The importance of the intestinal microbiome for health and disease of animals

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How much do we know about the physiology and pathology of THIS organ?
Intestinal microbiome

- $10^{12} - 10^{14}$ bacterial cells inhabit the mammalian intestine

- more than 50% of cells in the body are bacteria

- several hundred bacterial phylotypes

- intestinal microbes contain 100-fold more genes than the host genome

Human microbiome: 1,000,000+ genes

Human genome: 23,000 genes
development of the intestine

germin-free rodents have

➢ thinner intestinal wall
➢ thinner villi

stimulates immune system
germin-free rodents have

➢ Peyer’s patches ↓
➢ serum IgA & IgG concentrations ↓

Intestinal Microbiota

provides nutritional benefit to host by

➢ production of short-chain fatty acids and vitamins

protects the host against pathogenic bacteria

➢ competition for adhesion sites, luminal substrates
Overstreet et al., The Role of the Microbiota in Gastrointestinal Health and Disease. InTech 2012
Host-microbe communication modulated through immune system and bacteria-derived metabolites
<table>
<thead>
<tr>
<th>metabolic end-products</th>
<th>metabolic activities of intestinal microbiota</th>
<th>effect on host health</th>
</tr>
</thead>
<tbody>
<tr>
<td>propionate, acetate, butyrate</td>
<td>carbohydrate fermentation</td>
<td>anti-inflammatory, energy source of enterocytes, regulation of intestinal motility, amelioration of leaky gut barrier</td>
</tr>
<tr>
<td>Vitamin K2, B12, biotin, folate</td>
<td>vitamin synthesis</td>
<td>important co-factors for various metabolic pathways</td>
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<tr>
<td>indole</td>
<td>degradation of the amino acid tryptophan</td>
<td>increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation</td>
</tr>
<tr>
<td>ceramide</td>
<td>induces degradation of sphingomyelin via alkaline sphingomyelinase</td>
<td>significant role in apoptosis and in the prevention of intestinal epithelial dysplasia and tumourigenesis</td>
</tr>
</tbody>
</table>
Gut Microbiota in Disease

Inflammatory Bowel Disease (IBD)
Metabolic syndrome
Cancer
Asthma
Diabetes
Obesity
Stress related disorders
Healthy dogs have a highly individual microbiota
Healthy dogs have a highly individual microbiota.
DYSBIOSIS IN GI DISEASE
Acute diarrhea

Suchodolski et al., 2012, PLOS ONE
Dog and human inflammatory bowel disease rely on overlapping yet distinct dysbiosis networks

Yoshiki Vázquez-Baeza, Embriette R. Hyde, Jan S. Suchodolski and Rob Knight
PCR based Dysbiosis Index in dogs with Chronic Enteropathies
After FMT

Dysbiosis Index

tylosin
METABOLIC CONSEQUENCES OF GI INFLAMMATION AND DYSBIOSIS
IS DYSBIOSIS CAUSE OR EFFECT OF GI DISEASE?
Dysbiotic gut microbiota causes transmissible Crohn’s disease-like ileitis independent of failure in antimicrobial defence

Monika Schaubeck, Thomas Clavel, Jelena Calasan, Ilias Lagkouvardos, Sven Bastiaan Haange, Nico Jehmlich, Marijana Basic, Aline Dupont, Mathias Hornef, Martin von Bergen, André Bleich, Dirk Haller

Transfer of dysbiotic microbiota to germ-free recipients causes Crohn’s disease-like inflammation in genetically susceptible recipients.

Loss of Paneth cell-related antimicrobial defence is subsequent to the development of ileitis in TNF$^{\text{deltaARE}}$ mice.

Conclusions We provide clear experimental evidence for the causal role of gut bacterial dysbiosis in the development of chronic ileal inflammation with subsequent failure of Paneth cell function.
Winter et al., EMBO 2013
METABOLIC CONSEQUENCES OF GI INFLAMMATION AND DYSBIOSIS
Microbiota in GI health and disease

Intestinal microbiota
in health

Intestinal microbiota
in CE

Figure 3: duodenum – severe and extensive infiltration by small to medium lymphocytes into the epithelium and lamina propria.
Microbiota in GI health and disease

*Intestinal microbiota in health*

*Intestinal microbiota in CE*
Healthy dog

Dog with chronic enteropathy
ABNORMALITIES IN BIOCHEMICAL PATHWAYS

- Carbohydrate metabolism
  - Glycolysis
  - Pentose phosphate pathway
  - TCA cycle

- Lipid metabolism
  - Primary bile acid synthesis
  - Secondary bile acid synthesis
  - Sterol absorption
  - Lipid Oxidation

- Amino acid metabolism
  - Lysine metabolism
  - Aromatic amino acid metabolism
  - Redox homeostasis

- Nucleotide metabolism

- Cofactors and vitamins

- Xenobiotics

Based on analysis of 800 biochemical markers
Honneffer et al, DDW 2015
Feedback from the intestines regulates production of bile acids from the liver.

Secondary bile acids inhibit TNF-α, IL-1β, and IL-6 through activation of TGR5.

Primary bile acids promote germination.

Secondary bile acids inhibit germination of *C. difficile* spores.

