy, or both. We washed mixed microbes from the rumen with N-free buffer and dosed them with glucose. We measured heat production and partitioned it across (i) endogenous metabolism (heat production before dosing glucose), (ii) synthesis of reserve carbohydrate (from enthalpy of relevant ATP-yielding and ATP-utilizing reactions), and (iii) energy spilling [total heat production minus (i) and (ii)]. For cells dosed with 5 m*M* glucose, synthesis of reserve carbohydrate and endogenous metabolism explained nearly all heat production (94.1%); no spilling was detected ($P = 0.254$). For cells dosed with 20 m*M* glucose, energy spilling was not detected immediately after dosing, but it became significant ($P < 0.05$) by approximately 3000 s. Energy spilling accounted for as much as 38.3% of heat production in one incubation. Energy recovery (97.6%) and carbon recovery (99.9%) were complete, providing evidence that spilling was not a methodological artifact of incompletely measuring reserve carbohydrate. As documented for some pure cultures, we confirmed mixed microbial communities from the rumen can respond to large excesses of carbohydrate by spilling energy, although synthesis of reserve carbohydrate may predominate under small energy excesses.

Immunogenic inhibition of mixed culture ruminal bacteria as a means to reduce lipolysis and fatty acid biohydrogenation *in vitro*

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Dietary lipids are readily hydrolyzed and the resulting fatty acids are extensively biohydrogenated by the microbiome in ruminants. For this reason, only a small proportion of ingested polyunsaturated fatty acids are absorbed and assimilated into the meat and milk of ruminants. In this study, an antibody was generated against the lipase-producing bacterium *Butyrivibrio fibrisolvens H17C* and was tested for its ability to inhibit lipolysis. *Butyrivibrio fibrisolvens* also contributes to the first isomerization step of microbial fatty acid metabolism and thus the anti-*B. fibrisolvens* antibody was evaluated for its effects against biohydrogenation in a separate study. Rumen fluid was collected from a cannulated cow and 6 mL was distributed anaerobically into tubes containing 21 g of glass beads and 1,130 μmol olive oil to measure lipolysis, or 1,130 μmol of linseed oil or corn oil to measure biohydrogenation. Tubes were treated without (controls) or individually with 0.6 mL of the anti-bacterial antibody and were incubated for 12 h at 39°C under CO_2. Lipolysis of olive oil was measured colorimetrically as the accumulation of free fatty acids, and biohydrogenation was determined by measuring fatty acid products of linseed oil or corn oil by gas chromatography after 0, 3, 6 and 12 h. Antibody against *B. fibrisolvens* caused the greatest decrease ($P < 0.05$) in lipolytic activity (30%) compared to the controls (39 ± 1 vs 28 ± 1 μmol total free fatty acids per incubation period; mean ± SEM). In cultures incubated for 12 h with linseed oil, α-linolenic acid decreased from initial concentrations by 132 ± 2 μmol in tubes without antibody and by 124 ± 2 μmol in antibody-treated samples (time x treatment $P = 0.02$). Total *trans*-fatty acid isomers increased over 12 h by 126 ± 2 μmol in control samples and by 109 ± 2 μmol in antibody-treated cultures (time x treatment $P < 0.001$). There was no measureable lipolysis or hydrogenation in mixed cultures incubated with corn oil. This study demonstrated that the hydrolysis and hydrogenating ruminal fatty acids by

mixed cultures can be immunologically inhibited *in vitro*. Further research is warranted to establish the efficacy of immunizing ruminants against bacteria responsible for the saturation of dietary fatty acids.

POSTER ABSTRACTS (with board number)

Immunology

1. Progressive changes in gut microbes, mucosal immune responses and barrier functions in dairy calves

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Gut microbial establishment plays a vital role in development of the host mucosal immune system. However, there is limited knowledge on how establishment of the gut microbiome during early life influences development of mucosal immune responses and barrier functions in dairy calves. This study investigated the relationship between the gut microbiome and mucosal immune and barrier functions and how these relationships may change in newborn dairy calves. Small intestinal (mid jejunum, distal jejunum, ileum) tissue and digesta samples were collected from neonates (n = 3), 1 (n = 6), 3 (n = 6) and 6 (n = 6) week old calves and were analyzed to quantify total bacteria, *Lactobacillus* sp. and *Bifidobacterium* sp. as well as mucosal expression of bovine toll-like receptors (TLR) and tight junction (TJ) genes. The lowest bacterial density was observed in neonatal intestine (10^8 copies of 16S rRNA gene/g), which increased within one week to 10^{10} copies of 16S rRNA gene/g. The prevalence of *Lactobacillus* sp. was lowest in neonates and 6-week-old calves (>1%) compared to 1 (7.1%) and 3 week (5.7%) old calves. In contrast, the prevalence of *Bifidobacterium* sp. was always greater than *Lactobacillus* sp. at all ages with peak prevalence (21.2%) observed in 3-week-old calves. Nevertheless, the age-related changes in bacterial densities were not significantly different among age groups due to high individual animal variation. Expression of all the TLRs, except for TLR9, was different among age groups and expression of most TLRs was lowest in the neonates. A strong correlation was observed between total bacterial density and TLRs expression and these associations varied with age. TJ genes, claudin 1, claudin 4 and occludin, also displayed an age-dependent expression pattern in the small intestine. Expression of TJ genes was lowest in newborn calves and expression was up-regulated in 1-week-old calves. Temporal changes in TJ gene expression are consistent with high gut permeability at birth and decreased permeability in older animals. The present study revealed that gut microbial density, mucosal immune responses and barrier functions rapidly changed within the first week of life and the gut microbiome might influence these mucosal responses during the early life of dairy calves.