Primary BA promote germination.
**BILE ACID DIARRHEA (BAD)**

- under recognized in humans (limited diagnostic testing)
  - approx. 30% of patients with IBS
  - 40% of patients with Crohn’s Disease
  - 1% of total population

- type 1: bile acid malabsorption, secondary to ileal resection or inflammation

- type 2: idiopathic bile acid malabsorption, primary bile acid diarrhea

- type 3: secondary to various GI diseases including small intestinal dysbiosis, radiation enteropathy, celiac disease, chronic pancreatitis, etc.
• bile acid transporter in the **ileum** - 90% of bile acid reabsorption

ASBT IHC expression in brush border in canine ileum
Fecal bile acids in dogs

Blake Guard et al, ACVIM 2016
Ratio of primary to secondary bile acids in feces regulate microbiota in dogs

50% Total Sec BA%

R=0.413
P=0.001
• some dogs with chronic enteropathy (CE) have increased fecal fatty acid due to malabsorption

• bacteria convert fat into hydroxylated, which cause diarrhea

• highly digestible diets with moderate to low fat often useful
**POTENTIAL MECHANISMS**

- Reduction in total bacterial load
- Increases in “beneficial” bacterial groups
  - Metronidazole increases Bifidobacteria
  - Tylosin increases Enterococci
- Direct immunomodulatory effects
  - Metronidazole, tylosin
- Increase in mucus layer thickness
  - Metronidazole
Healthy dogs on various diets switched to Purina HA (hydrolyzed protein)
Metronidazole 14 days

Dysbiosis Index

Baseline 1 week Purina HA 4 weeks HA 6 weeks HA Metro (day 7) Metro (Day 14) 14 days after Metro 28 days after Metro
Taxonomic changes over time for healthy dogs receiving metronidazole
ALTERED PATHWAYS DUE TO METRONIDAZOLE

- Tyrosine metabolism
- Glycine, serine and threonine metabolism
- TCA cycle
- Pyruvate metabolism
- Tryptophan metabolism
- Thiamine metabolism
- Propanoate metabolism
- Glycerolysis/Gluconeogenesis
- Lysine degradation
- Aminoacyl-tRNA biosynthesis
- Galactose metabolism
- Cysteine and methionine metabolism
Conversion from primary to secondary bile acids is disrupted by metronidazole.
Antibiotics Associated With Increased Risk of New-Onset Crohn’s Disease But Not Ulcerative Colitis: A Meta-Analysis

Ryan Ungaro, MD, Charles N. Bernstein, MD, Richard Garry, MB ChB, PhD, Anders Hviid, MSc, Kaija-Leena Koelho, MD, PhD, Matthew P. Kronman, MD, MSCE, Souradot Shaw, MSc, Herbert Van Kuurningen, MD, Jean-Frédéric Colombel, MD, PhD and Ashish.

RESULTS: A total of 11 observational studies (8 case-control and 3 cohort) including 7,208 patients diagnosed with IBD were analyzed. The pooled odds ratio (OR) for IBD among patients exposed to any antibiotic was 1.57 (95% CI 1.27–1.94). Antibiotic exposure was significantly associated with CD (OR 1.74, 95% CI 1.35–2.23) but was not significant for UC (OR 1.08, 95% CI 0.91–1.27). Exposure to antibiotics most markedly increased the risk of CD in children (OR 2.75, 95% CI 1.72–4.38). All antibiotics were associated with IBD, with the exception of penicillin. Exposure to metronidazole (OR 5.01, 95% CI 1.65–15.25) or fluoroquinolones (OR 1.79, 95% CI 1.03–3.12) was most strongly associated with new-onset IBD.

CONCLUSIONS: Exposure to antibiotics appears to increase the odds of being newly diagnosed with CD but not UC. This risk is most marked in children diagnosed with CD.
Fecal Microbiota Transplantation
antibiotics promote \textit{C. difficile} infection by decreasing hydrolysis of bile salts and inhibiting conversion of primary bile acids to secondary bile acids

normal bile acid composition in the colon prevents germination of \textit{C. difficile} spores

antibiotics allow increased levels of bile salts and primary bile acids in the colon, which promote germination of \textit{C. difficile} spores and growth of vegetative forms of bacteria

\textbf{FMT restores normal microbiota and the levels of secondary bile acids}
Fecal microbiota transplantation (FMT) as enema
Dysbiosis index in 16 dogs with chronic diarrhea (after chronic AB use) pre and 1 and 4 weeks post single FMT (enema)

P<0.05

Fecal Score

Total primary BA
p=0.0465
FMT in dogs with chronic intestinal inflammation

Gerbec Ziga, MS thesis Helsinki Univ
SUMMARY

- FMT can improve microbiota
  - correlates with secondary bile acid conversion in subset of dogs

- FMT effect may depend on underlying disease
  - if initial trigger gone (e.g., dysbiosis due to antibiotic use), then lasting improvement of microbiota
  - in chronic enteropathies possible a need for repeated FMT
**Genetics**
- Breed predisposition
  - German shepherd dogs
  - Boxer
  - Others
- SNPs: TLR4, TLR5, NOD2, NCF2