Microbial Physiology and Genomic Analysis

2. *Bacteroides* isolated from four mammalian hosts lack host specific patterns in carbon and nitrogen metabolism

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Within the distal gut of mammals are found trillions of microbes that utilize nutrients from diet, intestinal mucosa, and other gut microbes. 402 isolates of *Bacteroides ovatus*, *B. thetaiotaomicron*, and *B. xylanisolvens* were recovered from cow, goat, human and pig fecal enrichments with cellulose or xylan/pectin. These isolates were compared using 16S rRNA gene sequencing and repetitive sequence

based-PCR (rep-PCR), using BOX and ERIC rep-PCR primers. Phylogenetic analysis of 16S rRNA gene sequences revealed high sequence homology in this Bacteroides clade, except for 3, all was 97% similar. 16S rRNA gene sequences formed distinct phylogenetic groupings by bacterial species but not host origin. With both rep-PCR methods, isolates did not segregate completely by *Bacteroides* species type. Grouping the isolates at 90% similarity of banding patterns resulted in the majority of groups containing isolates from just one host. Using 16S rRNA gene sequences and rep-PCR banding patterns 103 isolates and species type strains were selected for further analysis using phenotypic microarrays for carbon and nitrogen substrate utilization. These isolates represented genetic diversity across *Bacteroides* species and host origin to assess inter-and intra-host and *Bacteroides* species differences. These *Bacteroides* species shared the ability to utilize many of the same carbon substrates and were indicative of their broad carbohydrate fermentation abilities. Limited nitrogen substrates were utilized, ammonia was only utilized by 2 *B. xylanisolvens*. Guanine and xanthine, purine derivatives, were the most utilized nitrogen sources, followed by a few amino sugars and amino acids. This is the first report of *B. ovatus, B. thetaiotaomicron*, and *B. xylanisolvens* isolated from a range of mammalian hosts, preferential utilization of purine derivatives and amino sugars as nitrogen sources, and to suggest conservation of these organisms and their functions across mammals.

3. Fermentation gone awry: How microbial communities in the horse gut respond to starch overload associated with laminitis

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Laminitis is a systemic disease that can be triggered by a sudden influx of dietary starch, resulting in inflammation and degradation of the laminar layers of the hoof. Details of the molecular mechanisms underlying laminitis are not well understood, but it is clearly correlated with fermentation in the hindgut of the horse. Excess starch reaching the hindgut results in enrichment of lactic acid bacteria, overproduction of lactate, and a drop in pH. These events set into motion a cascade of systemic histological disruptions. Since lactate levels in the hindgut are normally low as lactate utilizing microbes convert it to short chain fatty acids readily absorbed by the horse. It is unclear why this mechanism fails when lactate levels are high, and how communities respond to the challenge of starch induction over time. Fecal samples collected from 3 healthy, adult horses eating an identical pasture based diet provided bacterial communities for this in vitro study. Triplicate microcosms of fecal slurries were enriched with lactate and/or starch. Metabolic products (short chain fatty acids, gases, and hydrogen sulfide) were measured and microbial community compositions were determined using Illumina 16s rRNA amplicon sequencing over 12-hour intervals. Here we report that as lactate levels build during starch induction, hydrogen gas accumulates, and levels of methane, acetate and hydrogen sulfide drop, suggesting an inhibition of lactate utilizers. In trials where lactate levels recover, acetate and propionate levels rise correspondingly and communities change, pointing to bacteria that may be most able to attenuate lactate under these conditions. Variation between cultures with disparate abilities to clear excess lactate suggest community structure with less tolerance for conditions accompanying starch induction.

4. Multiple starch binding modules involved in *Bacteroides thetaiotaomicron* starch metabolism
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Degradation of indigestible polysaccharides is one of the most important functions performed by the human gut microbiota. The Bacteroidetes, one of the dominant taxa in the human gut, are able to degrade a large number of carbohydrates via expression of unique multi-protein complexes, each targeting a different glycan. The first such system described was the starch utilization system (Sus) in *Bacteroides thetaiotaomicron* (*Bt*), an eight protein system required for the bacterium to metabolize starch. Homologous systems, termed Sus-like systems, have been found in all sequenced gut Bacteroidetes with certain species devoting up to 20% of their genome to encoding them. The *Bt* Sus has become a paradigm for glycan acquisition by the Bacteroidetes, yet questions regarding its mechanism remain unanswered. We solved the crystal structures of two Sus outer-membrane proteins (OMPs) of unknown function, SusE and SusF. SusE and SusF contain two and three starch binding sites respectively. This brings the total number of binding sites within the Sus OMPs to nine, only two of which are essential for *Bt* growth on starch. I demonstrated that loss of the non-essential binding sites caused a severe substrate-dependent growth defect as well as a deficiency in responding transcriptionally to available starch.

5. Hyper ammonia-producing bacteria and their interactions with ciliate protozoa from the rumen
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Excessive ammonia excretions are a major concern for the dairy industry due to reduced nitrogen utilization efficiency and the detrimental impact excreted ammonia has on the environment. Hyper ammonia-producing bacteria (HAB) and protozoa in the rumen contribute to excessive ammonia excretions from cattle. Besides *Clostridium aminophilum, C. sticklandii, and Peptostreptococcus anaerobius*, little is known about the HAB present in the rumen. In addition, rumen protozoa prey on bacteria and other microbes, excreting considerable amounts of amino acids and/or peptides that could promote the growth of HAB. In this study, experiments were undertaken to enrich and isolate new strains of HAB as well as characterize the relationships between HAB and protozoa. Fresh rumen fluid was collected from three ruminally fistulated dairy cows and combined as the source inoculum of HAB. HAB were enriched in a medium containing casamino acids as the sole carbon, nitrogen, and energy source. Following successful enrichment, the enrichment cultures were plated and individual colonies displaying rapid rates of ammonia production were isolated and characterized in terms of morphology, growth rate, optimal growth temperature, and ammonia production. Co-cultures were also performed for HAB enrichment and an *Entodinium caudatum* culture that had been maintained with a ground wheat mix substrate and mineral medium containing clarified rumen fluid. The co-culturing experiment was conducted with or without the substrate and *Monococcus luteus* (a strictly anaerobic bacterium) to assess the impact of feeding the protozoan. Strains with different rates of ammonia production (2.10 - 4.97 mg N/dL $24hr^{-1}$, when incubated at 39°C with an initial pH of 6.5) were isolated. Two of the strains exhibited high 16S rRNA gene sequence similarity to *Fusobacterium ulcerans* and *P. anaerobius.* In the co-cultures of HAB enrichment and *E. caudatum*, more ammonia was produced from casein compared with HAB enrichment alone or protozoa alone when the ground wheat substrate was provided. The addition of *M. luteus* to the co-cultures also increased ammonia production when the ground wheat substrate was not provided. These results suggest that protozoa and HAB interact in the rumen and are affected by the presence of feed, either plant materials or microbial biomass.