**GI microbiota**
- Dysbiosis
  - ↓: Bacterial diversity
  - ↓: Firmicutes
  - ↑: Proteobacteria

**Immune system**
- Reduced bacterial clearance
- Cytokine imbalance
  - IL-1β vs. IL-1Ra
  - ↑: IL-17, IL-2, TNF-α
  - ↓: CD11c⁺

**Environment/Food**
- Gluten-sensitive enteropathy
- Response to hydrolyzed & protein eliminated diet
ACKNOWLEDGMENTS

- Julia Honneffer, Amanda Blake, Anitha Isaiah, Blake Guard, Paula Giaretta, Yasushi Minamoto
- Members of the TAMU GI Lab
- Stefan Unterer, LMU Munich
- Nicole Luckschander-Zeller, VetMed Vienna, Austria
- Craig Webb, Sara Wennogle, Alison Manchester, Colorado State University
- Steve Hill, VSHSD

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- Nutramax Laboratories
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- Winn Feline Foundation

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- Rob Knight, Yoshiki Vazques, Embriette Hyde, University of San Diego
- Scot Dowd, MR DNA Laboratory
- Jaana Harmoinen, Elias Westermark, Thomas Spillmann, University of Helsinki

AKCCHF
US Dept. of Defense – Office of Naval Research
USDA – NIFA
DHHS-Food and Drug Administration
US Navy
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Effects of the Gastrointestinal Microbiome on Animal Nutrition and Health

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Department of Animal Sciences, Division of Nutritional Sciences, and Department of Veterinary Clinical Medicine
University of Illinois at Urbana-Champaign, USA
Physiologic Importance Known

- Host-microbe symbiosis
  - Fermentation of roughages
  - Gastrointestinal development and immunity
  - Pathogen resistance
  - Energy harvest
  - Host metabolism

Hampered by Methodology

- Limitations of traditional methods
  - How to culture?
  - Identification?
  - Functions?
  - Sampling?

- Coccus
- Coccobacillus
- Vibrio
- Bacillus
- Spirillum
- Spirochete
Dramatic Change in Past Decade

- Cost and speed of next-generation sequencing
- Computing power/bioinformatics
- Ground-breaking data
Current Methodologies

(a) Functional Analysis
- metatranscriptomics, metabolomics, metaproteomics

(b) Genomic Analysis
- high-throughput culturing

(c) Culturomics
- whole genome sequencing

Metagenomics

16S rRNA Gene Sequencing

Functional Inference
- protein/pathway prediction

Phenotyping
- in vitro and in vivo

Taxonomic/Phylogenetic Analysis

Walker, 2016
Microbiome Projects and Beyond

- Reference set of microbial genomes (catalogs)
- Handling of big data
- Microbe-host relationships and disease
- Dietary/drug interventions

Candela et al., 2010; Gevers et al., 2014
Human Data

• Gene catalogs of microbiome

Qin et al., 2010; Nature
Human Data

- Dietary effects

Wu et al., 2011; Science
Ongoing Research Worldwide

• Federal, industry, and private foundations all in
  – Identification, dynamics, and functions
  – Microbe-microbe interactions
  – Microbial metabolism
  – Influence of environment
  – Microbe-host interactions
  – Healthy vs. diseased
Microbial Metabolism

**Host substrates**
- Bile
- Mucus and saliva
- Digestive fluids

**Sugars**
- Lactose
- Sucrose
- Fructose
- Oligosaccharides

**Dietary fiber**
- Cellulose
- Inulin
- Resistant starch

**Proteins and lipids**
- Amino acids
- Peptides
- Saturated lipids
- Unsaturated lipids

**Redox potential**

**Families**
- Bacteroides
- Alistipes
- Parabacteroides
- Prevotella
- Dialister
- Veillonella
- Bifidobacterium
- Atopobium
- Eggerthella
- Fusobacterium
- Methanobrevibacter

**Species**
- Enterococcus
- Streptococcus
- Lactobacillus
- Escherichia coli
- Enterobacteria
- Anoerotruncus
- Catenibacterium
- Bryantella
- Holdemania
- Gordonibacter
- Cl. ramnosome
- Cl. perfringens
- Cl. difficile

Harmsen and de Goffau, 2016
Microbial Metabolism

• Available substrates?
  – Dietary macronutrients
    • Dietary carbohydrates
      – Fibers, RS, oligosaccharides
    • Dietary proteins and lipids
  – Dietary phytonutrients
  – Endogenous secretions

• Factors involved?
  – Raw ingredients; processing; food consumption rate; transit time; etc.

Oberbach et al., 2017
Focus on Fibers

• Diverse and complex group
  – Pectins, cellulose, hemicelluloses, etc.

• Complex polysaccharide breakdown requires metabolic syntrophy
  – Primary degraders
  – Secondary colonizers

Candela et al., 2010
Carbohydrate-Active Enzymes (CAZy)

- CAZy gene families
  - Glycoside hydrolases (>150)
  - Glycosyl-transferases (~105)
  - Polysaccharide lyases (~30)
  - Carbohydrate esterases (~15)
  - Carbohydrate-binding molecules (~80)
  - Auxillary functions (~15)

- Detect, bind, degrade, and import carbohydrates

- Capacity to degrade glycans that hosts cannot

www.cazy.org; Flint et al., 2012
Microbial Metabolism of Carbohydrates

- Gut signaling
- Energy
- pH
Microbial Metabolism of Proteins

- Odor
- Stool quality issues
- Health implications?

Nyangale et al., 2012; Flint et al., 2015
Microbe-Host Co-Metabolism

Claus et al., 2008; Zheng et al., 2011
Host Species Matters

• Goals:
  – Humans/pets: health; length of life; quality of life
  – Livestock: safe food source; health; efficient production

• Categories:
  – Non-ruminant carnivorous and omnivorous mammals
  – Non-ruminant herbivorous mammals
  – Ruminants
  – Poultry
Host Species Matters

- Microbiota:
  - Host genotype
  - Location in gut
  - Life stage
  - Environment
    - Diet
    - Water
    - Antibiotics

Yeoman and White, 2014
Host Species Matters

• Many similarities
  – Functions of gut microbiota
  – Microbial taxa
  – Functional capacity
  – Fermentation patterns

<table>
<thead>
<tr>
<th>Item</th>
<th>Species</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Dog</td>
</tr>
<tr>
<td>OMD, %</td>
<td>36.9^d</td>
</tr>
<tr>
<td>SCFA production</td>
<td></td>
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<tr>
<td>Acetate</td>
<td>1.70^d</td>
</tr>
<tr>
<td>Propionate</td>
<td>.51^d</td>
</tr>
<tr>
<td>Butyrate</td>
<td>.23^d</td>
</tr>
<tr>
<td>Total SCFA^c</td>
<td>2.44^d</td>
</tr>
<tr>
<td>Lactate production</td>
<td>.15^d</td>
</tr>
</tbody>
</table>

Sunvold et al., 1995
Unique Aspects of Host Species

• Living environment
• Diet type
• Metabolic capabilities
• Physiological adaptations
• Gastrointestinal transit rate/time
• Response to fibers/prebiotics fermentation and as energy source
Dogs and Cats – Food Options

• Wide variety
  – Ingredients and diet formats
  – Nutrient composition
    • Macro- and micronutrients
    • Phytochemicals

• Market drivers
  – Health (perceived)
  – Humanization
  – Convenience
Fibers and Prebiotics

• Microbial activity often altered
  – Stool characteristics
  – ↑ fecal SCFA and ↓ fecal pH
  – ↓ fecal protein catabolites
  – Moderate shifts in microbiome

Beloshapka et al., 2014: Panasevich et al., 2015
Macronutrient Ratios

- Kittens
  - ↑ HP = ↑ diversity
  - 324 bacterial genera altered
  - 2,000 genes and 194 networks altered
Macronutrients and Diet Form

- Diet composition (% DM basis)
  - Kibble: 24% protein; 12% fat; 12% fiber
  - Raw meat: 24-32% protein; 50-60% fat; 1-5% fiber

Beloshapka et al., 2011
Effects Beyond the Gut

Color by Group
- CD
- HC
- HF
- HP

HF Diet
HC Diet
HP Diet

Deng et al., 2014
Effects Beyond the Gut

• Shift, in part, by microbe-derived metabolites

Deng et al., 2014
Cattle – Rumen Microbiome

Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases

Jennifer M. Brulc\textsuperscript{a}, Dionysios A. Antonopoulos\textsuperscript{b}, Margret E. Berg Miller\textsuperscript{a,c}, Melissa K. Wilson\textsuperscript{a}, Anthony C. Yannarell\textsuperscript{a,c}, Elizabeth A. Dinsdale\textsuperscript{d,e}, Robert E. Edwards\textsuperscript{d,f,g,h}, Edward D. Frank\textsuperscript{i}, Joanne B. Emerson\textsuperscript{l}, Pirjo Wacklin\textsuperscript{l}, Pedro M. Coutinho\textsuperscript{l}, Bernard Henrissat\textsuperscript{l}, Karen E. Nelson\textsuperscript{l}, and Bryan A. White\textsuperscript{a,c,l}

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Table 2. Comparative analysis of cellulosome-based systems between the \textit{Clostridium thermocellum} (C.T.) genome and other metagenomic microbiomes

<table>
<thead>
<tr>
<th>CAZy family*</th>
<th>C.T. genome\textsuperscript{1}</th>
<th>C.T. 454 model</th>
<th>Pooled liquid</th>
<th>Fiber-8</th>
<th>Fiber-64</th>
<th>Fiber-71</th>
<th>Termite hindgut\textsuperscript{2}</th>
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<tbody>
<tr>
<td>GH1</td>
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<td>7</td>
<td>4</td>
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<td>ND</td>
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<td>Total CBM</td>
<td>62</td>
<td>331</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>3</td>
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<td>Total CE</td>
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<tr>
<td>Total GH</td>
<td>70</td>
<td>1099</td>
<td>1108</td>
<td>1005</td>
<td>1105</td>
<td>610</td>
<td>636–703</td>
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</table>

\textsuperscript{1}NR: Not Relevant. \textsuperscript{2}ND: Not Determined.
Rumen Microbiome Functionality