6. Exploring the bovine rumen bacterial community from birth to adulthood

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The mammalian gut microbiota is essential in shaping many of its hosts' functional attributes. One such microbiota resides in the bovine digestive tract in a compartment termed the rumen. The rumen microbiota is necessary for the proper physiological development of the rumen and for the animal's ability to digest and convert plant mass into food products, making it highly significant to humans. The establishment of this microbial population and the changes occurring with the host's age are important for understanding this key microbial community. Despite its importance, little information about colonization of the microbial populations in newborn animals, and the gradual changes occurring thereafter, exists. Here, we characterized the overall bovine ruminal bacterial populations at five ages, from 1-day-old calves to 2-year-old cows. We describe the changes occurring in the rumen ecosystem after birth, reflected by a decline in aerobic and facultative anaerobic taxa and an increase in anaerobic ones. Some rumen bacteria essential for mature rumen function could be detected as early as 1 day after birth, long before the rumen is active or even before ingestion of plant material occurs. The diversity and within-group similarity increased with age, suggesting a more diverse but homogeneous and specific mature community, compared to the more heterogeneous and less diverse primary community. In addition, a convergence toward a mature bacterial arrangement with age was observed. These findings have also been reported for human gut microbiota, suggesting that similar forces drive the establishment of gut microbiotas in these two distinct mammalian digestive systems.

7. Effects of shortening dry period and using single close-up diet on rumen microbiome of Holstein dairy cows

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The objective of this study was to compare the long-term effects of two dry period management strategies on the rumen microbiome of dairy cows. Twenty-four multiparous cows were paired based on expected calving date, and randomly assigned to treatment within each pair. Treatments were a 60-d dry period with separate far-off and close-up diets and a 40-d dry period during which only the close-up diet was fed. The 60-d dry period was divided into a 39-d far-off and a 21-d close-up period. The far-off diet contained 1.28 Mcal/kg net energy for lactation (NEL), 14.7% of crude protein (CP), and 50% of neutral detergent fiber (NDF) on a DM basis. The close-up diet contained 1.43 Mcal/kg NEL, 14.6% of CP, and 38% of NDF. A common diet was fed to all cows after calving, which contained 1.69 Mcal/kg, 17.6% of CP, and 31% of NDF. Rumen samples were taken at wks -2, -1, 1, 2 and 7 relative to calving. Relative quantifications of ciliate protozoa, methanogenic archaea and 14 rumen bacteria were performed in triplicate using real-time PCR. There was no difference in the populations of ciliate protozoa, *Fibrobacter succinogenes, Megasphaera elsdenii, Prevotella ruminicola, Prevotella albensis, Prevotella bryantii, Ruminococcus albus, Ruminococcus flavefaciens, Selenomonas ruminantium, Succinimonas amylolytica, Succinovibrio dextrinosolvens* and *Treponema bryantii* between treatments at the 5 sampling times. However, cows on the 40-d dry period had higher number of methanogenic archaea at wks -1 and 7 compared to cows with the 60-d dry period. Additionally, the number of *Ruminobacter amylophilus* was higher in the 40-d treatment at wk 1 postpartum compared to the 60-d treatment. *Anaerovibrio lipolytica* and *Prevotella brevis* population were higher at wk 7 postpartum in the 60-d compared to the 40-d treatment. Cows in the 40-d treatment were fed an extra 20 days of a high energy and protein diet, which could have resulted in higher rumen adaptation and consequently higher fermentation. Higher hydrogen produced in the rumen of these cows may have promoted the growth of methanogenic archaea. We

assume that the availability of high-energy diet for a longer period in 40-d treatment resulted in a greater number of *Ruminobacter amylophilus* at wk 1 compared to 60-d treatment. The increase in the number of *Anaerovibrio lipolytica* and *Prevotella brevis* at 7 wk post-calving could be due to the steadily higher feed intake and consumption of high energy and protein diet during the 60-d treatment.

8. Temporal Dynamics of the Prokaryotic Community in the Cow Rumen During Biomass Degradation Determined by 16S rRNA Pyrosequencing

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The microbial community that inhabits the cow rumen is composed of Archaea, Bacteria and Eukarya and is well known for its biomass-degrading ability. In order to understand this ecosystem at the whole-systems level it is important to monitor the dynamics of the individual community members. To obtain insights into the ecology of the prokaryotic fraction of the rumen community and its dynamics during biomass-degradation, we amplified a short region of the 16S rRNA gene from the prokaryotic population that colonized switchgrass during rumen incubation. Amplicons were generated and fiber analysis was performed at nine different time points to monitor the community dynamics and the biomass degradation process. Sequencing on Roche's Titanium platform resulted in a total of >1/4 million sequences with an average read length of ~500 bp amounting to a total of >130 Mbp of sequence information. Succeeding sequence analysis revealed that the microbial community associated with the dried switchgrass was dominated by members of the Bacilli (contributing 62% of the community) and was replaced within 30 min by rumen microorganisms belonging to the Bacteroidia and Clostridia (contributing 34 and 15% of the community respectively). A second significant shift in the community composition was observed after 4 hr of rumen incubation, during which members of the Spirochaetes and Fibrobacteria became more abundant in the fiber adherent community. Between these two distinct shifts of the bacterial population, members of the Archaea (i.e. Methanobacteria) increased up to 5-fold. Interestingly, no significant degradation of biomass was observed during the time when the archaeal population was more abundant, suggesting that the archaeal population was not involved directly in biomass degradation. It appears likely that the metabolic activity of the archaeal population was necessary before the second shift within the bacterial population and further biomass degradation could occur. In summary, results presented here suggest that rumen prokaryotes consistently colonize lignocellulosic substrates and that distinct bacterial and archaeal populations contribute during different stages of the degradation process of recalcitrant biomass in the rumen ecosystem.