Rumen Cellulosomics: Divergent Fiber-Degrading Strategies Revealed by Comparative Genome-Wide Analysis of Six Ruminococcal Strains

Bareket Dassa¹, Ilya Borovok², Vered Ruimy-Israeli¹, Raphael Lamed², Harry J. Flint³, Sylvia H. Duncan³, Bernard Henrissat⁴, Pedro Coutinho⁴, Mark Morrison⁵,⁶, Pascale Mosoni⁷, Carl J. Yeoman⁸, Bryan A. White⁹,¹⁰, Edward A. Bayer¹

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Figure 1. Blueprints of the cellulosome-related proteins

- Cohesin
- Dockerin
- X-Dockerin
- CBM
- Enzyme
- Sortase motif

Dassa et al., 2014
Whole Body Metabolism

McCann et al., 2014
Swine

• Dual purpose
  – Swine industry
    • Piglet mortality/morbidity
    • Ingredient evaluation
    • Animal performance

  – Biomedical model
    • Gastrointestinal health/disease
    • Nutritional intervention
    • Brain development

The Suckling Piglet as an Agrimmedical Model for the Study of Pediatric Nutrition and Metabolism

Jack Odle, Xi Lin, Sheila K. Jacobi, Sung Woo Kim, and Chad H. Stahl

Laboratory of Developmental Nutrition, Department of Animal Science, North Carolina State University, Raleigh, North Carolina 27695; email: jack_odle@ncsu.edu, lin_xi@ncsu.edu, sheila_jacobi@ncsu.edu, sungwoo_kim@ncsu.edu, chad_stahl@ncsu.edu

Odle et al., 2014
Swine

Dietary Fat Content and Fiber Type Modulate Hind Gut Microbial Community and Metabolic Markers in the Pig

Hui Yan¹, Ramesh Potu¹, Hang Lu¹, Vivian Vezzoni de Almeida², Terry Stewart¹, Darryl Ragland³, Arthur Armstrong⁴, Olayiwola Adeola¹, Cindy H. Nakatsu⁴, Kolapo M. Ajuwon¹*

1 Department of Animal Sciences, Animal Production Management, Purdue University, Lafayette, Indiana, United States

RESEARCH ARTICLE

The impact of high dietary zinc oxide on the development of the intestinal microbiota in weaned piglets

Ingo C. Starke¹, Robert Pieper¹, Konrad Neumann², Jürgen Zentek¹ & Wilfried Vahjen¹

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²Department of Animal Sciences, Animal Production Health Management, Purdue University, Lafayette, Indiana, United States

Early Postnatal Diets Affect the Bioregional Small Intestine Microbiome and Ileal Metabolome in Neonatal Pigs

Brian D Piccolo¹,², Kelly E Mercer¹,², Sudeepa Bhattacharyya¹,², Anne K Bowlin¹,², Manish K Saraf¹,², Lindsay Pack¹, Sree V Chintapalli¹,², Kartik Shankar¹,², Sean H Adams¹,², Thomas M Badger¹,², and Laxmi Yeruva¹,²,³

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³Department of Animal Science, Purdue University, Lafayette, Indiana, United States
Swine Gut

Piccolo et al., 2017
Poultry

- Main gut microbial phyla similar to other vertebrates

Waite and Taylor, 2014
Poultry

• Carnivorous vs. herbivorous birds?
  – Fusobacteria ↑ carnivores (similar to dogs/cats)
Chicken Cecal Microbiome

- >200 genes capable of degrading non-starch polysaccharides
  - Many glycoside hydrolase (GH) families

Deusch et al., 2015
In Ovo Injections

- Various biologics commonly used
  - Additional nutrients for embryo
    - Carbohydrates
    - Amino acids
  - Immunostimulants
  - Probiotics, prebiotics, and synbiotics

Roto et al., 2016
Equine

• Health and longevity
• Key diseases

Dougal et al., 2013; Ericsson et al., 2016
Equine

- Modern feeding practices
  - Concentrate feeds
  - Meal feeding
    - Black (1X/d); red (2X/d); blue (3X/d)

Venable et al., 2017
Equine

- Travel stress

Perry et al., 2018 (in press)
Summary Points and Future Needs

• Emerging view of microbiome and host health
  – New tools = new opportunities

• Wide-reaching implications
  – Humans, livestock, & companion animals
  – Host similarities and differences

• Microbiome still a secondary outcome; much is largely unknown
  – Healthy vs. disease
  – What diet-induced changes are clinically relevant?
  – Microbial taxa vs. biological potential vs. activity
Summary Points and Future Needs

• Future needs?
  – Molecular microbiology
  – Host-microbe relationships
  – Longitudinal sampling
  – Appropriate sampling
  – Microbial activity

• Bottlenecks
  – Data handling
  – Brain power
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Questions?
WHY THE INTERSECTION OF NEUROBIOLOGY AND MICROBIOLOGY MATTERS TO ANIMAL NUTRITION

Mark Lyte, Ph.D., M.S., MT(ASCP)
Iowa State University, College of Veterinary Medicine

April 4, 2018
Mid-Atlantic Nutrition Conference
What I have learned...

- Purpose of talk is to not review this literature, but instead introduce a new perspective that may lead to innovative ways of utilizing nutrition to benefit animal health.