Nutrition of Livestock, Humans and Companion Animals

9. Effects of corn ceramide on growth of mixed microflora and on experimental enterocolitis in mice

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Enterocolitis occurs frequently in humans and animals, but the pathogenesis of inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, remains unknown. Although corticosteroids and sulfasalazine are currently used commonly for the treatment of patients with IBD, the utility of these agents is limited by their adverse effects. Therefore, alternative therapies are needed. Ceramide is an important bioactive substance to exert various effects on host animals, such as the modulation of immune system pathways and the induction of apoptosis. Ceramides are rarely found in nature, but glucosylceramide, the precursor substance of ceramide, can be obtained from grains and fruits. In this study, our aim was to investigate using corn ceramide to alleviate the symptoms of enterocolitis,

including IBD. Ceramides were prepared by hydrolyzing glucose from corn-extracted glucosylceramide by using the novel bacterium isolated in this laboratory. The growth of some *Clostridium* sp. was inhibited by ceramide. However, some *Bifidobacterium* sp. were less sensitive to ceramide than *Clostridium* sp. Since some *Clostridium* strains, such as *C. difficile* or *C. perfringens*, are thought to be potential pathogens associated with IBD or necrotic enteritis, ceramide might be useful as an antibacterial substance. IBD was experimentally induced by administering dextran sulfate sodium orally to mice. Oral administration of ceramide alleviated the symptoms of colitis including body weight loss, diarrhea, and bloody stool. In addition, myeloperoxidase activities in colonic tissue were reduced, suggesting that ceramide mitigates bowel inflammation. Bacterial community fingerprints supported the conclusion that caecum bacterial populations shifted as a result of DSS addition. However, ceramide administration changed bacterial population toward that of the control. In qPCR monitoring, ceramide administration increased *Bifidobacterium* sp. and decreased *Clostridium* sp., suggesting that dietary ceramide might affect caecum microflora composition. These results suggest that dietary ceramide supplementation can alleviate the symptoms of IBD in mice and alter gut microflora composition to maintain colonic health in host animals.

10. Effect of level and source of supplemental protein on rate of ruminal ammonia production and concentrations of amino acid-utilizing and trypticase-metabolizing bacteria in *Bos taurus* and *Bos indicus* steers fed low-quality forage

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Five ruminally cannulated *Bos taurus* (Bt, 303 ± 10 kg initial BW) and five ruminally cannulated *Bos indicus* (Bi, 323 ± 28 kg initial BW) steers were used to quantify differences in source and level of protein on rate of ammonia production and concentrations of amino acid-utilizing and trypticase-metabolizing bacteria in Bt and Bi cattle. *Bos taurus* and Bi steers were assigned to concurrent 5 × 5 Latin squares and fed low-quality forage (4.5% CP). Treatments were a 2 × 2 factorial plus a control: the first factor was level of protein 60 or 120 mg N/kg BW daily; and the second was source DIP (degradable intake protein; 72% DIP) or UIP (undegradable intake protein; 72% UIP). Rumen fluid was collected prior to feeding and 4h after feeding and processed according to standard laboratory protocols. There were no significant differences between breeds for any of the measured variables ($P \geq 0.21$). There was a main effect of protein supplementation on *in vitro* NH_3–producing activity for both Bt ($P = 0.04$) and Bi ($P = 0.03$) steers. In both cases, rates of NH_3 production were higher in steers fed 120 mg N/kg BW DIP (0.053 ± 0.02 and 0.048 ± 0.02 μmol NH_3/mL per h, respectively) and lower in steers receiving 0 mg N/kg BW (0.031 ± 0.01 and 0.033 ± 0.01 μmol NH_3/mL per h, respectively). For Bt steers there was a treatment × time interaction ($P = 0.04$) for most probable number (MPN) of amino acid-utilizing bacteria resulting from decreases in MPN from h0 to 4 when DIP was supplied at both levels and increases for control and 60 mg N/kg BW UIP. More specifically, steers fed 60 or 120 mg N/kg BW DIP had decreased MPN with time after feeding (0 to 4h; from 5.96 to 5.37 and 6.41 to 5.85 $\log_{10}$ cells/mL, respectively) versus steers fed 60 mg N/kg BW UIP which increased MPN from 5.74 to 6.07 $\log_{10}$ cells/mL and 120 mg N/kg BW UIP which decreased slightly from 6.0 to 5.8 $\log_{10}$ cells/mL. In Bi steers there was no effect of dietary treatment ($P = 0.77$) or time after feeding ($P = 0.85$) on MPN. There was a main effect of time after feeding on recoverable trypticase-metabolizing bacteria for both Bt ($P \leq 0.01$) and Bi ($P \leq 0.01$) with $\log_{10}$ CFU mL^{-1} being lower at 4h after feeding (7.00 ± 0.77 and 7.12 ± 0.64) versus 0h (7.51 ± 0.88 and 7.53 ± 0.86) for Bt and Bi, respectively. Results indicate that irrespective of breed, microbial N-metabolizing populations responded more rapidly to diets supplemented with DIP than UIP.

11. Characterization of the bovine ruminal microbial community and its relation to energy harvest efficiency

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Plant degradation in cattle is performed by a highly specialized microbial community found in one of the compartments of the digestive tract of ruminants: the rumen. This community allows for the degradation of the most recalcitrant carbohydrates found in plants, and through this process, ruminants are able to harvest energy found in plants for growth, making cattle the main source of milk and meat for humankind. The improvement of feed efficiency, and through it, product yield in cattle remains one of the most sought after goal in agriculture today. Until now, this improvement was solely accomplished by selective breeding and improvement of feed composition. While the animals are completely dependent on their microbiota, a connection between the bovine capacity for improved energy harvest and its resident bacterial taxa has yet to be established. Using a pyrosequencing approach we characterized the bacterial community composition found in 16 dairy cows, analyzed the degree of divergence that can be found between different animals, and found significant correlations between metabolic parameters of the animals and the abundance of specific bacterial taxa. This work suggests the bacterial community may have a significant role in the efficiency of energy harvest, and a deeper understanding of this process may allow us to improve yield through community design.

12. Comparative Analysis of Cecal Microbiome between Chickens with High and Low Feed Conversion Ratios

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The microbiome in the poultry gastrointestinal tract functions as an interface between the host and the feed ingested and plays an important role in feed digestion and absorption, which can in turn affect feed utilization efficiency and growth performance of the host. The objective of this study was to investigate potential association between cecal microbiome composition and feed conversion ratio (FCR) in broiler chickens. Ninety six male Cobb 500 broiler chickens of similar genetics were fed the same diet, and their FCR was determined individually. On day 25 post-hatch the birds were euthanized by cervical dislocation and fresh cecal samples were collected. These chickens were ranked based on their FCR, and 24 chickens at both ends of the FCR spectrum were chosen for comparative analysis of their cecal microbiome using a poultry intestinal tract chip (PITChip, version 2). Briefly, metagenomic DNA was extracted from each cecal sample. 16S rRNA gene was amplified by PCR using universal bacterial primers. The amplicons were converted to complementary RNA (cRNA), which was labeled and hybridized to PITChip 2. The microarray signals were normalized and analyzed to determine differences in microbiome composition between chickens with low FCR and with high FCR. Based on 12 of the 24 chickens in each group, the microarray data indicated that there is great similarity in cecal microbiome composition between chickens with low FCR and with high FCR. However, chickens with low FCR had higher levels of bacteria in the genera *Barnesiella*, *Roseburia*, *Alcaligenes*, *Jeotgalicoccus*, *Pasteurella*, and in the family *Lachnospiraceae* than the chickens with high FCR. On the other hand, chickens with high FCR had higher levels of bacteria in the family *Coriobacteriaceae* than the chickens with low FCR. These results suggest that these bacteria might be associated with feed utilization by broiler chickens. Some of these bacteria may serve as indicator of feed conversion efficiency.