- Also learned how a "longer" perspective helps view the microbiome field and where it may be headed.

- Personal perspective – increasingly hearing it from others.
Structure of talk

- Introduction and recognition of bacteria as neuroendocrine organisms.
  - What prompted creation of field
    - Evolutionary vantage point

- What does this mean for understanding the complex interactions between host (stress, etc.), diet and microbiota that may influence disease progression and health in a wide range of animal species from companion to farm production.
  - Consideration of microbiota from neuroendocrine vantage point allows introduction of new approaches to nutrition and health maintenance.

- Concept of neuroendocrine-bacterial interactions has been termed Microbial Endocrinology.
What is happening...

- We are at a critical juncture concerning microbiome and health.
  - Human-related is in bubble phase
- Animal nutrition offers unique opportunity to explore microbiome-health interactions.
  - Especially given the removal of antibiotics

Microbial communities and how they interact with each other and bi-directionality with host.

With removal of antibiotics everything has changed – tremendous opportunities exist in forging new paradigms.
Research has established the extent of microbiome effects on host health. But what does this mean for the animal farm production industry? How do these findings impact the interaction of the microbiome with nutrition and your specific product or practice? All of this applies to farm animal health as well.

Nutrition and the microbiome

- Large, ever-expanding literature that extends back decades.
  - Not the purpose of this talk to serve as a review.
- Talk will cover way to interpret the data based on evolutionary-based approach linking the components of nutrition with the microbiome and its interface with the host.
- Use of neurochemistry as a “common evolutionary language” in which all elements, host, microbiota and nutritive, interact.
- It is fully recognized that microbial endocrinology is one of the possible mechanisms and that a vast array of other possibilities, such as the production of short chain fatty acids, exist and need to be explored.
Ever before anything gets “into” your animal, it first comes in contact with the microbiota.

Having talking about the role of neurochemicals in food as regarding the microbiota, but they can also directly influence the host immune response.

Not saying there is no role for the immune response, quite the contrary.

Use of neurochemistry as a “common evolutionary language” in which all elements, host, microbiota and nutritive, interact.

It is fully recognized that microbial endocrinology is is one of the possible mechanisms and that a vast array of other possibilities, such as the production of short chain fatty acids, exist and need to be explored.
What do we really know about what is in animal feeds?

But what else could be present that influences the animal — either directly or indirectly?

Feeds that appear to be the same may result in different biological effects in animals.
Microbial endocrinology defined

Microbiology

Neurobiology

Evolution as a theme throughout

Disease

Behavior

GUT

NUTRITION

HEALTH

STRESS

MICROBIAL ENDOCRINOLOGY
Where are areas that microbiota and host interact?

So, why should a hormone that is produced in the milk influence the functional activity of a member of the human GI tract?

And more importantly, melatonin is not secreted into milk to benefit the human population – is there an effect meant for the calf microbiome?

Swarming behavior in E. aerogenes is induced by melatonin and occurs with a circadian frequency.

Control Melatonin (1 nM)
Stress-immune interactions

STRESS → IMMUNITY → DISEASE

IS THIS THE COMPLETE STORY?
Free range is not necessarily stress free

- Mortality was twice as high in aviary reared hens compared to cage reared hens during their laying period
  - Tahamtani et al. 2014 Sep 17;9(9):e107357014
- Mortality due to injurious pecking which is a social stress

https://commons.wikimedia.org/w/index.php?curid=3981994
What led to development of microbial endocrinology?

Phagocytosis increased >500%
Does stress protect from infection?

- Social conflict stress and then gave orally the common food pathogen *Y. enterocolitica*.

*Does this make evolutionary sense??* *For whom??*
Stress-immune interactions - reconsidered

Whose survival are we talking about?
Are microorganisms neurochemical responsive organisms?
Ubiquity of neurochemicals in food chain:

Potato plants contain the catecholamines norepinephrine and dopamine

- Szoba et al., Phytochemistry, 58:315-20, 2001
Presence of neurochemicals in food

- **Bananas**
  - 700 µg/g dopamine and 70 µg/g NE
  - Division between peel and pulp

- **Tribal pulses**
  - 8 g of L-Dopa per 100 g of flour
  - Resistant to destruction by autoclaving and boiling

- **Other common foodstuffs**
  - Tomatoes - dopamine, tyramine
    - Use of psychoactive drugs and MAOI restrictive diets
  - Cheese - tyramine

- And various neurochemicals present in lab animal feeds
What’s the Connection?
- Pigs are used to hunt truffles.
  - Why?
- Androsterol
  - But who came first?
Presence of neurochemicals in the microbial world

- **Bacteria**
  - Insulin-like material - present in all strains examined
  - GABA – Clinical bacterial pathogens
  - Somatostatin - *Bacillus subtilis*
  - Catecholamines – *E. coli*
  - Specific receptors have been demonstrated – 100% homology of *E. coli* EnvY gene for high affinity opioid binding site.
  - Probiotics produce neurochemicals such as GABA
  - Tryptamine – Human microbiota

- **Protozoa**
  - Catecholamines - *Crithidia fasciculata, Paramecium*
  - Serotonin - *Tetrahymena pyriformis*

- **Fungi**
  - Sex pheromone - Truffles (Androsterol)

Do neurochemicals affect bacteria?
Relevance of microbial endocrinology to infectious disease

Each condition possesses common sets of conditions applicable to microbial endocrinology.