13. **Amino acids metabolism by bacteria in pig small intestine depends on their niche compartments**
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Previous studies showed that pig intestinal microorganisms can actively metabolize amino acids (AA). However, little research has focused on the AA metabolism by bacteria in different compartments. This study was conducted to compare the metabolism of free AA by microorganisms derived from luminal and epithelial wall of the pig intestine, aiming to test the hypothesis that the metabolic fate of AA was influenced by bacterial ecological niches. Samples from the digesta, gut wall washes and gut wall of the jejunum and ileum were used as inocula, representing luminal bacteria, loosely-attached adherent bacteria and firmly-attached bacteria in the small intestine, respectively. These samples were inoculated into anaerobic media containing 10 mmol/L one of the selected AA (L-glutamate, L-glutamine, L-histidine, L-arginine, L-threonine, L-valine, L-methionine, L-isoleucine, L-leucine, L-phenylalanine or L-lysine) and cultured for 24 h. The 24 h culture served as inocula for the following 30 subcultures, which were also incubated for 24 h for each generation. The 24 h incubation showed that 60-95% of glutamate, glutamine, lysine and arginine were utilized by luminal bacteria. With tightly attached bacteria from the jejunum, AA except glutamine were constantly synthesized for the first 12 h and afterwards were utilized to varying degrees. For loosely-attached bacteria, AA synthesis dominated in the jejunum while catabolism dominated in the ileum. After culturing for 30 generations, both anabolism and catabolism of AA by microbes from each compartment decreased, and the metabolic rate was lower than 50%. Denaturing gradient gel electrophoresis revealed that the diversity of luminal bacteria community was similar to that of tightly-attached bacteria but significantly higher than that of loosely-attached bacteria. Bacteria loosely attached to the epithelium exhibited the lowest degree of diversity than the other two communities. These findings suggested that AA metabolism by bacteria in small intestine depends on their ecological niche compartments.

Prebiotic, probiotic and DFM Development

14. Effects of Antibiotic and Direct-Fed Microbial Supplementation on Gut Lactic Acid Bacteria Populations and Growth Performance of Broiler Chickens
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Many non-nutritive dietary additives affect the gut microbial ecosystem by shifting the balance of beneficial and harmful organisms. The use of direct-fed microbials (DFM) and antibiotics have been reported to shift the microbial populations towards beneficial bacteria, specifically toward lactic acid bacteria (LAB) like those of the *Lactobacillus* and *Enterococcus* genera. A study was conducted to evaluate the differences in selected gastrointestinal microbiota in conjunction with growth performance of broiler chickens administered a three strain *Bacillus* DFM, a virginiamycin antibiotic, and a combination of the two in the diet. A 28-day battery trial using Cobb 500 broiler chickens was designed as a 2 x 3 factorial arrangement of treatments. Treatments consisted of a non-medicated corn-soy basal diet with two levels of a *Bacillus*-based DFM at 0 and 500 g/ton of feed, which is equivalent to 1.5×10^5 cfu of *Bacillus* per gram of finished feed, and three levels of virginiamycin antibiotic included at 0, 10 and 20 g/ton. Trial design allowed for eight replicate cages per treatment with 8 birds each. Three birds per replicate were randomly selected, pooled together, and gut LAB populations assessed. Antibiotic supplementation improved body weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) compared to birds fed diets devoid of antibiotic, and the same response was observed with DFM inclusion. Birds fed diets containing DFM had a greater ($P < 0.01$) proportion of *Lactobacillus* and lower ($P < 0.01$) proportion of *Enterococcus* when compared to birds fed diets without DFM inclusion. Specifically, birds fed the DFM had an 8% greater proportion of *Lactobacillus* and an 8% lower proportion of *Enterococcus* compared to

birds fed diets devoid of the DFM. Furthermore, *Lactobacillus* populations were linearly regressed and correlated to growth performance of birds, and *Lactobacillus* populations in the gut tended (P = 0.07) to be positively correlated to feed efficiency. These results indicate that growth performance of broiler chickens was improved by the inclusion of either the DFM or the antibiotic; however, only the administration of the DFM enhanced populations of *Lactobacillus* in the gastrointestinal tracts of birds.

Research

15. Colonic Microbial Fermentation and Hydrogenotrophic Microbiota Dynamics Under Short-Term Reciprocal Diet Exchanges for Native Africans and African Americans

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Compared to other U.S. racial and ethnic groups, African Americans (AA) have a higher incidence and mortality from sporadic colorectal cancer (CRC). In contrast, native Africans (NA) are rarely diagnosed with colonic diseases. Reduced susceptibility to CRC in Africans is associated with low consumption of animal products, a CRC risk factor and higher consumption of complex polysaccharides and starch, which decrease CRC risk. Thus, we hypothesize that differences between the Western and African diet drives differences in the colonic microbiota composition and function linked to the differential CRC risk. Here, 20 NA and 12 AA were subjected to reciprocal diet exchange over two weeks. NA were fed a Western-style diet and AA were fed a South African style diet. Diets were fed under strict in-house controlled conditions and energy intake was adjusted to maintain body weight. The extent of fermentation was measured directly with measurements of short-chain fatty acids by LC-MS and qPCR targeting the bacterial butyrate production gene; and indirectly by qPCR of hydrogenotrophic microbiota and measurement of breath methane. For NA a significant decrease in the number of methanogenic Archaea and acetate and butyrate production genes was observed at the end of the two-week diet exchange, consistent with decreasing levels of stool acetate and butyrate (91.8±16.3 to 20±4.7 p=; 25.7±7.2 to 4.9±1.7 μmole/per g respectively). Despite the high amounts of meat, sulfate-reducing bacteria were found to decrease, except for *Bilophila wadsworthia*, which has been shown to be stimulated by a high-fat diet. Conversely for AA, a significant increase in the number of methanogenic Archaea and acetate and butyrate producers was observed at the end of the two-week diet exchange accompanied by a significant increase in breath methane and increasing levels of stool acetate and butyrate (31.3±6.2 to 58.1±9.8; 7.5±2.1 to 14±3 μmole/per g respectively). The present data provide evidence that the human colonic microbiota and corresponding metabolic pathways are shaped by diet in a dynamic manner. Importantly, these changes result in alterations in the balance of beneficial and detrimental microbial metabolism, which may explain in part the differences in CRC risk among populations.

16. Increased Abundance of Sulfidogenic Bacteria in the Colonic Mucosa of Patients with Ulcerative Colitis and Crohn's Disease

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The gut microbiota is increasingly suspected as a key factor in the etiology of inflammatory bowel disease (IBD). However, it is most often characterized in stool samples, which are only partially representative of the mucosal micro-environment where the inflammation originates. Here, a molecular-based approach was used to examine mucosa-associated microbiota in ileal, ascending and descending colon and rectal biopsies from ulcerative colitis (UC) and Crohn's disease (CD) patients, and healthy control subjects. We

focused on sulfidogenic bacteria producing hydrogen sulfide, which is proinflammatory and a potent genotoxic agent. Targets included sulfate-reducing bacteria (SRB: dissimilatory sulfite reductase (*dsrAB*) gene of SRB and 16S rRNA genes of *Desulfovibrio*, *Desulfobulbus*, *Desulfobacter* and *Desulfotomaculum*), *Bilophila wadsworthia* (taurine:pyruvate aminotransferase (*tpA*)) and *Fusobacterium nucleatum* (Fn1419 and Fn1055 encoding enzymes that convert cysteine to hydrogen sulfide). These targets were quantified through qPCR targeting 16S rRNA genes and functional genes. A total of 321 biopsies from 28 CD and 19 UC patients as well as 40 healthy subjects were quantified for the abundance of sulfidogenic microbes. A 10-fold greater abundance of SRB, in particular the genus *Desulfotomaculum*, was detected in inflamed tissue of CD and UC. Strikingly, all functional genes for sulfidogenic bacteria and SRB genera except *Desulfobulbus* were significantly more abundant in biopsies from IBD patients compared to healthy controls (at least 10 times more abundant for the more abundant genes). In addition, *Bilophila wadsworthia* was ubiquitous in IBD patients (99.5%) but much less prevalent for healthy controls (48%). This increased prevalence of SRB and genes mediating sulfide production from organic sulfur sources in IBD patients is consistent with the proinflammatory properties of hydrogen sulfide, which may contribute to chronic inflammation. As many of the patients examined were in remission, it can be hypothesized that they may be deficient in sulfide detoxification or other host pathways that provide tolerance to bacterial-produced sulfide. Together the data indicate an urgent need for systematic study of the various microbial and host metabolic pathways involved.

17. Evaluation of PCR-DGGE as a Method to Recapitulate Host Phylogeny by Fecal Microbial Community Fingerprint

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Recent studies indicate that host animal could be the primary factor determining the composition of the gastrointestinal microbiome. If host phenotype dictates microbiome composition, then composition should recapitulate host phylogeny. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was evaluated as a means to compare the fecal microbiomes of horses to those of other herbivores. The hypothesis was that the PCR-DGGE banding patterns from horses would be more similar to each other than to those of other animals. Fecal samples were collected from horses (*Equus ferus caballus*; n=30), Hartmann's mountain zebras (*Equus zebra hartmannae*; n=2), white rhinocerous (*Ceratotherium simum*; n=2), Malayan tapirs (*Tapirus indicus*; n=2), domestic goats (*Capra aegarus hircus*; n=2), African elephant (*Loxodonta africana*; n=1) and Asian elephant (*Elephas maximus*; n=1). PCR was performed on extracted DNA to amplify the 16S rRNA gene (primers: 341F-GC, 901R). Amplicons were separated on an acrylamide gel (urea-formamide 40-60%, 56° C, 69 V, 17 h). Gel images were normalized with external standards included in every gel and analyzed with BioNumerics software (AppliedMaths). The similarities of lanes were compared as both bands and densitometric curves. Band-based analysis (Dice's algorithm, UPGMA) indicated little similarity between the two zebra samples (31.9%) and between the two rhinocerous samples (47.1%), which resulted in mixed-species groups. However, in curve-based analysis (Pearson's *r*, UPGMA) each sample was most similar to a sample from the same species. One exception was a horse sample that grouped with zebra. The highest similarity between any two samples was between two horses (94.8%). Overall similarity between all samples was 7.5%. Statistical resampling (Jackknife, mean similarity) indicated confidence values of 76.7% and 93.3% for associating horse samples with the correct species and Order, respectively. These results indicate that comparisons based on densitometric curves are more useful than binary data from called bands when evaluating fecal microbial communities with PCR-DGGE. Note that the error in the curve-based analysis was a horse sample that grouped with zebras, the two most closely related host species in the study.

18. Changes in the activity of lactase and lactobacilli in ileum and colon in offspring nursed by lead intoxicated mother

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Increasing the concentration of lead ions in food and water is a threat to human and animal health, especially for a growing organism, in industrialized regions. This is because the developing organism is more susceptible to various negative environmental factors compared with adults. Lead salts get to an organism, mainly through the digestive system. The aim of this research is to investigate the effect of lead poisoning of the dam on the activities of lactase and lactobacilli in the ileum and the colon of growing rats. Toxicity in Wistar rats was performed by replacing the dam's drinking water with a 0.01% solution of lead acetate from the first day of lactation until the end of the experiment. Intact rats served as the control. Rats were sacrificed on the 24th day of postnatal life. The results showed that the lactobacilli activity was 30.5±1.2 and 117.0±7.2 °T, and the lactase activity was 25.0 ± 1.4 and 7.8 ± 0.5 mmol / g protein / min in the ileum and colon, respectively, in control rats. In fed dams that drank a 0.01% solution of lead acetate instead of drinking water, the growing rat pups' activity of lactobacilli was 28.5 ± 1.2 ° T in ileum and 87.0 ± 7.2 ° T in the colon, whereas lactase activity in the experimental group of rats was 44.1 ± 2.5 and 11.8 ± 0.5 mmol / g protein / min in the colon and ileum, respectively. Thus, the activity of lactase more evident in the ileum in comparison with the colon; in contrast, the activity of lactobacilli in the colon is stronger than in the ileum. At the same time, lead intoxication of nursing dams increased the lactase activity in the intestine of offspring, accompanied by a decrease in the activity of lactobacilli. Consequently, the amount of lactose is decreased due to increased lactase activity in the intestine resulting from lead intoxication. Reducing nutrient supply possibly suppressed the activity of lactobacilli.