Campylobacter jejuni
Staphylococcus epidermidis

Microbiota - gut - brain axis
First observation: *Yersinia enterocolitica*

Lyte & Ernst, Life Sci. 50:203-12, 1992
Pre-treatment with norepinephrine increases virulence of Y. enterocolitica

One week post-challenge Y. enterocolitica pre-treated with norepinephrine 50,000X more infective.

Clinical relevance of microbial endocrinology

MECHANISMS OF DISEASE

Mechanisms of disease

Lancet 361:130-5, 2003

Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes
“Low hanging fruit” – Campylobacter spp.

- What do we know about the neuroendocrine environment of the gut, especially in farm production animals?

Common theoretical thread:

- There is an evolutionary relationship between microorganisms and host.
- Evolution of cell-cell signaling in animals may be due to late horizontal gene transfer from bacteria.  
- Microorganisms, such as those in the gut (really everywhere), do not simply rely on traditional nutritive (energy) sources for their survival and behavior.
- Concept of direct neuroendocrine-bacterial interactions means bacteria interactive player.
Gut – Where neurochemicals and bacteria meet

- Production and metabolism of norepinephrine and dopamine within mesenteric organs over 50% total body.

- Within lumen of GI tract physiologically relevant levels of neurochemicals:
  - Serotonin.
  - Norepinephrine and dopamine.
    - Produced by luminal bacteria

- Dietary sources:
  - Foods are a rich source of neurochemicals
Microbiota-gut-brain axis

1. Food-derived substrates and neurochemicals
2. Enteric nervous system (ENS)
3. Uptake into portal circulation
4. Behavior and cognition
5. Correlation and causation

Lyte, Gut Microbes, 5:381-9, 2014
Differential innervation along the intestinal tract means the microbial biogeography of the alimentary canal is not continuous as well.

What do we know about the innervation of the alimentary canal of poultry?

How extensive is the innervation in the ENS where bacteria are in close proximity?

Vagal villus afferents

Question is where does information flow and possible bi-directionality

Powley et al. Journal of Comparative Neurology 519:644-60, 2011
Intimate associations: Gut to brain

- Enteroendocrine cells can connect to sensory neurons to create a neuroepithelial circuit.

Gut vagal afferents modulate behavioral responses

- What is consequence of this “bottom-up” information flow from gut-to-brain?

- Klarer et al. performed sub-diaphragmatic deafferentation

Take home message: Emphasizes an important role of afferent visceral signals in the regulation of emotional behavior

- Klarer et al. Journal of Neuroscience 34:7067-76, 2014

Anxiety-like behavior

Anxiolytic effect
Where we have come from...

- Emergence of human microbiome and what it means for behavior in a microbiota-gut-brain axis:

  - From 1914: "The control of man’s diet is readily accomplished, but mastery over his intestinal bacterial flora is not... They are the cases that present...malaise, total lack of ambition so that every effort in life is a burden, mental depression often bordering upon melancholia...A battle royal must be fought and when this first great struggle ends in victory for the Bacillus bulgaricus it must be kept on the field of battle forever at guard...”

  - Stow, Medical Record Journal of Medicine and Surgery, 1914
Microbial endocrinology-based mechanisms mediating the bi-directional effects of social stress.
Bacteria in the gut are “seen” by the brain

- First “modern-era” demonstration of microbiota-gut-brain

- Introduction of novel bacterial species
  - Critical that bacterial species chosen does not cause overt immune response or systemic infection
  - Use of live, replicating organism instead of killed or antigen
    - Campylobacter jejuni – infection/diarrhea not produced

- Natural infection route
  - Per oral for C. jejuni

- Measure behavior
  - Apparatus used in psychopharmacology
  - Anxiety-like behavior

Bacteria in gut induce anxiety-like behavior:

Bacteria in gut can activate neurons in brain:

Nutrition and the microbiome through the lens of microbial endocrinology

- Neuroendocrine-bacterial interactions imply a long evolutionary pathway where mammalian neurochemicals evolved first in bacteria.
- Evolutionary symbiosis implies that clinical conditions involving both the gut (where the bugs are) and brain (which interprets gut signals) can be viewed in a new paradigm.
- Can nutritive approaches be used as a means to alter the microbiota and thereby influence production of neurochemicals within the gut?
- If so, a new spectrum of nutritive approaches to benefit the animal can be identified which utilize microbial endocrinology as a governing mechanism.
Experimental design

- Use of meat-based diet to alter microbial flora
  - Literature shown mice readily consume meat-based diet with consistent changes in composition of bacterial flora
    - Past literature solely culture-based
  - Effects of diet on behavior always ascribed to direct effect on neural substrates
- Feed mice meat-based diet and use pyrosequencing to examine bacterial diversity
- Measure behavior
  - Anxiety-like behavior
  - Memory and learning

CAVEAT: CORRELATIONAL STUDY – Not direct cause and effect

FIRST STEP
Effect of diet on bacterial diversity

Control (PP) Diet

Meat (BD) Diet

Li et al., Physiology and Behavior 96:557-67, 2009
Memory testing – Barnes maze

Animal 13 (Test 1)

Animal 22 (Test 5)
Short-term memory: stress-microbiome-diet interactions

Barnes maze

Microbiome currently being sequenced
Returning back to farm animal nutrition and the central questions

What is the way forward? What is the science telling us? Where do we need to go?