19. Naïve analysis of global diversity of anaerobic gut fungi

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Anaerobic fungi play a key catalytic role in digesting fibrous feeds in the gut of herbivores, but little is known about their global diversity. In this study, the collective diversity of gut anaerobic fungi was examined using all the curated internal transcribed spacer 1 (ITS1) sequences of anaerobic fungi deposited in the GenBank database. As of April 2012, 30,362 chimera-free ITS1 sequences of anaerobic fungi were found and analyzed in this study. The fungal sequences were assigned to 68 operational taxonomic units (OTUs) at species-level (0.07 genetic distance) and 19 genus-level (0.14 genetic distance) OTUs. Of the 6 known genera, *Piromyces* (17,784 sequences) and *Neocallimastix* (3,672 sequences) were the most predominant. while *Cyllamyces* (7 sequences) the least predominant. Four new genera each represented by a large number of sequences have been identified, with each forming a distinct lineage than the existing genera in a global phylogenetic tree of anaerobic fungi. Seven possible new genera represented by less than 5 sequences each have also been identified. Rarefaction analysis indicated that 83% of the maximum number of species has been captured so far. The results of this study may help guide future studies involving targeted isolation and characterization of new anaerobic fungi. Culture and evaluation of metabolic or enzymatic potential of the members of new genera need to be undertaken to gain insight into their role in degradation of plant cell wall materials.

20. Bacterial and protozoal communities: Equine hindgut *versus* faeces

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Alterations of equine hindgut microbiota potentially lead to colics. The large colon is the most commonly affected segment. Since starchy particles of digesta flow quicker through the caecum to the right ventral

colon (RVC), that part of the colon could be particularly susceptible to dysmicrobism. For studying the hindgut ecosystem, faeces are easily collected and data extrapolated to the hindgut. Previous data concluded that fecal microbiota was representative of right dorsal colon (RDC) one (Dougal *et al.*, 2012). However it is not well established if it is representative of the RVC one. In the present study, the faecal microbiota was compared to those of the caecum and RVC. Faeces, caecal and colonic contents were collected from six adult fistulated horses fed a similar diet (75 forage : 25 concentrate) 4h after the morning meal. Total DNA was extracted and PCR primers were used to amplify and quantify specific sequences of total bacterial (16S rRNA) and total protozoal (18S rRNA) communities. Total bacterial and protozoal densities were expressed in number of target gene copies/g fresh matter. Caecal, colonic and fecal contents pH and volatile fatty acids (VFA) were also determined. No segment effect was observed on total bacterial density. Protozoal density was significantly lower in the caecal and faecal samples than in the RVC samples ($P<0.001$). pH values and total VFA concentrations were significantly higher in the RVC (pH: 6.48 ; VFA: 75.2 mM) than in the caecum (pH: 6.41 ; VFA: 69.8 mM) and faeces (pH: 6.05 ; VFA: 51.0 mM) ($P<0.001$), and higher in the caecum than in the faeces ($P<0.001$). A higher proportion of acetate was measured in faecal samples (74.2%), a higher proportion of propionate in caecal contents (20.8%), and a higher proportion of butyrate in colonic contents (8.3%) ($P<0.0001$). The present results confirmed that the equine faecal microbiota was different from the caecal one and showed that the faecal microbiota was also different from the RVC one. Combined with the previous data (Dougal *et al.*, 2012), these results suggest a gradual shift of microbiota along the hindgut (caecum, RVC, RDC and faeces). More studies are needed to assess the microbiota diversity in different parts of the large colon in comparison to the faeces.

21. Anaerobic Oxalate-Degrading Bacteria in Avian Cecal Contents

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Herbivorous birds, like mammals, benefit from microbial detoxification of plant compounds such as oxalate. Given the ubiquity of oxalates in plants, and the diet of many birds, it is likely that oxalate-degrading bacteria reside in the avian intestinal tract. However, the presence and identity of these bacteria are unknown. The purpose of this study was to survey the ceca of birds for the presence or absence of anaerobic oxalate-degrading bacteria. Gastrointestinal tracts from three domestic chickens (*Gallus domesticus*), three wild turkeys (*Meleagris gallopavo*), and three white-plumaged ring-necked pheasants (*Phasianus colchicus*) were obtained from a local poultry processing plant. The chickens were raised indoors while the turkeys and white pheasants were raised outside on wood shavings. All birds were fed a standard pelleted diet. Cecal contents from a single bird were added to triplicate serum bottles (1 g contents/bottle) each containing 24 ml of anaerobic oxalate medium (AOM; 20 mM oxalate, minerals, metals, 0.1% yeast extract, 0.01% acetate, 0.05% cysteine, bicarbonate/100% CO_2 buffer system; and pH 6.6) and incubated at 38°C. Samples of cultures were removed during incubation (every 2-3 days for 15 days), and oxalate levels in cultures were monitored by a $CaCl_2$ precipitation test. Cultures positive for oxalate degradation were transferred to AOM, and oxalate-degrading bacteria were isolated by streaking onto roll tubes of calcium oxalate agar (AOM with 0.2% $CaCl_2$ and 1.5% agar). Oxalate-degrading bacteria were detected in streaked roll tubes by the appearance of colonies with clear zones. All AOM cultures initiated with cecal contents from chickens and pheasants tested positive for oxalate degradation. In contrast, none of the cecal contents obtained from wild turkeys yielded an AOM culture positive for oxalate degradation. Oxalate-degrading isolates were obtained from the ceca of chickens and pheasants. All isolates were Gram-negative, curved rods and resembled *Oxalobacter formigenes*. This report presents the first evidence for the presence of anaerobic oxalate-degrading bacteria in the cecal contents of birds, thus extending the range of known gut habitats (human large intestine, sheep rumen, and ceca of wild and laboratory rats, guinea pigs, and swine) for these organisms.

22. The effects of bedding material on the gastrointestinal microbiota of the broiler chickens
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The objective of this study was to explore the possible effects of litter conditions (reused versus fresh) on the intestinal microbiota of broiler chickens. Two flocks of broiler chicken were used in this study, with one reared on fresh litter of pine shaving while the other reared on the same type of litter that had been used for five consecutive growth cycles. Samples of ileal mucosa and cecal digesta were collected at age of 10 and 35 days, and litter samples were also collected at day 35. Metagenomic DNA extracted from each samples, and amplicons of 16S rRNA genes were analyzed using both DGGE and pyrosequencing. Principal Component Analysis (PCA) was used to compare the community similarity between samples. The pyrosequencing reads were analyzed by the Qiime pipeline to identify the operational taxonomic units (OTU), which were assigned to putative species according to Greengenes database. RDP Libcompare was used to compare the differences between chickens reared on the two types of litters. The results showed that both the age of birds and litter conditions play key roles in modulating the microbial community of ileal mucosa and cecal lumen. Litter conditions affected the microbial composition in both the ileal mucosa and the cecal lumen. Comparison of cecal luminal microbiota revealed 9 and 4 OTUs that significantly differed in relative abundance between the birds reared on fresh vs reused litter at days 10 and 35, respectively. At the age of 10 days, birds reared on fresh litter have significantly greater increased population of *Lactobacillus*, *Escherichia/Shigella* group, *Bacteroides*, *Subdoligranulum*, *Clostridium* XIVb and *Lachnospiracea*_incertar_sedis group but deduced population of the *Blautia*, *Faecalibacterium* and *Anaerotruncus* in their cecal luminal microbiota than those reared on reused litter. In the 35-day-old birds, *Faecalibacterium* and *Oscillibacter* have higher abundance in the cecal luminal microbiota under fresh litter condition than reused litter while *Subdoligranulum* and *Clostridium* XVIII were on the contrary. Several species in the genera *Bacteroides*, *Lactobacillus* and *Faecalibacterium* showed significantly differential distribution in the cecal lumen between birds reared on the two litter conditions. These results suggest profound impact of litter conditions on the composition of intestinal microbiota, providing useful information on modulating intestinal microbiota through litter management to improve broiler chicken health.

23. Effects of Gas Phase and Formate on Nitropropionic Acid-Metabolizing Activity of Mixed Populations of Bovine Ruminal and Equine Cecal Microbes
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The nitro toxins 3-nitro-1-propionic acid (NPA) and 3-nitro-1-propanol are produced by a wide variety of leguminous plants, including over 150 different species and varieties of *Astragalus* potentially grazed by livestock. These toxins are known to be detoxified by at least one ruminal bacterium but detoxification by bacteria from other gut habitats is not known. In the present study, mixed populations of bovine ruminal and equine cecal microbes were enriched for NPA-metabolizing bacteria via consecutive 24 to 72 h culture in a basal minimal rumen fluid-based medium supplemented with 4 mM NPA and H_2:CO_2 (1:1) as the energy source. Rates of NPA metabolism by the respective populations increased (P<0.05) from 58.4±4.8 and 8.6±11.6 nmol NPA/mL per h during initial culture to 88.9±30.6 and 50.2±30.9 nmol NPA/mL per h following enrichment. Results from 3-tube most probable number tests indicated that numbers of NPA-degrading microbes increased 2.1 and 1.8 $\log_{10}$ units during enrichment from numbers measured pre-enrichment ($3.9x10^3$ and $4.3x10^1$ cells/mL for ruminal and equine cecal populations, respectively). To assess the effects of different H_2-containing gas phases, with or without the addition of formate, on NPA-metabolism by the different populations, the enriched ruminal (n=3) and equine cecal (n=2) cultures were grown in basal medium with H_2:N_2 (1:1) or H_2:CO_2 (1:1) each supplemented with or without 30 mM sodium formate. Results revealed that for ruminal populations, rates of NPA-metabolism (nmol NPA/mL per h) were slower (P<0.05) when cultured with H_2:N_2 as the only energy source

(9.3±8.5) than when cultured similarly with added formate (59.2±4.0) or with H_2:CO_2 alone or with added formate (54.6±12.1 and 60.6±11.4). Conversely, rates of NPA-metabolism by the equine cecal populations lacking added formate did not differ (P>0.05) due to gas phase (14.7±2.7 and 19.8±7.9 for cultures grown with H_2:CO_2 and H_2:N_2, respectively). Rates were higher (P<0.05) for the equine cecal populations cultured with added formate (51.6±4.2 and 37.4±20.7 for cultures grown with H_2:CO_2 and H_2:N_2, respectively). These results reveal that formate is an important energy source for equine NPA-metabolizing bacteria and that formate or H_2:CO_2 are important energy sources for more active NPA-metabolizing bovine ruminal populations.

24. **Newly isolated *Methanosphaera* sp. and *Methanoplasmatales sp.* from Australian bovine**

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Recent studies have validated the existence of a seventh order of methanogens, the *Methanoplasmatales,* and further, that heterotrophic methanogens play a quantitatively important role in livestock methane production. Here we characterise a co-culture comprised of a *Methanosphaera sp.* (strain BMS) and a *Methanoplasmatales sp.* (strain BMP), isolated from the bovine rumen using methanol/acetate as the principal carbon sources. Microscopic examination of the co-cultures showed there appeared to be no clumping or coaggregation of the two different Archaea, although the relative abundance of strain BMS (monitored by the abundance of autofluorescent cells during UV transillumination) was less than BMP. An axenic culture of BMS was produced with the addition of novobiocin to the medium, while a colony pick was needed to produce an axenic culture of BMP. Axenic cultures of both strains utilize methanol, which also results in the formation of methane. Like the human isolate (*M. stadtmanae* DSMZ3091) growth of strain BMS is hydrogen-dependent, but unlike the kangaroo isolate recovered by our group (WGK6), no growth was observed when ethanol was used in place of hydrogen gas. The findings for BMP are similar to reports for strains recently isolated from the human gut and oral cavity, but different to those observed for another bovine *Methanoplasmatales* strain, which showed only weak growth with methanol/H_2. The findings suggest that the ruminal/gut *Methanoplasmatales* and *Methanosphaera* spp. might be differentiated not only by their C-utilization profiles, but also by their metabolic versatility to reduce these carbon sources for methanogenesis and growth.

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