The study of microbial endocrinology will be crucial to improving poultry health from issues of well-being to infectious disease.

- What is the way forward? What is the science telling us? Where do we need to go?
Overriding issues...

- Mechanism, mechanism, mechanism.
  - Without it we will be groping in darkness.

- Any mechanism proposed must at some level include neurochemistry.

- It is a very long way from the gut (outside) to the brain (inside).
  - Need “touchable” means for veterinarians, nutritionists, etc. to utilize.
  - What about “microbial biogeography”?

- Use of microbial endocrinology as an evolutionary-based mechanism.
  - Almost certainly not the only mechanism.
Getting to causation

The Neurotransmitter Transporter Uptake Assay principle

Critical: Need adherent cells as this is a bottom-read assay.

Bacteria possess biogenic amine-like transporters

- Biogenic amine transporters in mammalian cells
  - Two types:
    - PMAT: Plasma membrane monoamine transporter
    - OCT: Organic cation transporter
  - PMAT and OCT are main targets of psychotherapeutic drugs for depression and anxiety-related disorders

![Graphs showing 6 Hour Biofilm at 37°C and 24 Hour Biofilm at 37°C with RFUs on the y-axis and Hours on the x-axis, comparing L. salivarius - PMAT, L. salivarius - OCT, L. rhamnosus - PMAT, and L. rhamnosus - OCT](Lyte and Brown, PLoS ONE 13(1):e0191037)
Probiotics as neurochemical delivery system to influence behavior

<table>
<thead>
<tr>
<th>Genus</th>
<th>Neurochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus, Bifidobacterium</td>
<td>GABA</td>
</tr>
<tr>
<td>Escherichia, Bacillus, Saccharomyces</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>Candida, Streptococcus, Escherichia, Enterococcus</td>
<td>Serotonin</td>
</tr>
<tr>
<td>Bacillus, Serratia</td>
<td>Dopamine</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>Acetylcholine</td>
</tr>
</tbody>
</table>

*Neurochemical levels produced by probiotics are physiologically relevant – often in the mg/ml level*

Future – Where we are headed

We are at beginning of understanding microbiome-host interactions and what determines health and disease progression in poultry and other farm production animals, which will ultimately translate to human health.

- Microbiome

Microbial endocrinology provides for an evolutionary-based approach to understand and utilize shared neurochemistry between host and microbe to improve health.

- Non-nutritive

- Use in improving feed efficiency

Always keep in mind: CORRELATION vs. CAUSATION
As already mentioned...

Research is all well and good –

And what are the “take-home” lessons for your particular company if you are interested in the interaction of the microbiome with your specific product or practice?

What are you paying for?
Science isn’t necessarily helping…

“There’s a gap between the promise of the microbiome and the current science. It has been filled with misconceptions, snake oil and hype: microbiomania”

“Microbiome research is incomplete. We simply do not know what many microbial genes do”

In the wake of the White House’s latest “moonshot” – an initiative to understand the microbiome – New Scientist cuts through the crap about our internal flora and probiotics
Why the emergence?

- Due to technology
  - Prior we could only “see” what we could culture
  - Preponderance of work on pathogens

CAVEAT: Technology is rapidly changing which means what is there with one technology platform may not be there with another.
The obvious...

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type.1</td>
<td>Separate hard lumps, like nuts (hard to pass)</td>
</tr>
<tr>
<td>Type.2</td>
<td>Sausage-shaped but lumpy</td>
</tr>
<tr>
<td>Type.3</td>
<td>Like a sausage with cracks on its surface</td>
</tr>
<tr>
<td>Type.4</td>
<td>Like a sausage, smooth and soft</td>
</tr>
<tr>
<td>Type.5</td>
<td>Soft blobs, clear cut edges (passed easily)</td>
</tr>
<tr>
<td>Type.6</td>
<td>Fluffy pieces, ragged edges, mushy stool</td>
</tr>
<tr>
<td>Type.7</td>
<td>Watery, no solid pieces. Entirely liquid</td>
</tr>
</tbody>
</table>

What we think we know... And what we **really** know...

- So what does it say about uniting the two?
- "Computational Challenges to..."  
- Bioinformatics-based approaches may not yield same result with same set of data.
  - When experiments using only 11 known species were evaluated with programs using reference databases, "... dozens to hundreds of species were falsely predicted by the most popular programs."  
  - Peabody *et al.* BMC Bioinformatics (2015) 16:363
- It’s not as much as who are the players and their numbers, as what are the players doing
  - Otherwise known as “What are the mechanisms”
Probiotics – an instructive example

- Why this one, why not that one?
- You find one that works, but it is not consistent.
- How do you know the next one is not even better for your situation?
- Do you understand even how they are made?
And, whenever we still think we’re the first...